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VCE | BIOLOGY UNITS 3&4



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Cover Photograph

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Sugar gliders *(Petaurus breviceps)* are extremely active animals that can glide up to 45 metres. Groups of sugar gliders are mutually exclusive and territorial. Each group defends a certain number of eucalyptus trees which provide the group with its staple food source. https://animaldiversity.org/accounts/Petaurus_breviceps/

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BIOZONE Learning Media Australia

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

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

www.BIOZONE.com.au

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About the Authors



Dr Tracey Greenwood *Senior Author*

Tracey has been writing resources for students since 1993 when she joined BIOZONE and enjoys the challenge of curriculum analysis and content development. Tracey has a PhD in biology, specialising in lake ecology and has taught both graduate and undergraduate biology, including implementing courses for medical intermediate students and remedial and foundation programs for nursing students. Since joining BIOZONE, Tracey has been a team leader in the design, development and production of targeted curricula for students and teachers in Australia, New Zealand, the UK and the US.



Kent Pryor *Author*

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



Lissa Bainbridge-Smith *Author*

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

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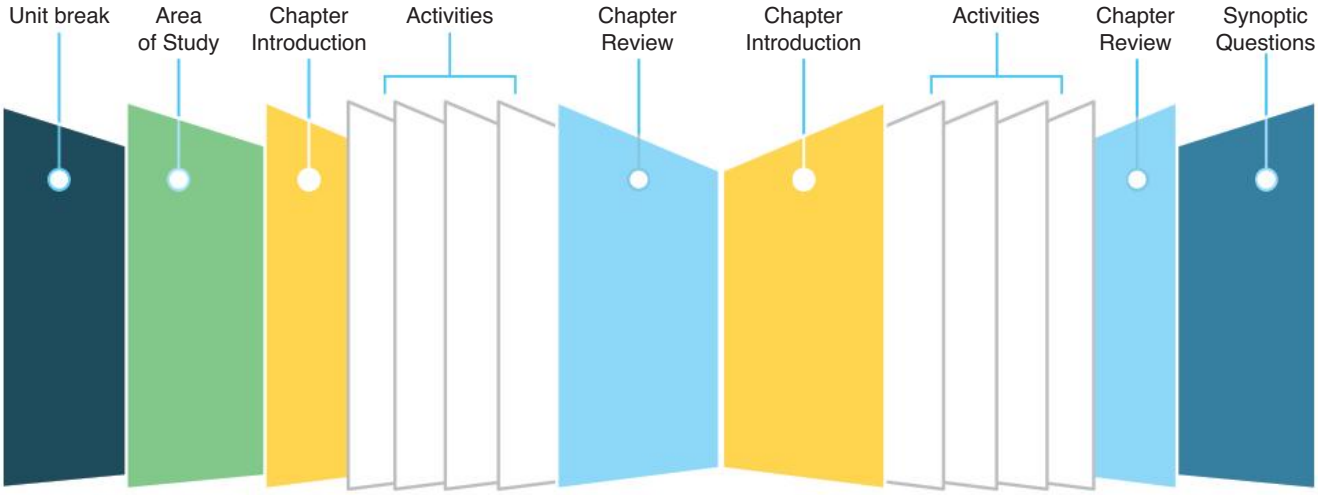
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Using This Workbook

This edition of Biology for VCE Units 3 & 4 has been specifically written to meet the content and skills requirements of the Victorian Certificate of Education, Biology, Units 3 and 4. The workbook follows the unit structure outlined in the Study Design, so it is easy for you to know where you are in the course. Unit and Area of Study breaks help you navigate through the content. Each chapter has an introduction page so you can see the key knowledge and skills requirements for each chapter. You can review and test your understanding, and prepare for assessments and exams by carrying out the Chapter Review and Synoptic Question activities.

- A structure of a unit is outlined below, it will help you identify the features within each unit.



Chapter introduction

- A check list of key knowledge.
- A list of key terms.

Activity pages

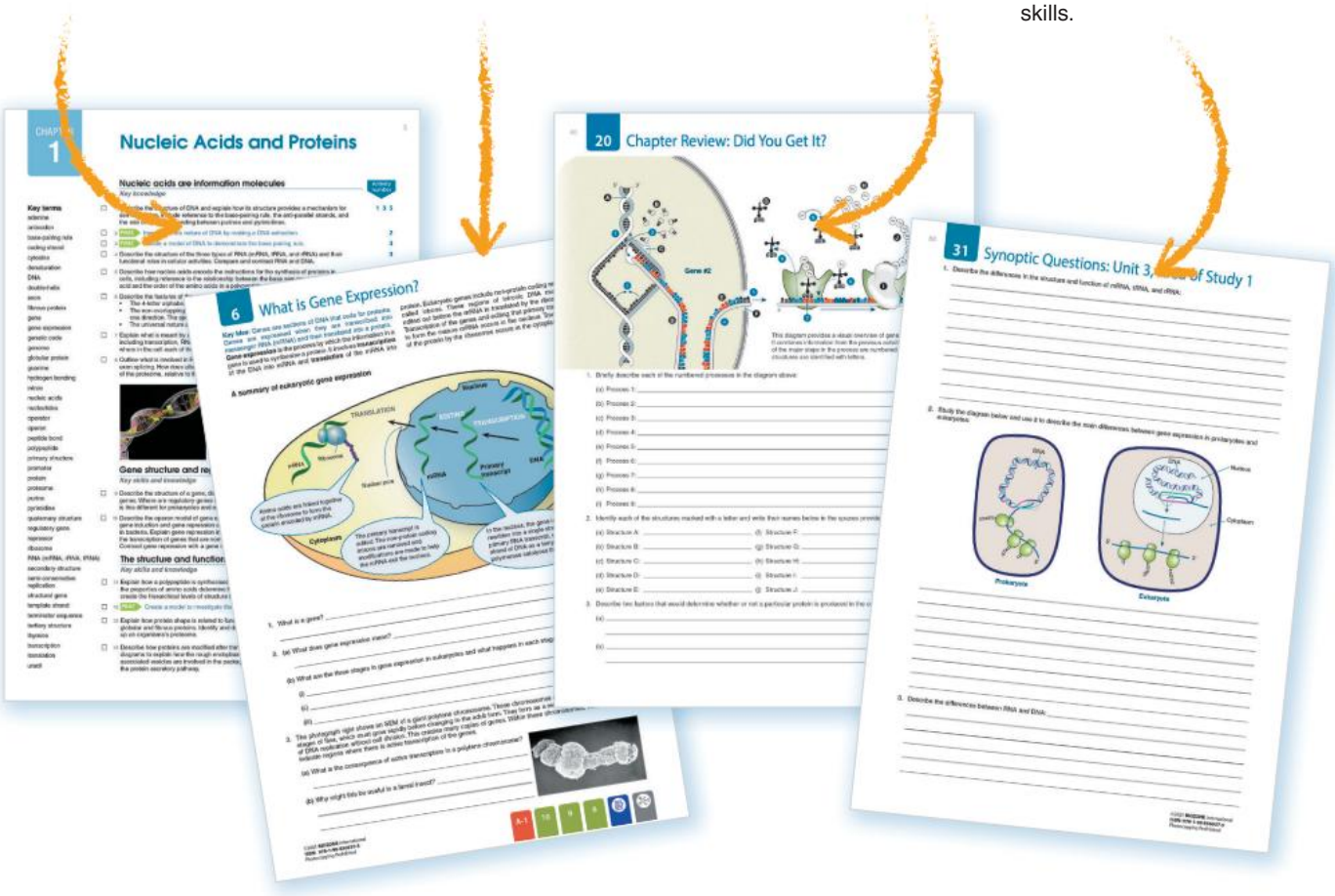
- Contain essential knowledge.
- Questions review the content of the page.

Chapter review

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

Synoptic questions

- Synoptic questions conclude the unit and area of study covered in the workbook.
- Practise your written exam skills.

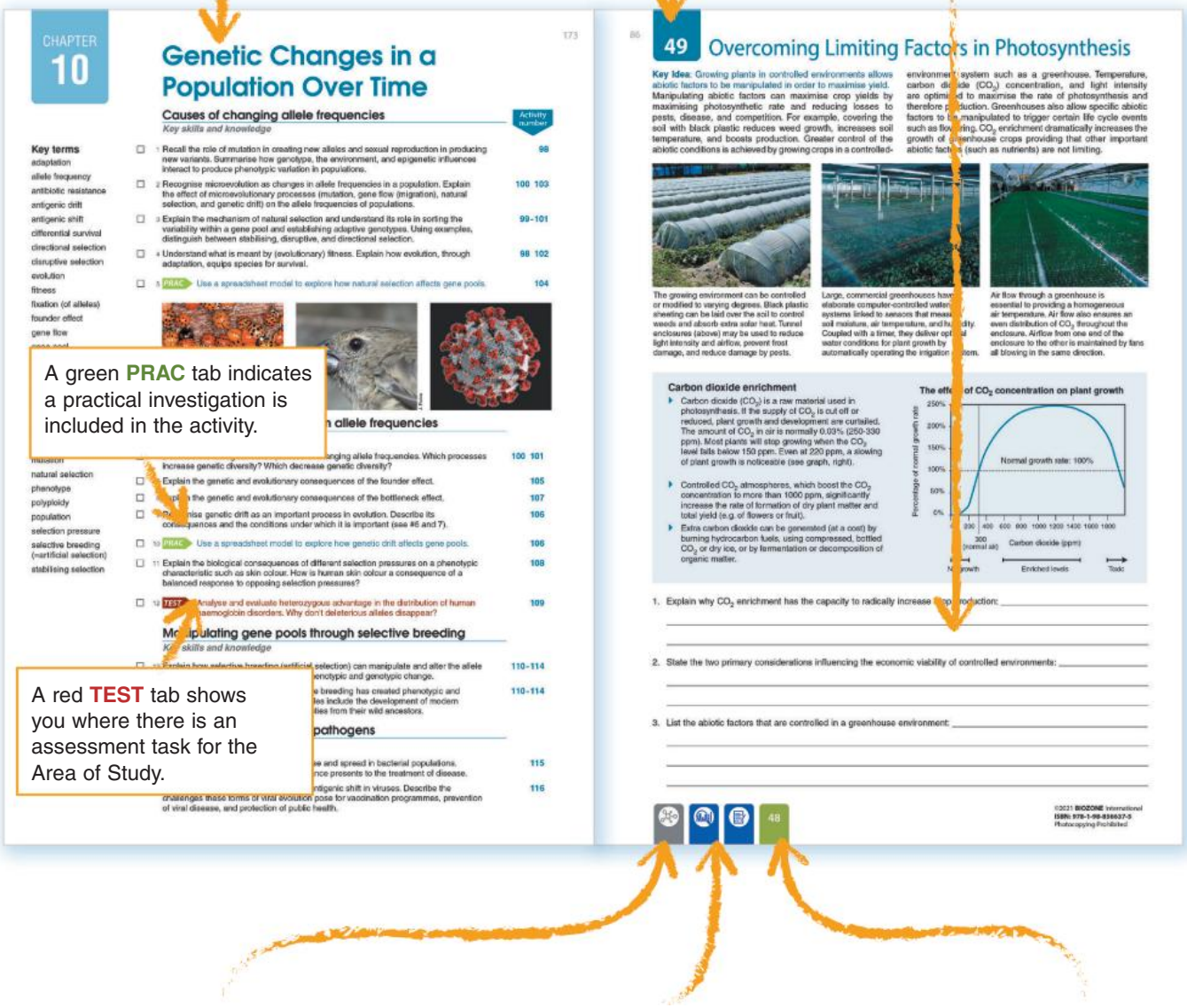


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The **chapter introduction** provides you with a summary of the knowledge and skills requirements for the topic, phrased as a set of learning outcomes. Use the check boxes to identify and mark off the points as you complete them. The chapter introduction also provides you with a list of key terms for the chapter, to help you practise using scientific language.

The **activities** form most of this workbook. The activity number is found at the to of the first page. Each activity has a short introduction with a key idea identifying the main message of the page. Most of the information is associated with pictures and diagrams.

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



Resource Hub tabs at the bottom of the activity page show where external, online support material is provided for an activity. The types of resources vary and include animations, video clips, photo libraries, articles, or quizzes. Bookmark the link to Resource Hub (see page ix) and visit it often as you progress through the workbook.

Pictograph tabs identify where a **key science skill** is covered. A full description of the pictograph codes is given on the following page.

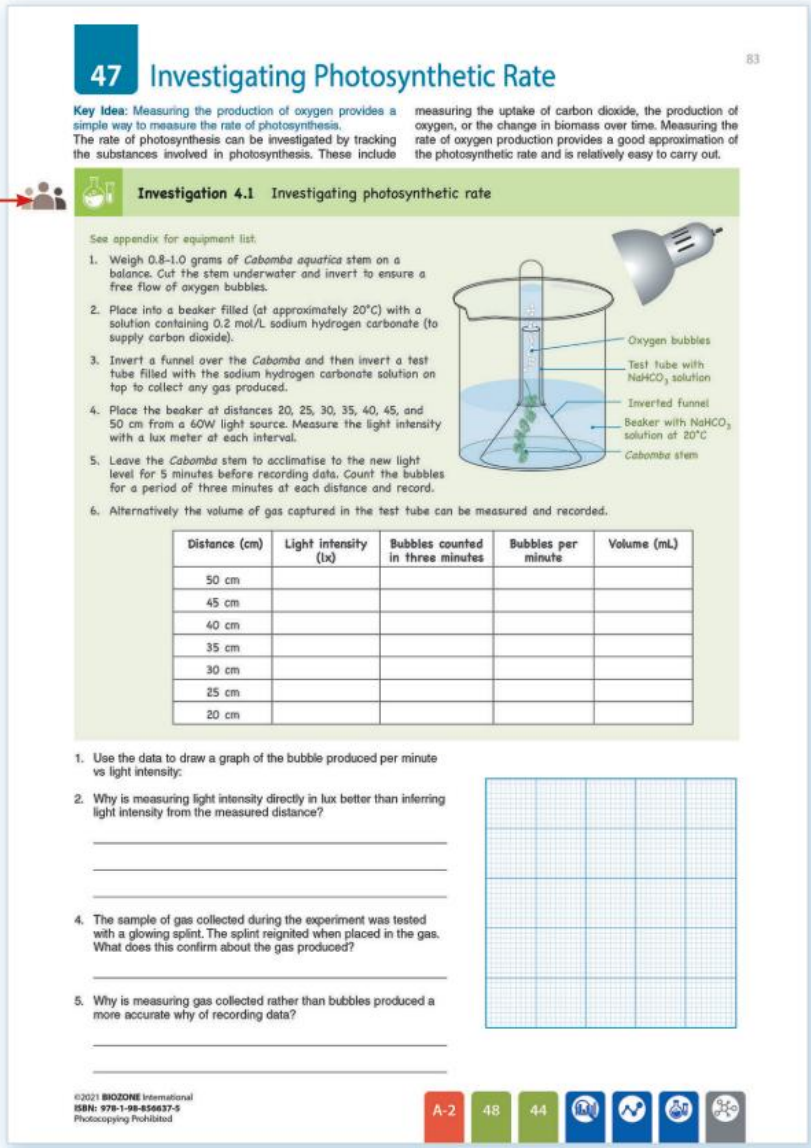
Connect tabs at the bottom of the activity page identify activities that are related in that they build on content or apply the same principles to a new situation.

Using the Tab System

- The tab system is a useful way to identify important parts of the VCE Biology course. The tabs also allow you to see at a glance if online support is provided and if there are content links with other activities.

A group icon in the margin shows where you can work in pairs or small groups to complete a task or activity.

Working in groups allows you to experience the benefits of collaboration. Scientific vocabulary is extended as you listen to the ideas of others and share and discuss your own ideas.



47 Investigating Photosynthetic Rate

83

Key Idea: Measuring the production of oxygen provides a simple way to measure the rate of photosynthesis. The rate of photosynthesis can be investigated by tracking the substances involved in photosynthesis. These include measuring the uptake of carbon dioxide, the production of oxygen, or the change in biomass over time. Measuring the rate of oxygen production provides a good approximation of the photosynthetic rate and is relatively easy to carry out.

Investigation 4.1 Investigating photosynthetic rate

See appendix for equipment list.

1. Weigh 0.8–1.0 grams of *Cabomba aquatica* stem on a balance. Cut the stem underwater and invert to ensure a free flow of oxygen bubbles.
2. Place into a beaker filled (at approximately 20°C) with a solution containing 0.2 mol/L sodium hydrogen carbonate (to supply carbon dioxide).
3. Invert a funnel over the *Cabomba* and then invert a test tube filled with the sodium hydrogen carbonate solution on top to collect any gas produced.
4. Place the beaker at distances 20, 25, 30, 35, 40, 45, and 50 cm from a 60W light source. Measure the light intensity with a lux meter at each interval.
5. Leave the *Cabomba* stem to acclimatise to the new light level for 5 minutes before recording data. Count the bubbles for a period of three minutes at each distance and record.
6. Alternatively the volume of gas captured in the test tube can be measured and recorded.

Distance (cm)	Light intensity (lx)	Bubbles counted in three minutes	Bubbles per minute	Volume (mL)
50 cm				
45 cm				
40 cm				
35 cm				
30 cm				
25 cm				
20 cm				

1. Use the data to draw a graph of the bubble produced per minute vs light intensity:
2. Why is measuring light intensity directly in lux better than inferring light intensity from the measured distance?
4. The sample of gas collected during the experiment was tested with a glowing splint. The splint reignited when placed in the gas. What does this confirm about the gas produced?
5. Why is measuring gas collected rather than bubbles produced a more accurate why of recording data?

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Page tabs show where key science skills are specifically addressed. Some activities may incorporate using more than one skill. The tabs also indicate where is online support, links to activities with related or background content, or useful reference material in an appendix.

Grey hub tabs indicate the activity is supported on the Resource Hub. See page v for details.

Green tabs make connections to related activities elsewhere in the book



Blue tabs indicate the activity covers the following key skills (L → R):

- Develop aims and questions, formulate hypotheses, make predictions
- Plan and conduct investigations
- Comply with safety and ethical guidelines
- Generate, collate, and record data
- Analyse and evaluate data and investigation methods
- Construct evidence-based arguments and draw conclusions
- Analyse, evaluate and communicate scientific ideas

Red tabs indicate appendices (L → R):

- A-1: Glossary
- A-2: Equipment list

See pages 311-315

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Using BIOZONE's Resource Hub

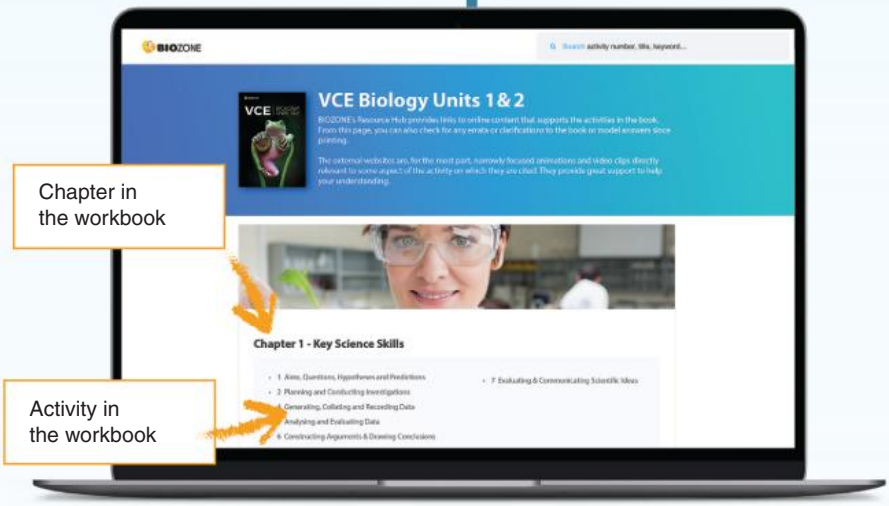
- BIOZONE's **Resource Hub** provides links to online content supporting the activities in the book. From this site (below), you can also explore BIOZONE's collection of annotated 3D models and check for any errata or clarifications to the book or model answers since printing. Activities with Resource Hub support are indicated by a **grey tab** (below) at the bottom of the activity page. Most activities have resources to support them.
- The external websites are generally narrowly focussed animations, illustrated content, or video clips relevant to some aspect of the activity on which they are cited. They provide great support to help your understanding.

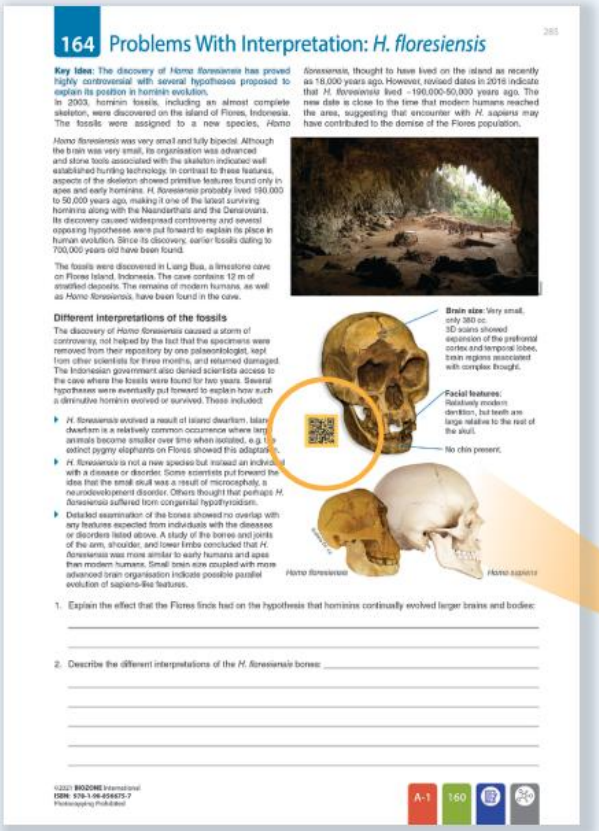
www.BIOZONEhub.com

Then enter the code in the text field

VCE12-2-6375

Or scan this QR code





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



Assessment Tasks and Key Science Skills

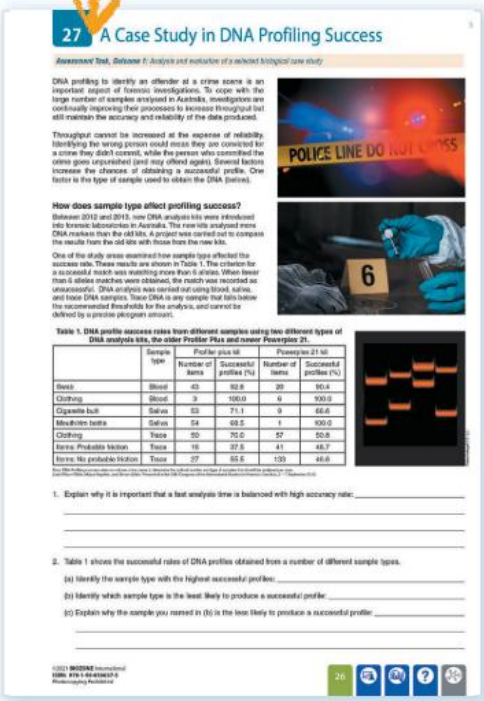
Assessment Tasks

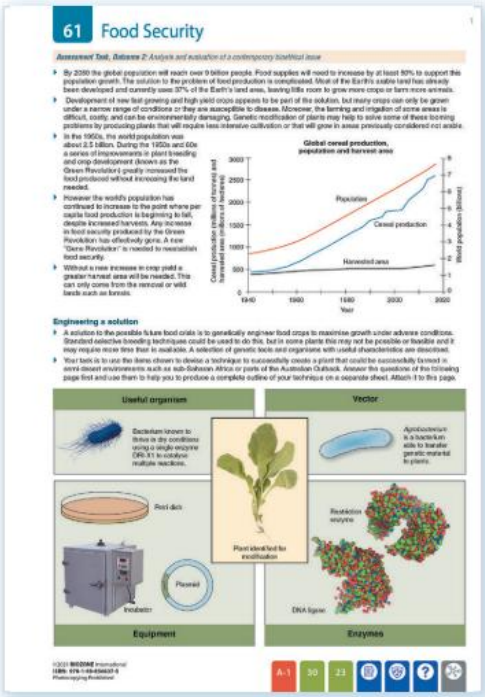
Assessment Tasks form an important component of the VCE Biology Study Design. BIOZONE's *VCE Biology* contains material in each Area of Study to support you in preparing for and completing these mandatory assessments. *Assessment Tasks* are identified in the table, right.

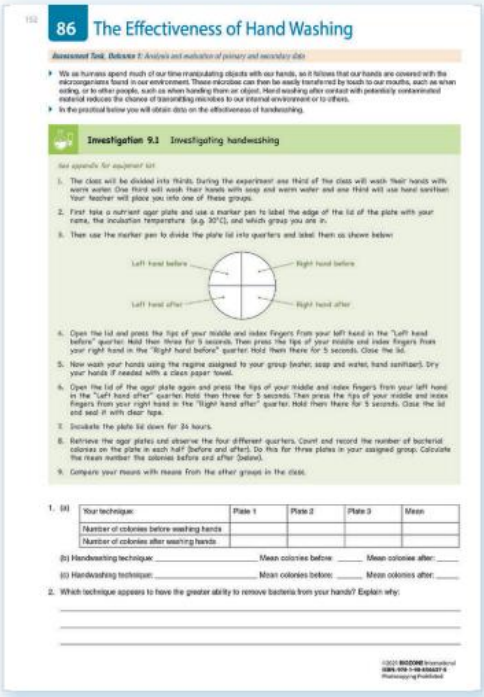
Unit 3		
Outcome 1	A Case Study in DNA Profiling Success	Activity 27
Outcome 2	Food Security	Activity 61

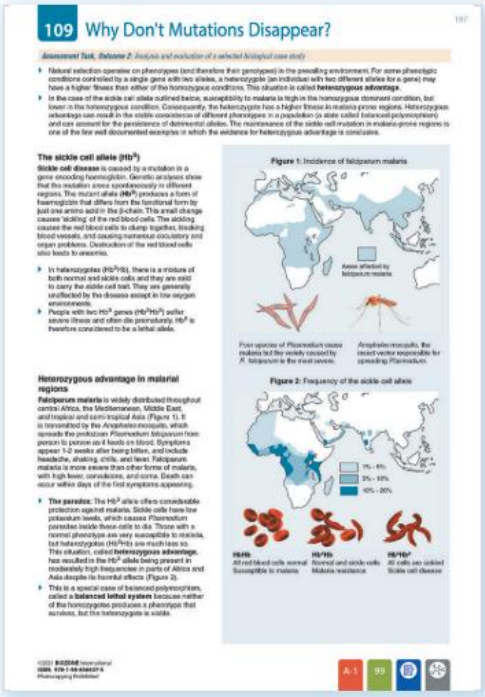
Unit 4		
Outcome 1	The Effectiveness of Handwashing	Activity 86
Outcome 2	Why Don't Mutations Disappear?	Activity 109

The assessment task is clearly identified on the page.


27 A Case Study in DNA Profiling Success


61 Food Security


86 The Effectiveness of Hand Washing


109 Why Don't Mutations Disappear?

Answering Exam Questions

- Exams require you to demonstrate your understanding of a particular concept by providing a written paragraph or essay.
- Open answer questions (meaning there is no definitive answer) are designed so that you can demonstrate your level of understanding. The question may give you some guidance as to what you should include in your answer, such as definitions of certain terms or to provide specific examples.
- In order to gain the highest possible mark in these questions, you need to organise your answer in a clear and logical way so that the examiner can easily see how you have demonstrated your understanding of the topic.
- The difference between you obtaining a low, mid, or high grade depends on how well you demonstrate your understanding of a concept.
  - Defining, drawing, annotating, or giving a description demonstrates a basic understanding of the material.
  - Explaining how a process works, why it works, and how changes to it may affect an outcome shows a deeper understanding of how the system works in that situation.
  - Linking biological ideas, comparing and contrasting, analysing, or justifying ideas shows both a deep understanding and an ability to translate that understanding to a new situation.
- The following example shows how an answer can be built up from a simple definition, through explanation, to comparisons and linking of ideas.

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

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

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

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

The advantage of a tiered defence system is explained.

Overview of the innate immune system is provided.

Overview of the adaptive immune system is provided.

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

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

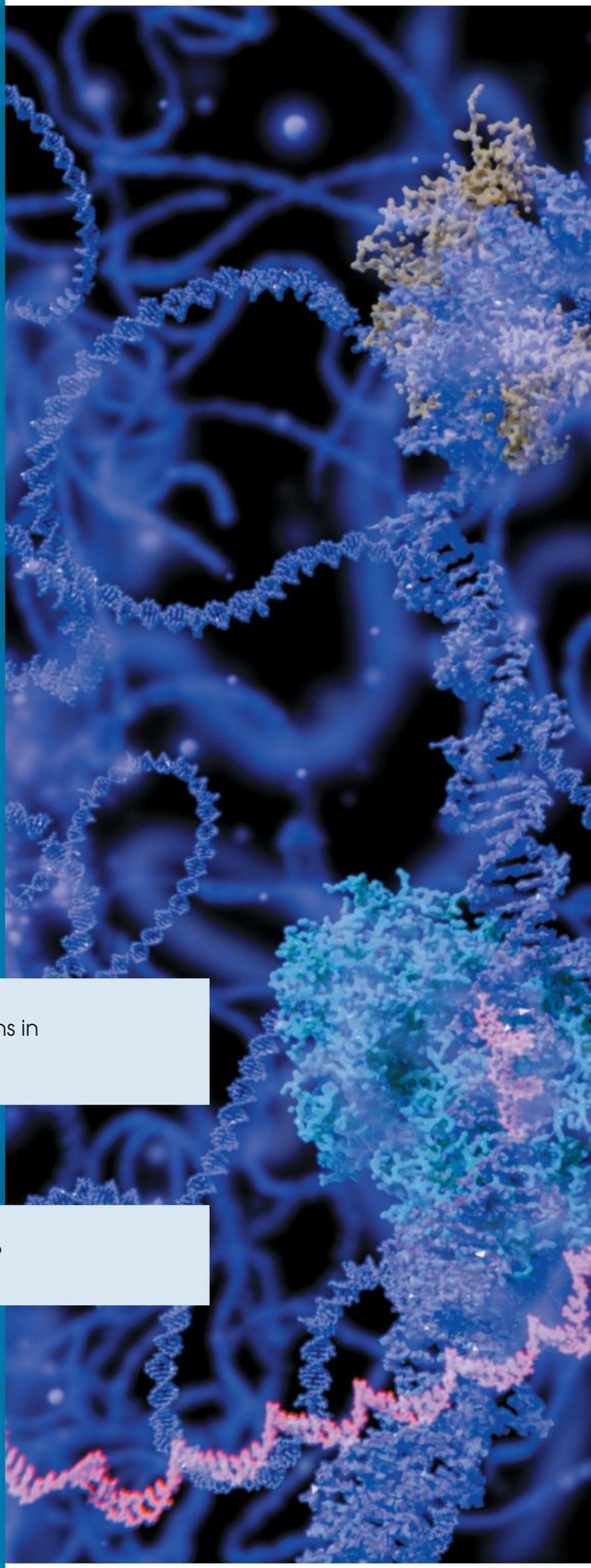
Specific details about the third line of defence (adaptive responses) are provided

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UNIT

03

How do cells maintain life?

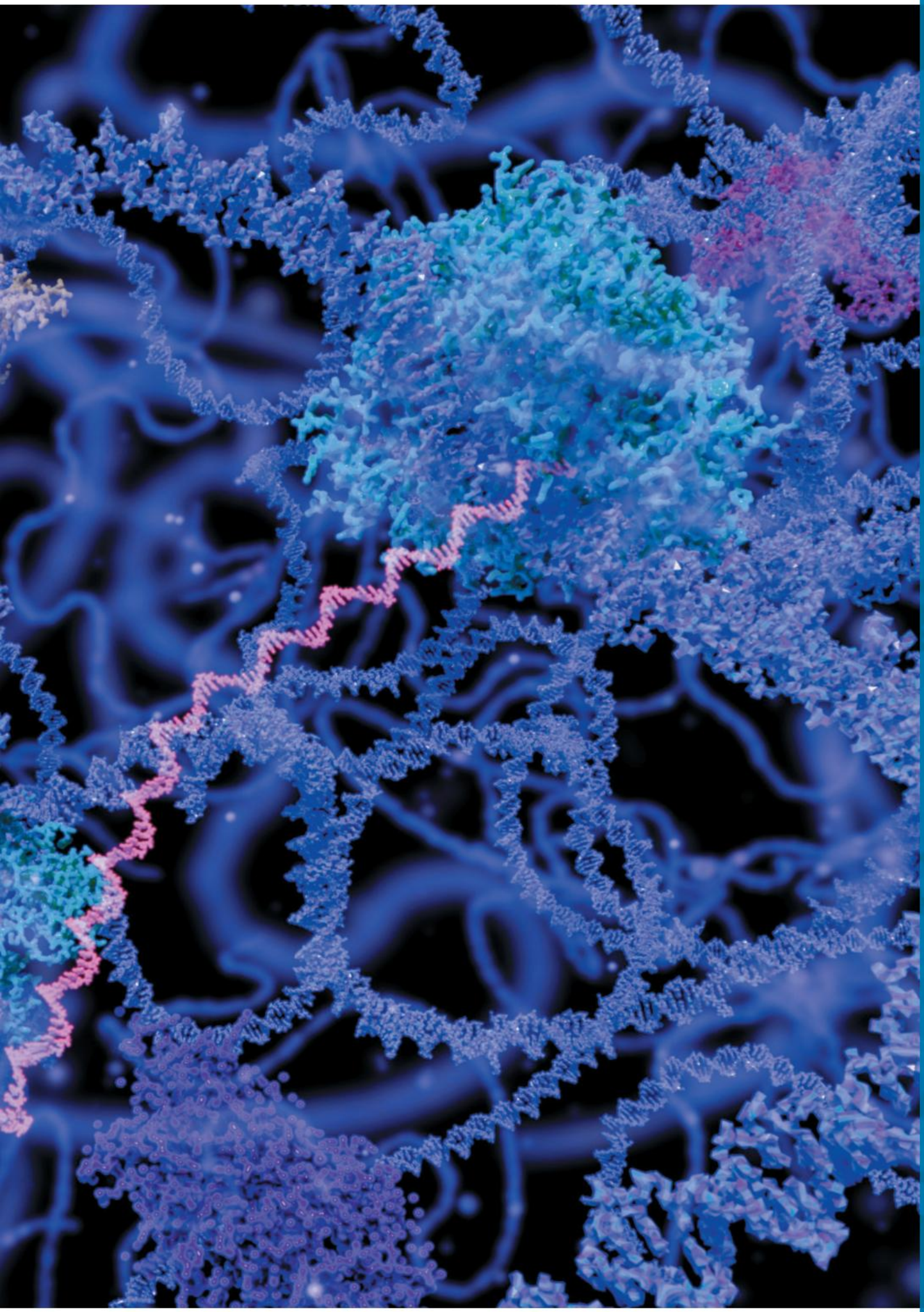


Area of Study 1

What is the role of nucleic acids and proteins in maintaining life?

Area of Study 2

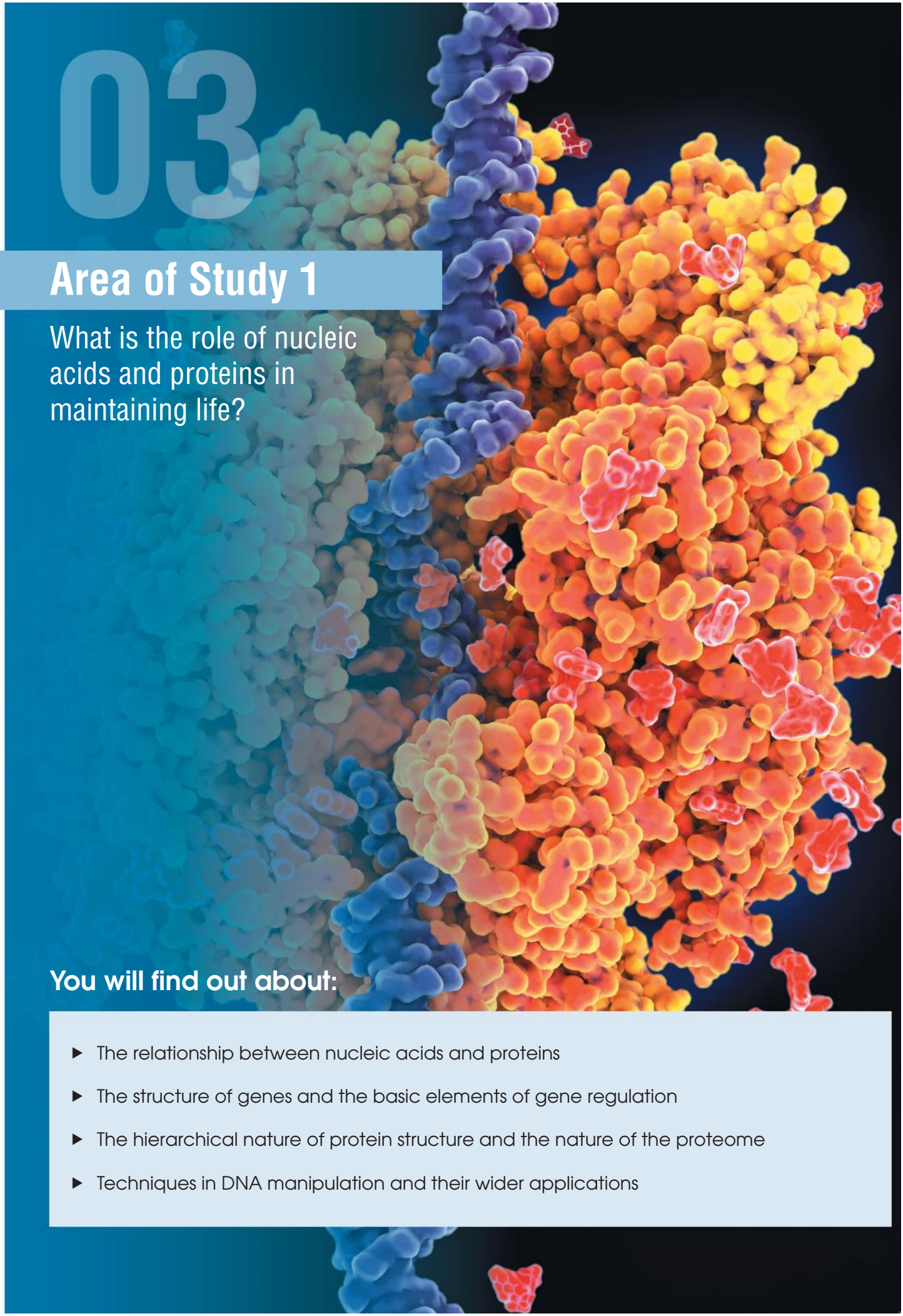
How are biochemical pathways regulated?



03

Area of Study 1

What is the role of nucleic acids and proteins in maintaining life?



You will find out about:

- The relationship between nucleic acids and proteins
- The structure of genes and the basic elements of gene regulation
- The hierarchical nature of protein structure and the nature of the proteome
- Techniques in DNA manipulation and their wider applications

CHAPTER 1

Nucleic Acids and Proteins

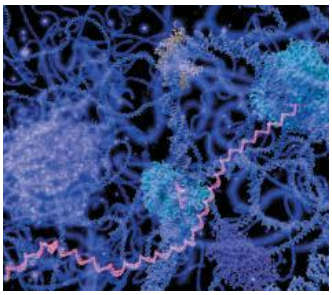
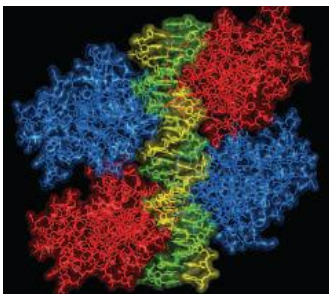
Key terms

adenine
anticodon
base-pairing rule
coding strand
codon
cytosine
denaturation
DNA
double-helix
exon
fibrous protein
gene
gene expression
genetic code
genome
globular protein
guanine
hydrogen bonding
intron
nucleic acids
nucleotides
operator
operon
peptide bond
polypeptide
primary structure
promoter
protein
proteome
purine
pyrimidine
quaternary structure
regulatory gene
repressor
ribosome
RNA (mRNA, rRNA, tRNA)
secondary structure
semi-conservative replication
structural gene
template strand
terminator sequence
tertiary structure
thymine
transcription
translation
uracil

Nucleic acids are information molecules

Key skills and knowledge

		Activity number
☐	1 Describe the structure of DNA and explain how its structure provides a mechanism for self-replication. Include reference to the base-pairing rule, the anti-parallel strands, and the role of hydrogen bonding between purines and pyrimidines.	1 3 5
☐	2 PRAC Investigate the nature of DNA by making a DNA extraction.	2
☐	3 PRAC Create a model of DNA to demonstrate the base pairing rule.	3
☐	4 Describe the structure of the three types of RNA (mRNA, tRNA, and rRNA) and their functional roles in cellular activities. Compare and contrast RNA and DNA.	3
☐	5 Describe how nucleic acids encode the instructions for the synthesis of proteins in cells, including reference to the relationship between the base sequence in a nucleic acid and the order of the amino acids in a polypeptide chain.	6
☐	6 Describe the features of the genetic code, including: • The 4-letter alphabet and the 3-letter triplet code (codon) of base sequences. • The non-overlapping, linear nature of the code, which is read from start to finish in one direction. The specific punctuation codons and their significance. • The universal nature and degeneracy of the code.	7
☐	7 Explain what is meant by a gene. Describe the steps involved in gene expression including transcription, RNA processing (eukaryotic cells), and translation. Identify where in the cell each of these steps occurs.	8-10
☐	8 Outline what is involved in RNA processing, including reference to intron removal and exon splicing. How does alternative exon splicing account for the difference in the size of the proteome, relative to the number of identified genes in the human genome?	9

Gene structure and regulation

Key skills and knowledge

☐	9 Describe the structure of a gene, distinguishing between structural and regulatory genes. Where are regulatory genes located in relation to the genes they control? How is this different for prokaryotes and eukaryotes?	11
☐	10 Describe the operon model of gene expression in prokaryotes, recognising that both gene induction and gene repression are involved in the regulation of gene expression in bacteria. Explain gene repression in prokaryotes (e.g. the *trp* operon) in which the transcription of genes that are normally transcribed all the time are switched off. Contrast gene repression with a gene induction model (*lac* operon).	12

The structure and function of proteins

Key skills and knowledge

☐	11 Explain how a polypeptide is synthesised from amino acid monomers. Explain how the properties of amino acids determine how they interact and how these interactions create the hierarchical levels of structure that produce a functional protein.	13 14
☐	12 PRAC Create a model to investigate the hierarchical nature of protein structure.	
☐	13 Explain how protein shape is related to function and compare the functional roles of globular and fibrous proteins. Identify and describe the diverse roles of proteins making up an organisms's proteome.	15-17
☐	14 Describe how proteins are modified after translation for different roles. Interpret diagrams to explain how the rough endoplasmic reticulum, Golgi apparatus, and associated vesicles are involved in the packaging and export of proteins from the cell via the protein secretory pathway.	18 19

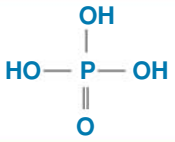
1 Nucelotides

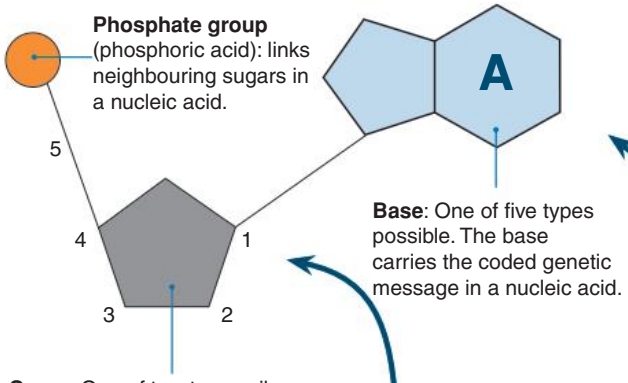
Key Idea: Nucleotides make up nucleic acids. A nucleotide is made up of a base, a sugar, and a phosphate group.

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

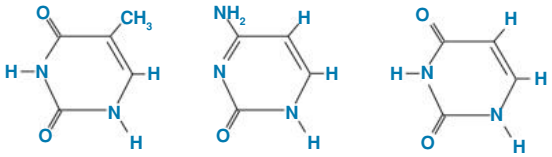
Phosphate

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



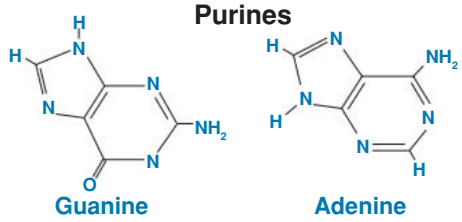


Pyrimidines



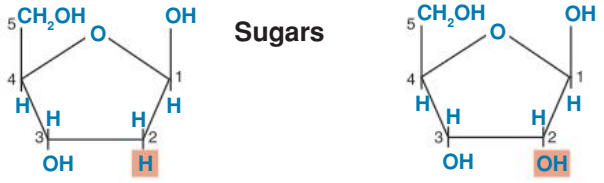
Pyrimidines are single ringed bases. DNA contains the pyrimidines cytosine (C) and thymine (T). RNA contains the pyrimidines cytosine and uracil (U).

Purines



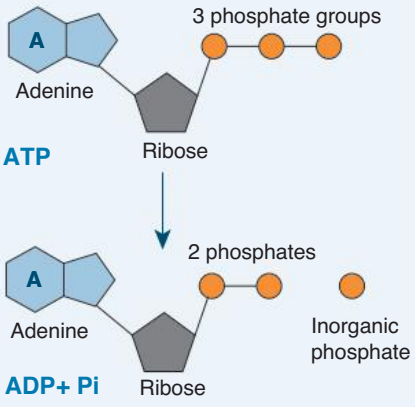
Purines are double ringed bases. Both DNA and RNA contain the purines adenine (A) and guanine (G).

Sugars



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

Nucleotide derivatives



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

Nucleotide formation



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

1. List the nucleotide bases present:

   (a) In DNA: _____

   (b) In RNA: _____

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

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

_____

_____

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2 What Does DNA Look Like?

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

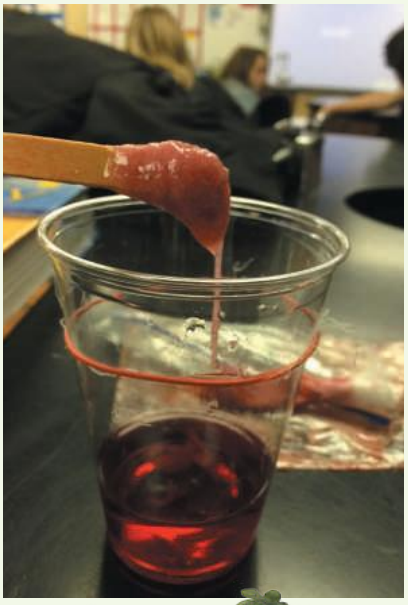
In a lab, scientists usually use extraction kits to separate DNA from cells. These will contain all the parts needed to accurately remove the DNA, but in general they are all based on the same technique. This includes breaking open the cells, precipitating the DNA, and removing contaminants. In a classroom,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

Investigation 1.1 Extracting DNA

See appendix for equipment list.

Work in pairs for this activity.

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



Yaminchhipa10 CC 4.0



1. In the extraction and isolation of DNA:

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

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

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

   (d) Why is salt added? _____

   _____

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

   _____

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

   _____

   _____

   _____

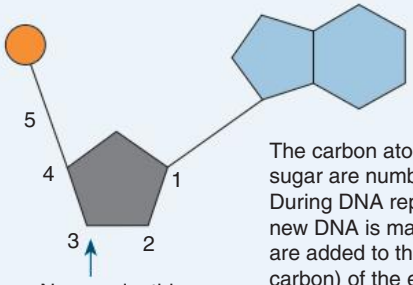
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3 Nucleic Acids

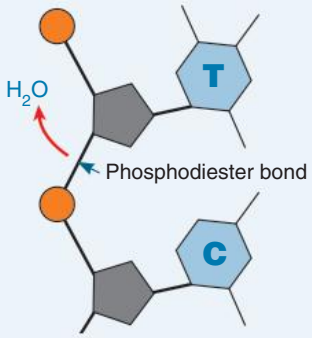
Key Idea: Nucleic acids are macromolecules made up of long chains of nucleotides, which store and transmit genetic information. DNA and RNA are nucleic acids.

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

Nucleotides are joined by condensation polymerisation

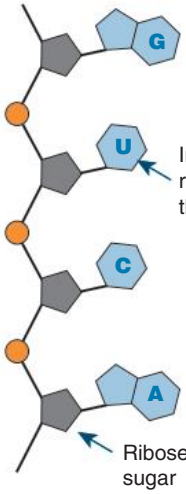


The carbon atoms on the pentose sugar are numbered one to five. During DNA replication (when new DNA is made) nucleotides are added to the 3' end (the third carbon) of the existing nucleotide chain. Therefore, DNA replication occurs in a 5' to 3' direction.



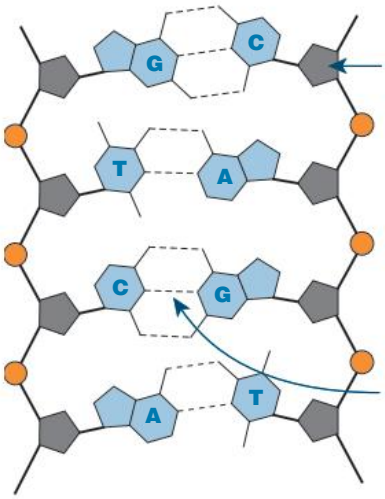
A **condensation** reaction (left) joins two molecules together with the loss of a water molecule. In the formation of nucleic acids, nucleotides are joined together into polymers through a condensation reaction between the phosphate group of one nucleotide and the sugar of another. Water is released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

RNA molecule

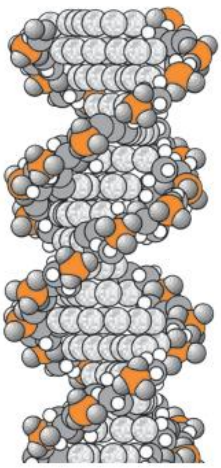


In RNA, uracil replaces thymine in the code.

DNA molecule



Symbolic representation



Space filling model

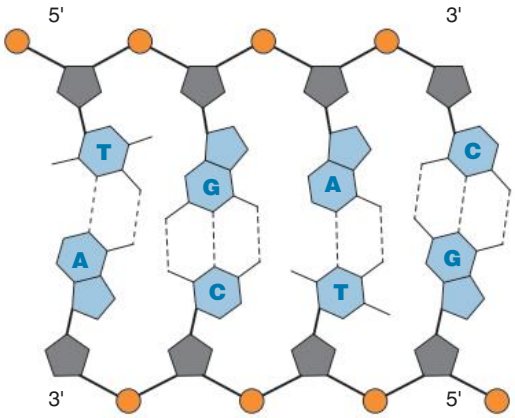
Ribonucleic acid (RNA) is made up of a single strand of nucleotides linked together. Although it is single stranded, it is often found folded back on itself, with complementary bases joined by hydrogen bonds.

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

Double-stranded DNA

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

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

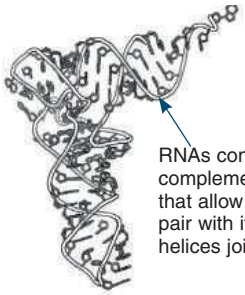


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RNAs are involved in decoding the genetic information in DNA, as messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA). RNA is also involved in modifying mRNA after transcription and in regulating translation.

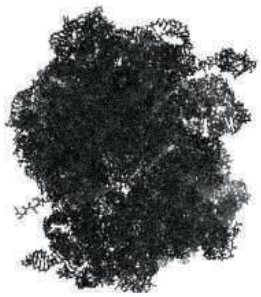


Messenger RNA (above) is transcribed (written) from DNA. It carries a copy of the genetic instructions from the DNA to ribosomes in the cytoplasm, where it is translated into a polypeptide chain.



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

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



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

1. (a) Use the diagrams opposite to describe the base-pairing rule that applies in double-stranded DNA: _____

   _____

   _____

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

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

   _____

2. Briefly describe the roles of RNA: _____

   _____

   _____

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

   _____

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

   _____

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

   _____

   _____

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

   _____

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

	DNA	RNA
Sugar present		
Bases present		
Number of strands		
Relative length		

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4 Creating a DNA Model

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

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



DID YOU KNOW?

Chargaff's rules

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

DNA base pairing rule

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





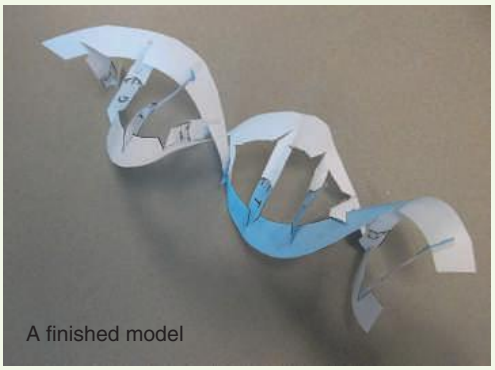
Investigation 1.2 Creating a model of a DNA molecule

See appendix for equipment list.

Work in pairs for this activity.

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

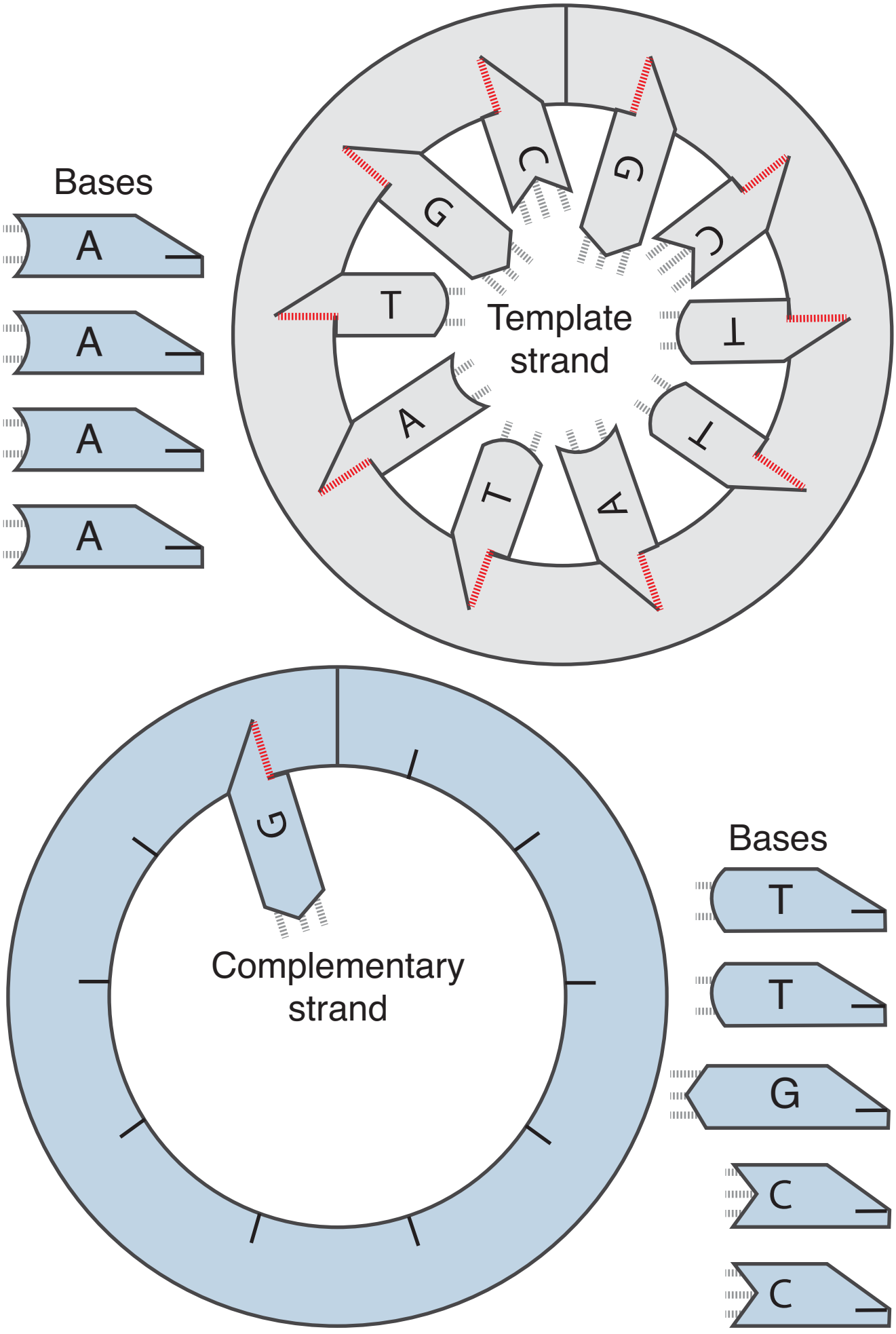
Template strand	Complementary strand
Cytosine (C)	Guanine (G)
Guanine (G)	(a)
Thymine (T)	(b)
Adenine (A)	(c)
Thymine (T)	(d)
Adenine (A)	(e)
Thymine (T)	(f)
Thymine (T)	(g)
Cytosine (C)	(h)
Guanine (G)	(i)



A finished model

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

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5 How Does DNA Replicate?

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

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

Step 1
Unwinding the DNA molecule

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

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

Step 2
Making new DNA strands

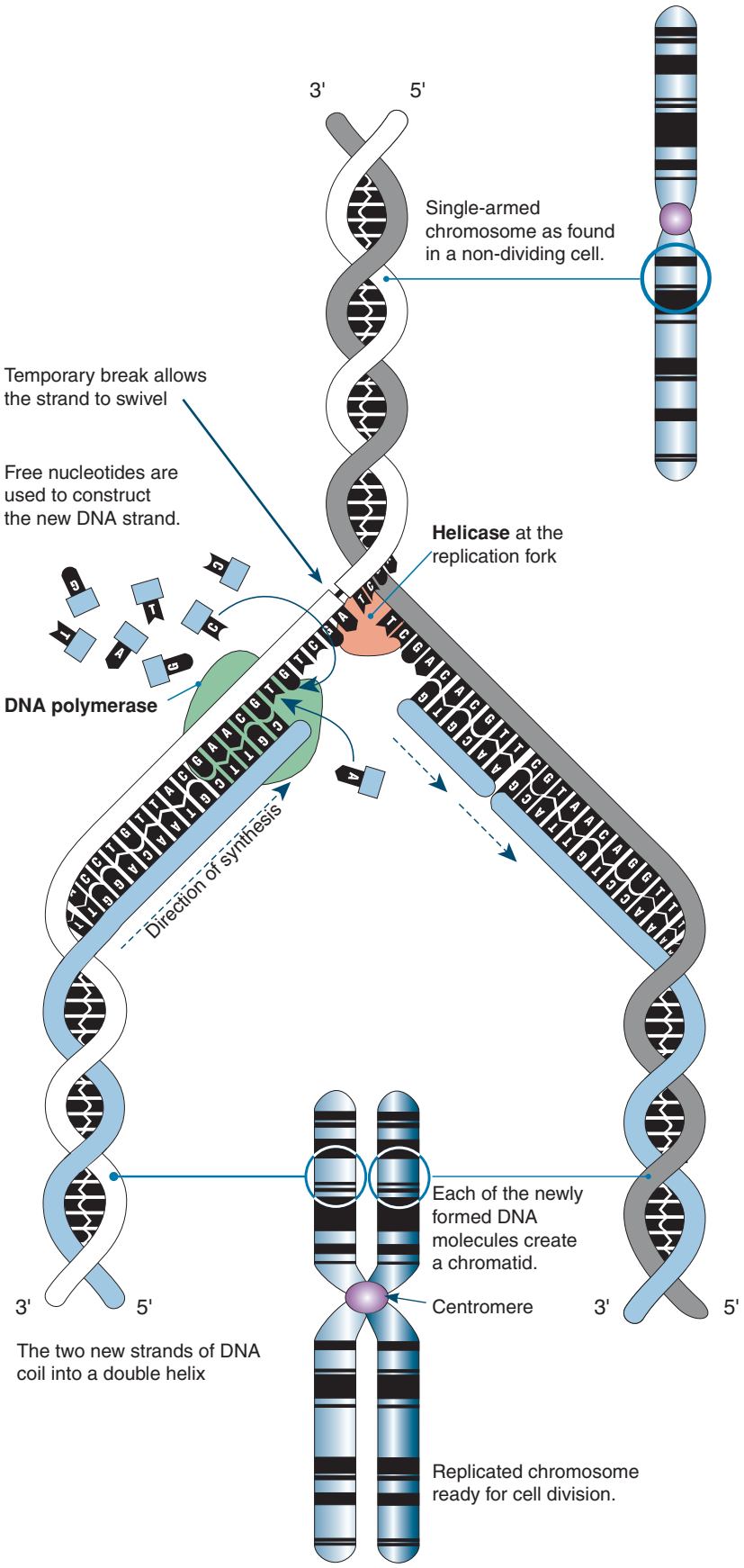
The formation of new DNA is carried out mostly by an enzyme complex called **DNA polymerase**.

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

Step 3
Rewinding the DNA molecule

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

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



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1. What is the purpose of DNA replication? _____

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

   (a) _____

   (b) _____

   (c _____

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

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

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

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

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

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

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

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

Write the complete paragraph here:

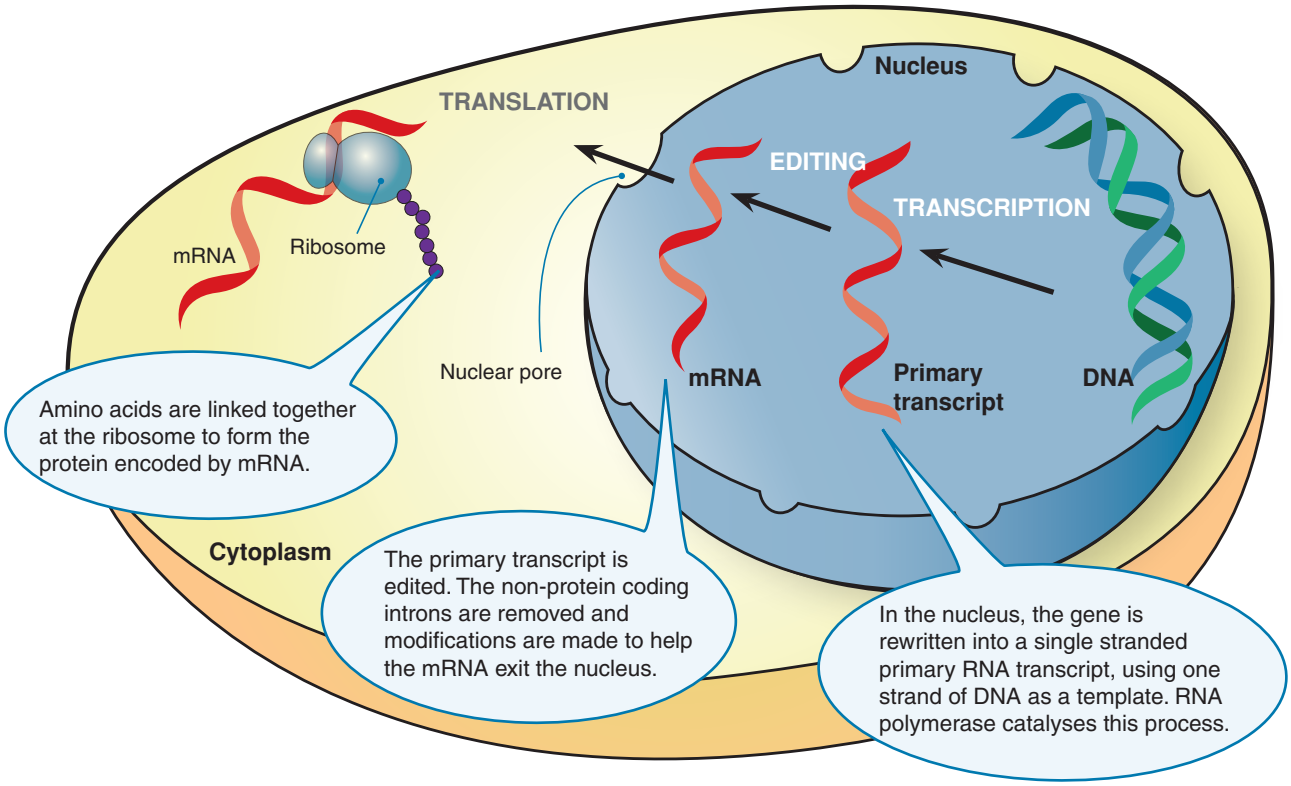
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6 What is Gene Expression?

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

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

A summary of eukaryotic gene expression



1. What is a gene? _____

_____

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

_____

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

(i) _____

(ii) _____

(iii) _____

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

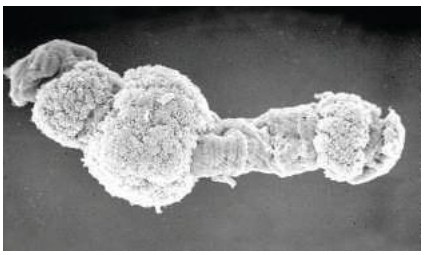
(a) What is the consequence of active transcription in a polytene chromosome?

_____

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

_____

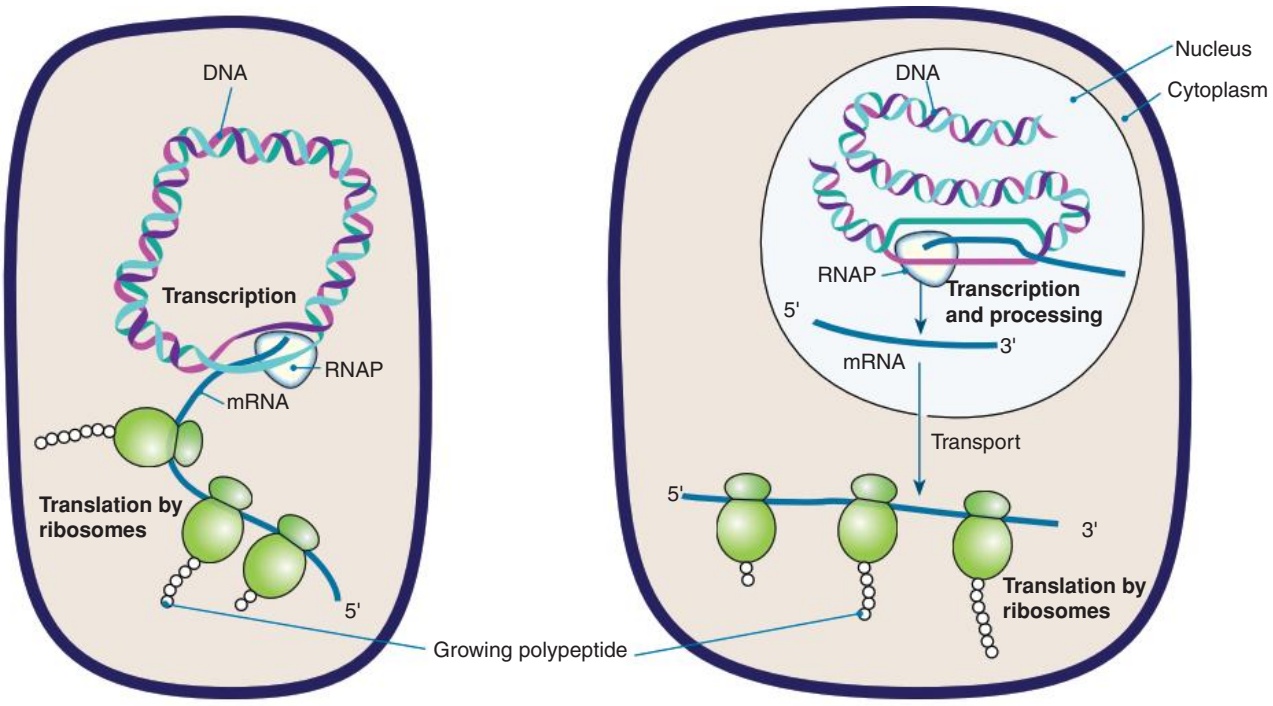
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Comparing gene expression in prokaryotes and eukaryotes

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



Prokaryote **Eukaryote**

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

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

   Codons on the mRNA: _____

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

   Codons on the mRNA: _____

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

   _____

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

   _____

   _____

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

   (a) _____

   _____

   (b) _____

   _____

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

   _____

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

   _____

   _____

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7 What is the Genetic Code?

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

The mRNA - amino acid table

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

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

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

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

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

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

Read second letter here

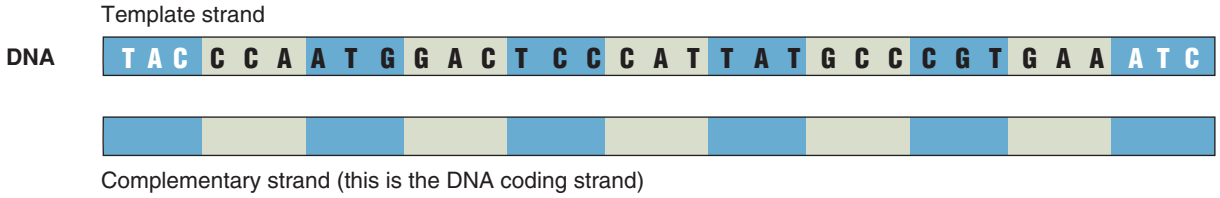
First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA STOP	UGA STOP	A
	UUG Leu	UCG Ser	UAG STOP	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC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

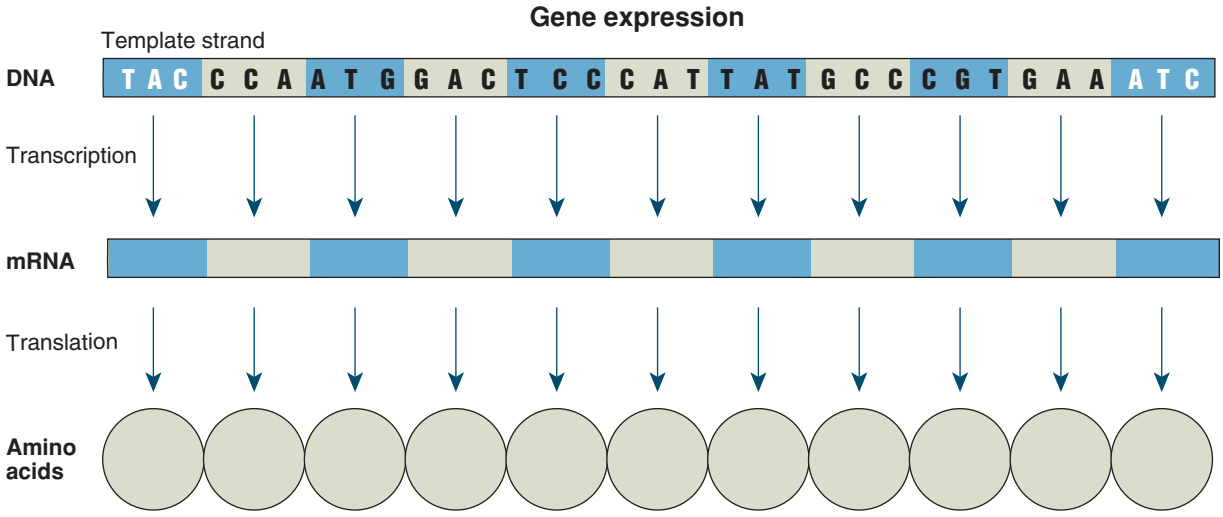
Read first letter here

Read third letter here

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

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





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

_____

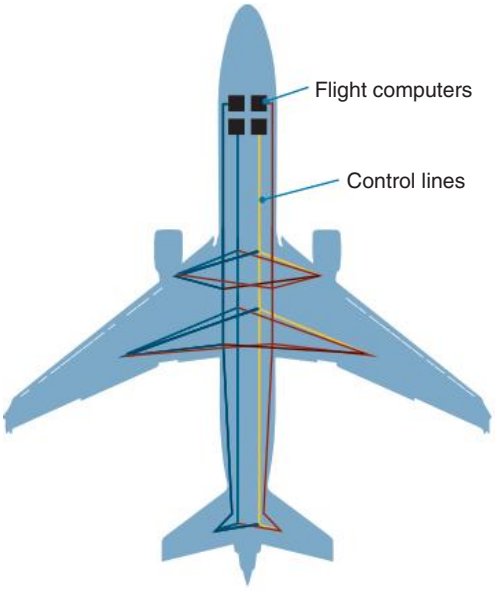
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Redundancy and degeneracy

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

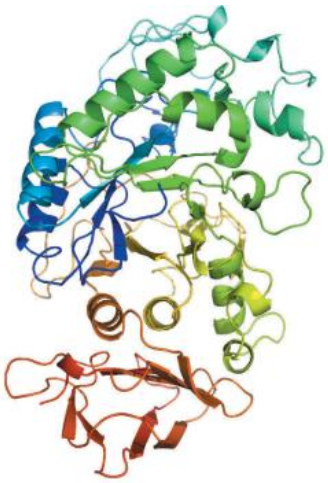
- **Redundancy** is when several situations code for or control the actions of one specific thing.
- **Degeneracy** is when a particular output can be produced by several different pathways.

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



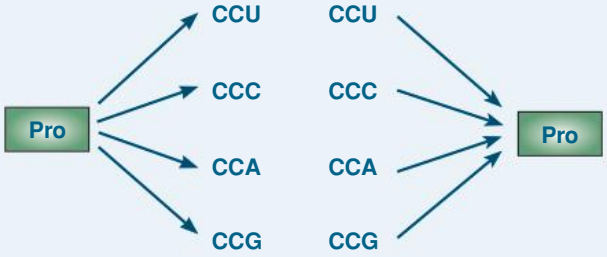
Modern aircraft (left) have multiple redundant features for safety. Often there are three or four flight computers linked independently to the flight surfaces and other input/output devices. If one computer or control line fails the others can continue to fly the plane normally.

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



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

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



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

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

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

5. Identify the following:

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

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

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

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

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

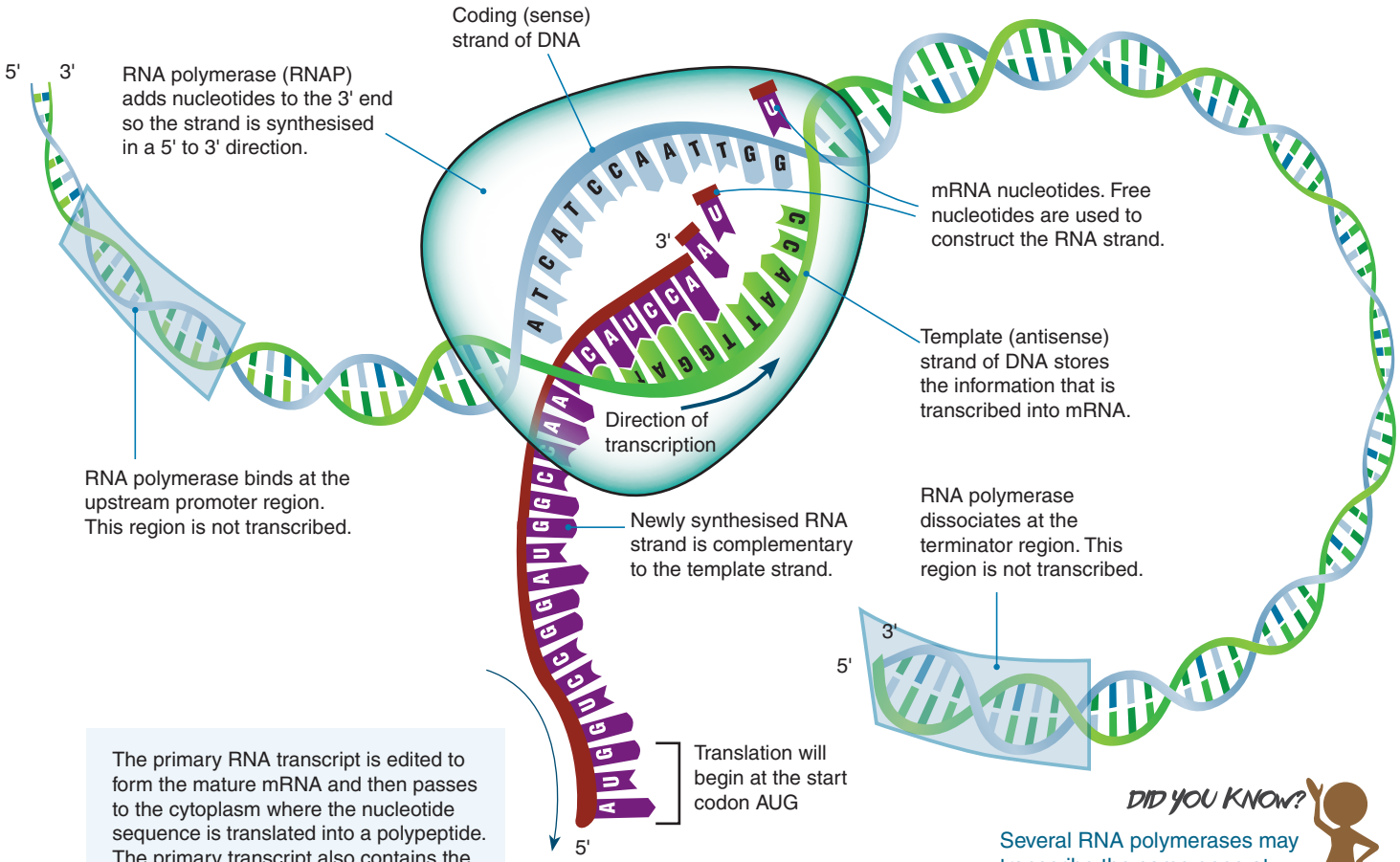
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8 Transcription in Eukaryotes

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

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

Transcription is carried out by RNA polymerase (RNAP)



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

DID YOU KNOW?

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

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

   (b) What strand of DNA does this enzyme use? _____

   (c) The code on this strand is the [same as / complementary to] the RNA being formed (circle correct answer).

   (d) Which nucleotide base replaces thymine in mRNA? _____

   (e) On the diagram, use a coloured pen to mark the beginning and end of the protein-coding region being transcribed.

2. (a) In which direction is the RNA strand synthesised? _____

   (b) Explain why this is the case: _____

   _____

   _____

3. (a) Why is AUG called the start codon? _____

   (b) What would the three letter code be on the DNA coding strand? _____

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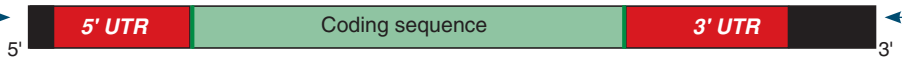
9 mRNA Processing in Eukaryotes

Key Question: How is the primary transcript modified after transcription and what do the modifications achieve?

Once a gene is transcribed, the primary transcript is modified to produce the mRNA strand that will be translated in the cytoplasm. Modifications to the 5' and 3' ends of the transcript enable the mRNA to exit the nucleus and remain stable long enough to be translated. Other post transcriptional modifications remove non-protein coding intronic DNA and splice exons in different combinations to produce different protein end products.

Primary RNA is modified by the addition of caps and tails

CAP
A guanine nucleotide cap at the 5' end of the primary transcript stops degradation during transport from the nucleus and helps in the first phase of translation.



POLY-A TAIL
Adenosine nucleotides are added to the primary transcript. These poly-A tails aid nuclear export, translation, and stability of the mRNA.

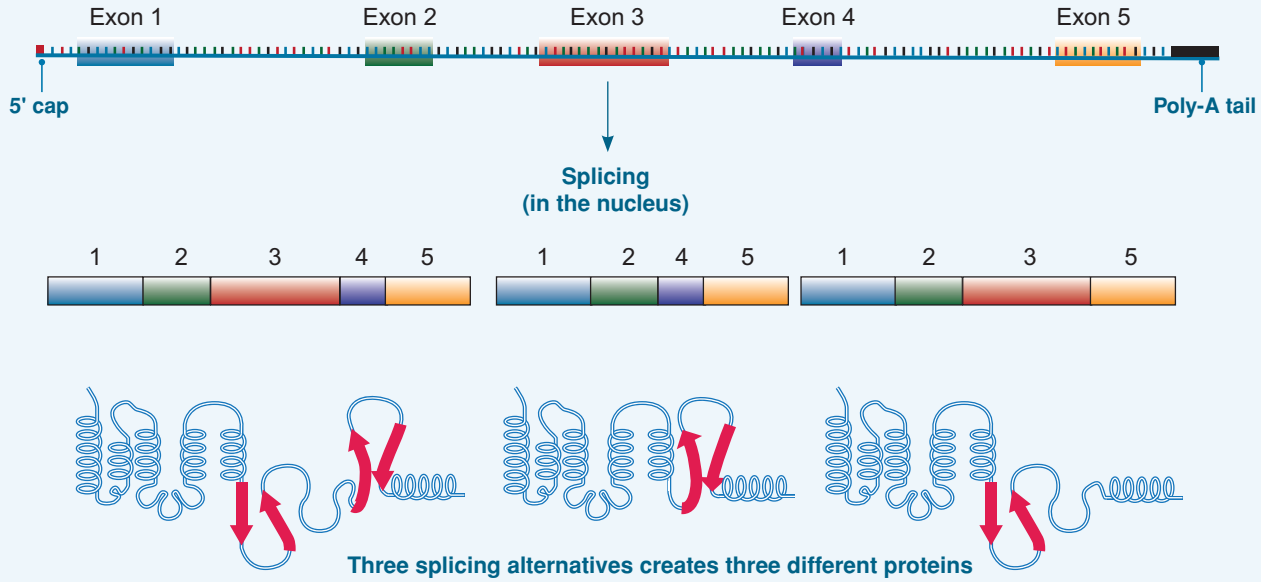
After transcription, the primary RNA transcript is modified by enzymes to create 'caps' and 'tails'. These modifications are part of the untranslated region (UTR) at each end of a gene. They stabilise the RNA, protect it from degradation, and help its transport through the nuclear pore. They are also important in translation although they are not translated themselves. The START and STOP points of translation are marked by darker green lines.

Modification after transcription

- As you have seen earlier, introns are removed from the primary mRNA transcript and the exons are spliced together. However, exons can be spliced together in different ways to create variations in the translated proteins. Exon splicing occurs in the nucleus, either during or immediately after transcription.
- In mammals, the most common method of alternative splicing involves exon skipping, in which not all exons are spliced into the final mRNA (below).

DID YOU KNOW?

Human DNA contains 25,000 genes, but produces up to 1 million different proteins. Modifications after transcription and translation allow several proteins to be produced from just one gene.



1. What is the purpose of the caps and tail on mRNA? _____

_____

2. (a) What happens to the intronic sequences in DNA after transcription? _____

   (b) What is one possible fate for these introns? _____

3. How can so many proteins be produced from so few genes? _____

_____

_____

4. If a human produces 1 million proteins, but human DNA codes for only 25,000 genes, on average how many proteins are produced per gene?

_____

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10 Translation

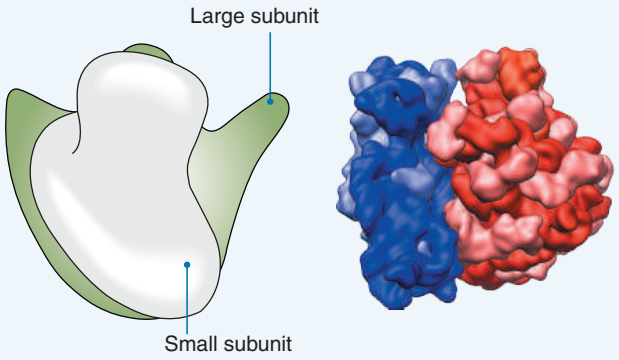
Key Idea: Translation is the final stage of gene expression in which ribosomes read the mRNA and decode (translate) it to synthesise a protein. This occurs in the cytoplasm.

In eukaryotes, translation occurs in the cytoplasm either at free ribosomes or ribosomes on the rough endoplasmic reticulum. Ribosomes translate the code carried in the mRNA molecules, providing the catalytic environment for the linkage of amino acids delivered by transfer RNA (tRNA) molecules. Protein synthesis begins at the start codon and, as the ribosome wobbles along the mRNA strand, the polypeptide chain elongates. On reaching a stop codon, the ribosome subunits dissociate from the mRNA, releasing the protein.

Ribosome structure

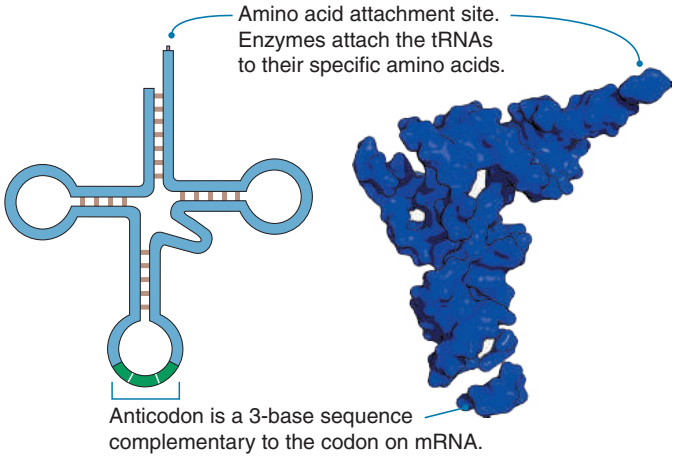
Ribosomes are made up of a complex of ribosomal RNA (rRNA) and ribosomal proteins. These small cellular structures direct the catalytic steps required for protein synthesis and have specific regions that accommodate transfer RNA (tRNA) molecules loaded with amino acids.

Ribosomes exist as two separate sub-units (below) until they are attracted to a binding site on the mRNA molecule, when they come together around the mRNA strand.



tRNA structure

tRNA molecules are RNA molecules, about 80 nucleotides long, which transfer amino acids to the ribosome as directed by the codons in the mRNA. Each tRNA has a 3-base anticodon, which is complementary to a mRNA codon. There is a different tRNA molecule for each possible codon and, because of the degeneracy of the genetic code, there may be up to six different tRNAs carrying the same amino acid.



1. Describe the structure of a ribosome: _____

_____

2. What is the role of each of the following components in translation?

   (a) Ribosome: _____

   (b) tRNA: _____

   (c) Amino acids: _____

   (d) Start codon: _____

   (e) Stop codon: _____

3. There are many different types of tRNA molecules, each with a different anticodon (HINT: see the mRNA table).

   (a) How many different tRNA types are there, each with a unique anticodon? _____

   (b) Explain your answer: _____

   _____

   _____

   (c) Determine the mRNA codons and the amino acid sequence for the following tRNA anticodons:

   tRNA anticodons: U A C U A G C C G C G A U U U

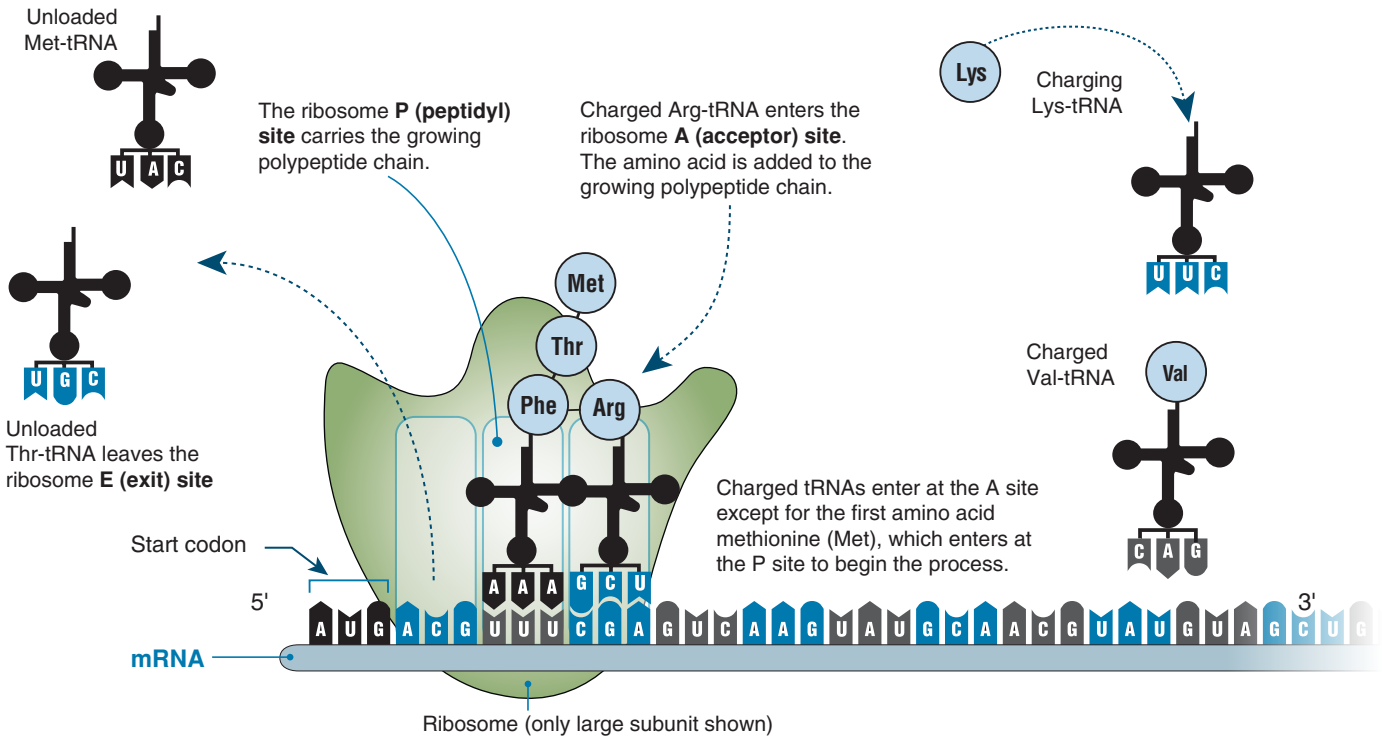
   Codons on the mRNA: _____

   Amino acids encoded: _____

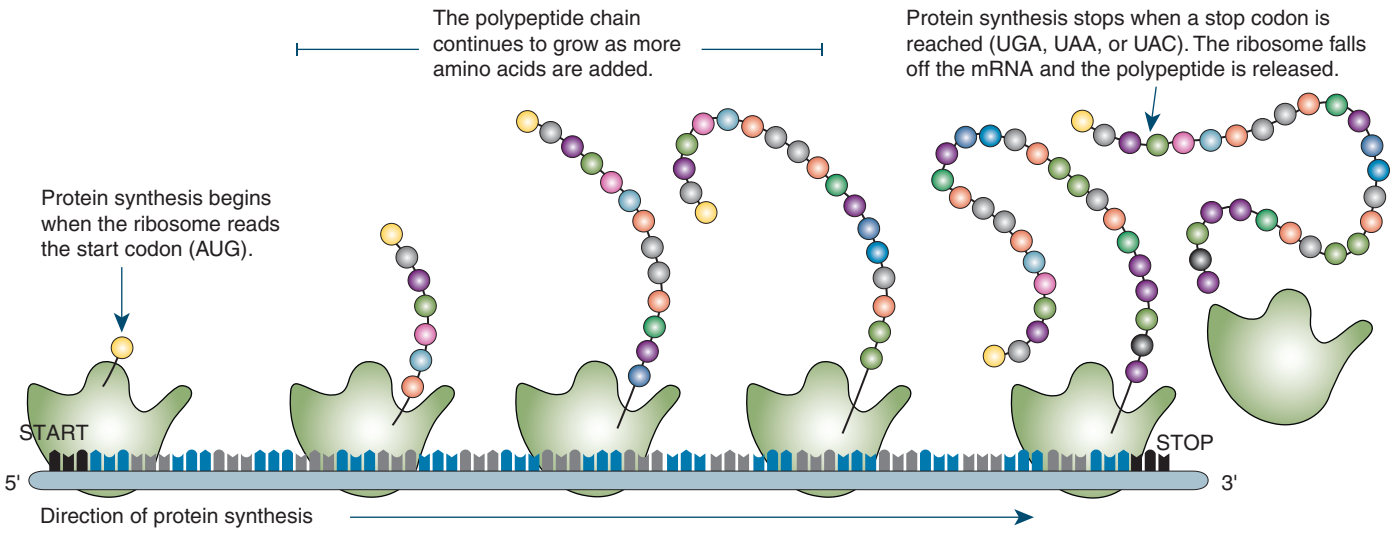
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tRNA molecules deliver amino acids to ribosomes

tRNA molecules match amino acids with the appropriate codon on mRNA. As defined by the genetic code, the anticodon specifies which amino acid the tRNA carries. The tRNA delivers its amino acid to the ribosome, where enzymes join the amino acids to form a polypeptide chain. During translation the ribosome "wobbles" along the mRNA molecule joining amino acids together. Enzymes and energy are involved in charging the tRNA molecules (attaching them to their amino acid) and elongating the peptide chain.



The polypeptide chain grows as more amino acids are added



4. Describe the events occurring during translation: _____

_____

_____

_____

5. Many ribosomes can work on one strand of mRNA at a time (a polyribosome system). What would this achieve?

_____

_____

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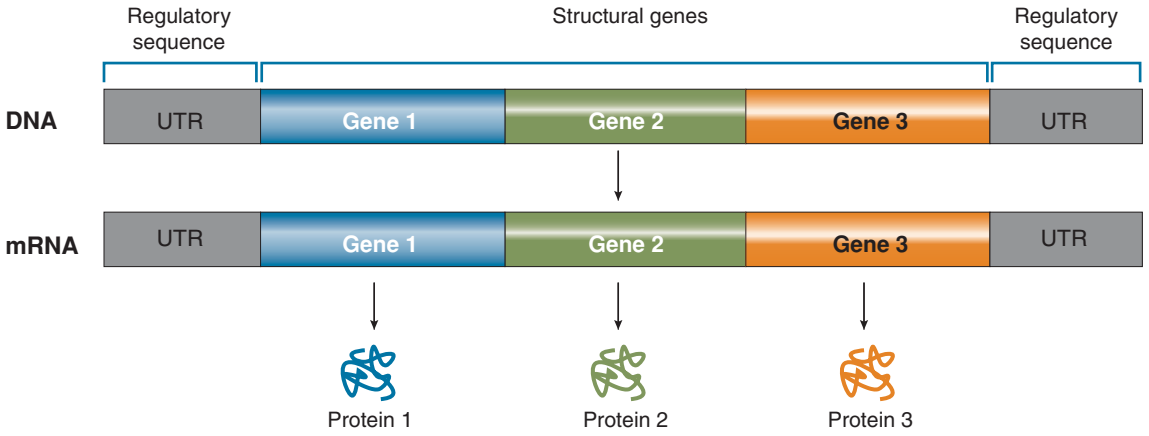
11 Structural and Regulatory Genes

Key Idea: Structural genes code for all proteins except for regulatory proteins. Regulatory genes code for the molecules involved in controlling the expression of structural genes.

Genes are sections of DNA that code for proteins. They are divided broadly into structural genes and regulatory genes. **Structural genes** code for any protein product other than a regulatory protein. The proteins encoded by structural genes are diverse and have roles in maintaining the structure or function of a cell. **Regulatory genes** code for proteins and other small molecules, such as microRNAs, that control the expression of structural genes. These regulatory genes may be some distance from the structural genes they control. Expressed structural genes are also bounded by untranslated regions (UTRs). UTRs contain regulatory sequences that directly control protein synthesis. These are arranged differently in prokaryotes and eukaryotes (below).

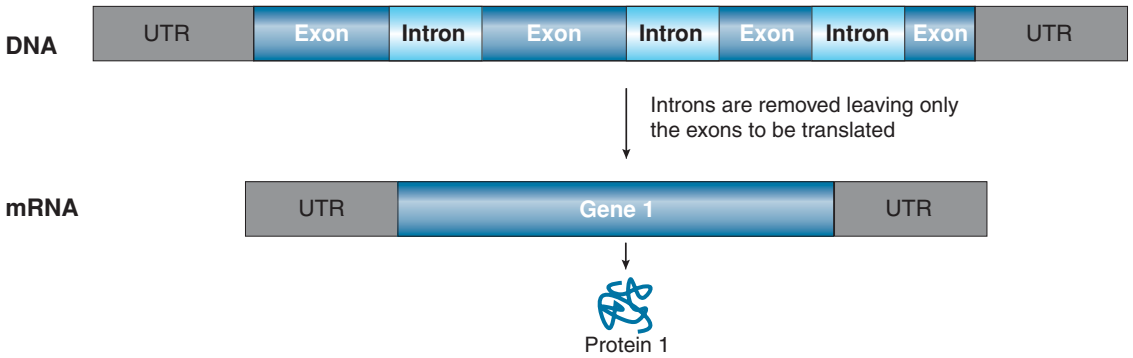
Prokaryotic gene structure

- In prokaryotes, several structural genes with related functions are grouped together between UTRs (below). These groupings of structural genes and their regulatory elements are called **operons**. The UTR upstream of the structural genes contains a regulatory sequence to initiate transcription of the structural genes. The downstream UTR stops transcription of the genes.
- In prokaryotes, the entire transcribed mRNA sequence for the structural genes is translated into proteins (there is no gene editing). Upstream of the operon, there is also a regulatory gene, which encodes a regulatory protein (not shown).



Eukaryotic gene structure

- In eukaryotes, structural genes are also under the control of regulatory sequences. However, only one structural gene is enclosed by UTRs and there is no 'bulk control' of a structural gene sequence as seen in prokaryotes (i.e. no operon).
- It is important to remember that before the primary RNA transcript is translated in eukaryotes, the non-protein coding introns are removed (only the protein-coding exons form the mature mRNA for translation).



1. What is the difference between a regulatory gene and a structural gene? _____

2. How does gene expression differ in eukaryotes and prokaryotes? _____

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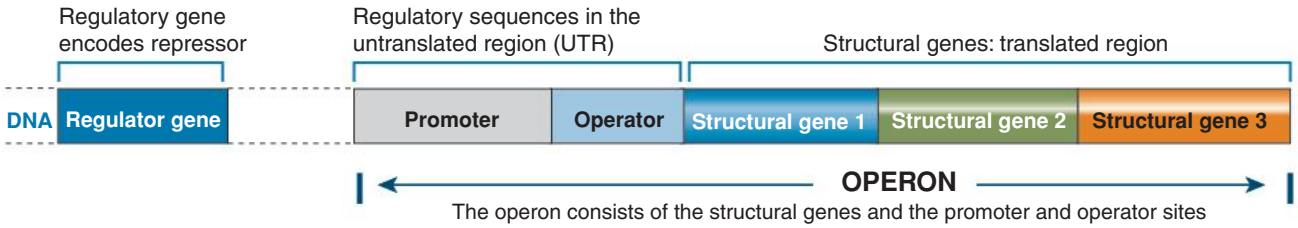
12 Models of Gene Regulation in Prokaryotes

Key Idea: Prokaryotic genes are organised as operons. An operon is a cluster of several structural genes under the control of the same regulatory genes.

Tryptophan is an amino acid used by cells to synthesise proteins. Tryptophan (Trp) is an essential amino acid for mammals meaning it cannot be synthesised and must be obtained from food. However, free-living bacteria, such as *E. coli*, are able to synthesise tryptophan and regulate its production via the trp operon, a group of structural genes encoding enzymes and their regulatory sequences. Transcription of the structural genes in an operon is controlled by the regulatory sequences. A regulator gene outside the operon encodes a repressor molecule, which can bind to the regulatory sequences and switch off the expression of the structural genes. The operon model applies only to prokaryotes because eukaryotic genes are not found as operons. Two mechanisms operate in this model: gene induction and gene repression.

Prokaryotic genes occur as operons

A number of structural genes encoding the enzymes for a metabolic pathway are under the control of the same regulatory elements.



The regulator is some distance from the operon. It codes for the repressor that prevents the expression of specific genes.

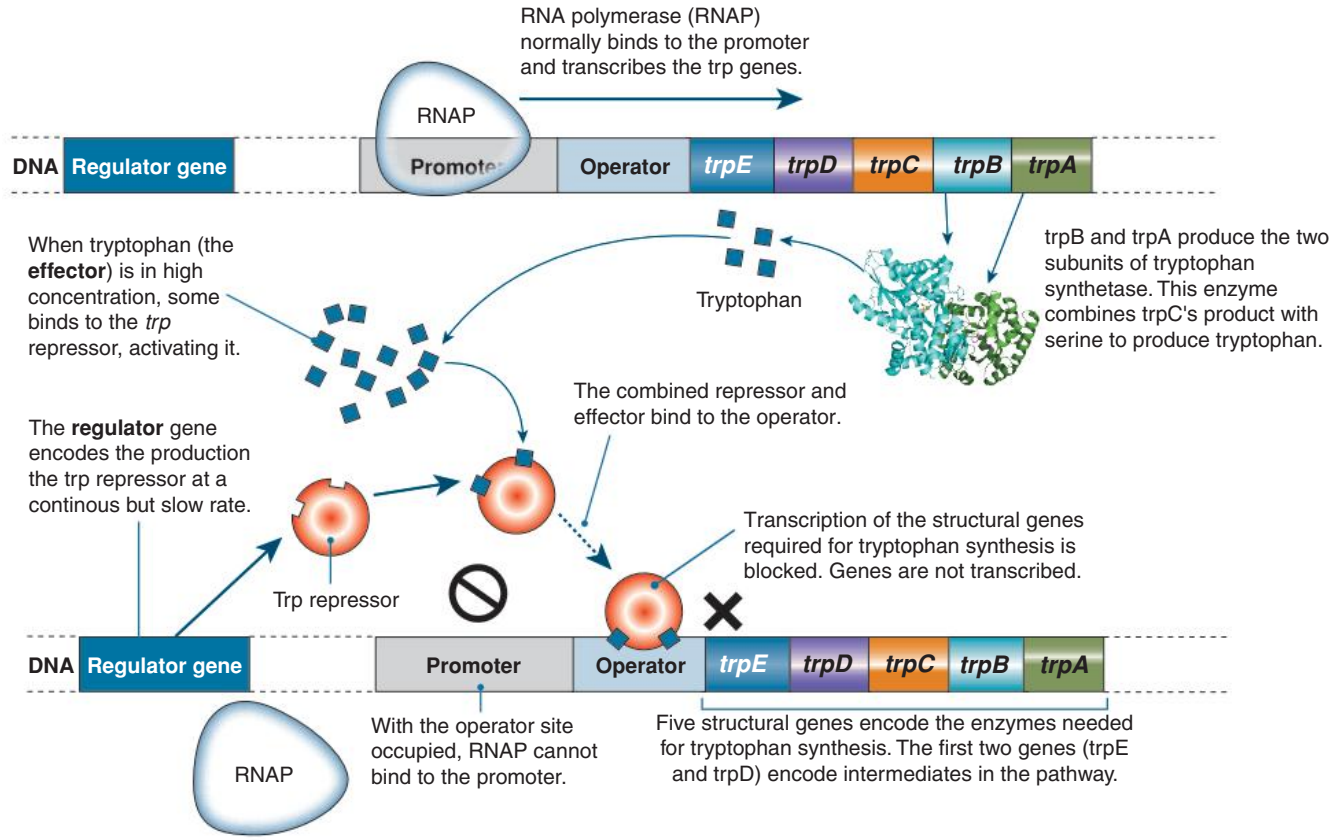
The promoter site is where the RNA polymerase enzyme first attaches itself to the DNA to begin synthesis of the mRNA.

The operator is an 'on-off' switch that controls RNA polymerase's access to the structural genes. It is the repressor binding site.

Structural genes. At least one structural gene is present in an operon but usually there are more. The *trp* operon (below) in bacteria has five. Structural genes code for the synthesis of enzymes in a metabolic pathway.

The *trp* operon: a repressible operon

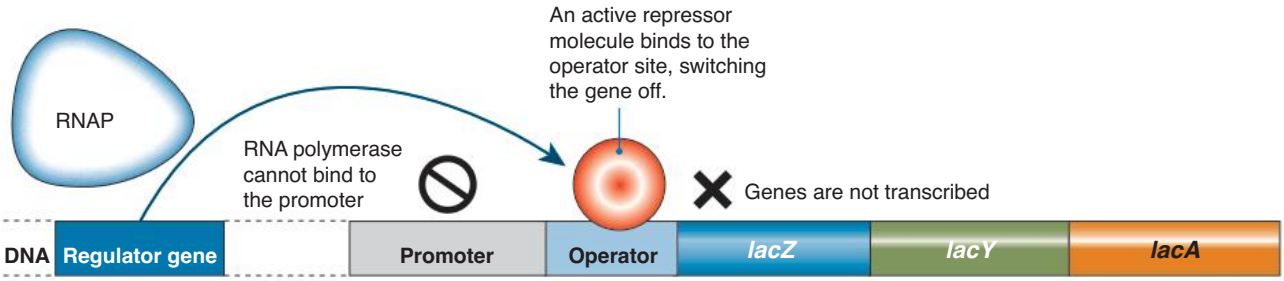
Transcription of the five structural genes in the trp operon is normally on. To stop transcription, the genes are switched off (repressed) by tryptophan itself, so this operon is called repressible. The genes trpA and trpB produce the enzyme tryptophan synthetase, which catalyses the formation of tryptophan from serine and trpC's product. When tryptophan is present in excess, some of it binds to and activates the trp repressor encoded by the regulator gene. The active repressor then blocks transcription of the structural genes. This mechanism allows tryptophan synthesis to be stopped when it is in plentiful supply (in the cell or environment).



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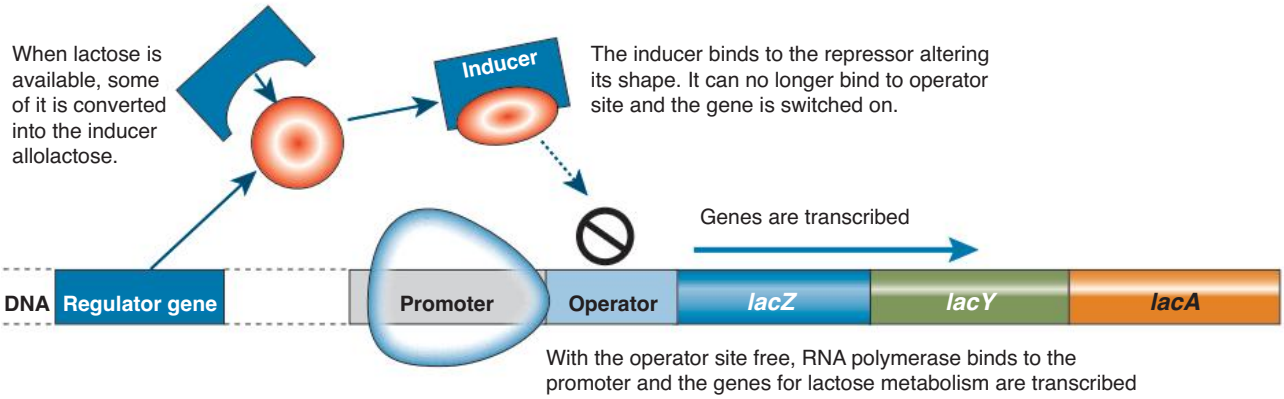
The *lac* operon: an inducible operon

The *lac* operon is an operon required for the transport and metabolism of lactose in many bacteria including *E. coli*. Normally the repressor is active and the genes are switched off. They are switched on (induced) by allolactose so the operon is inducible.



Lactose switches the *lac* operon on

Presence of lactose results in inactivation of the repressor so the genes can be transcribed.



1. Outline the role of each of the following components of an operon:

   (a) Promoter: _____

   (b) Operator: _____

   (c) Structural genes: _____

2. What is the role of the repressor molecule in operon function? _____

   _____

3. (a) In the trp operon, is the repressor normally attached or not attached to the operator? _____

   (b) What is the role of the effector in the production of tryptophan? _____

   _____

   _____

4. Describe how the trp operon is a self regulating system (a negative feedback loop): _____

   _____

   _____

   _____

   _____

5. Give a brief description of how repressible and inducible operons differ: _____

   _____

   _____

   _____

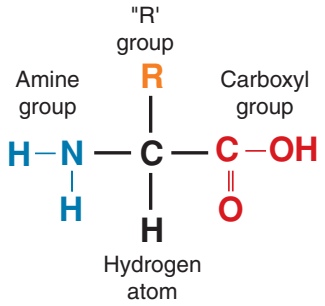
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13 Amino Acids Make up Proteins

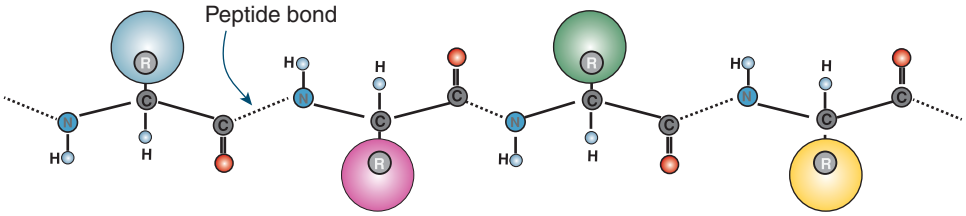
Key Idea: Amino acids join together in a linear chain by condensation reactions to form polypeptides. The sequence of amino acids in a protein is defined by a gene and encoded in the genetic code. Polypeptides are broken apart into amino acids by hydrolysis (the opposite reaction to condensation).

Amino acids are the basic units from which proteins are made. Twenty amino acids commonly occur in proteins and they can be linked in many different ways by peptide bonds to form a huge variety of polypeptides. Proteins are made up of one or more polypeptide molecules.

The structure and properties of amino acids

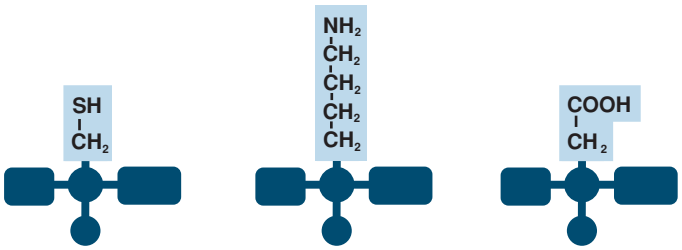


- Amino acids are the building blocks of proteins. They are linked by peptide bonds (below and opposite) for form long chains called polypeptides, which are the basis of proteins. All amino acids have a common structure (left) with an amine group (blue), a carboxyl group (red), a hydrogen atom, and a functional or 'R' group (orange).
- Each type of amino acid has a different functional R group (side chain). Each functional R group has a different chemical property.
- Amino acids are represented by a single upper case letter or a three-letter abbreviation. For example, proline is known by the letter P or the three-letter symbol Pro.



Different amino acids have different R groups

- The R group in the amino acid determines the chemical properties of the amino acid. Different amino acids have different R groups and therefore different chemical properties. Amino acids can be grouped according to these properties. Common groupings are nonpolar (hydrophobic), polar (hydrophilic), positively charged (basic), or negatively charged (acidic).
- The property of the R group determines how the amino acid will interacts with others and how the amino acid chain will fold up into a functional protein. For example, the hydrophobic R groups of soluble proteins will be folded into the protein's interior.



Cysteine
The 'R' group of cysteine forms **disulfide bridges** with other cysteines to create cross linkages in a polypeptide chain.

Lysine
The 'R' group of lysine gives the amino acid an **alkaline** property.

Aspartic acid
The 'R' group of aspartic acid gives the amino acid an **acidic** property.

1. What makes each of the amino acids in proteins unique and how does this uniqueness contribute to protein structure?

2. Do some research to assign each of the 20 amino acids found in proteins to one of the four groups below. Use a standard 3-letter code to identify each amino acid:

   (a) Nonpolar (hydrophobic): _____

   (b) Polar (hydrophilic): _____

   (c) Positively charged (basic): _____

   (d) Negatively charged (acidic): _____

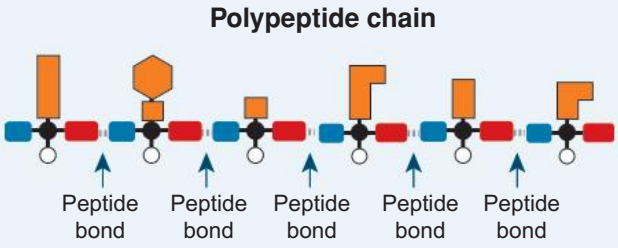
3. (a) Which type(s) of amino acids would you find on the surface of a soluble protein? Which type(s) would you find in the interior? Explain:

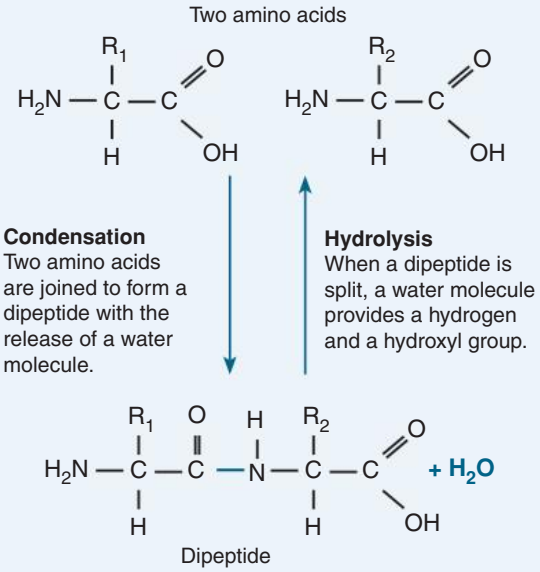
   (b) What distribution of amino acids would you expect to find in a protein embedded in a lipid bilayer?

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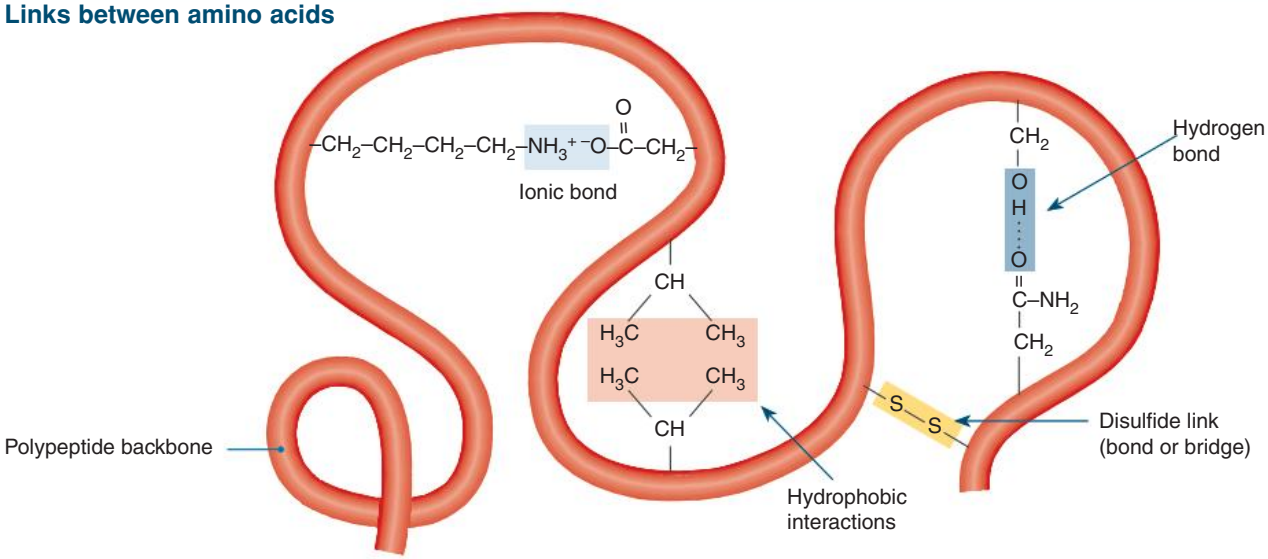
Polypeptides are made by condensation and broken down by hydrolysis

- Amino acids are linked by **peptide bonds** to form **polypeptide chains** of up to several thousand amino acids.
- Peptide bonds form between the carboxyl group of one amino acid and the amine group of another (right). Water is formed as a result of this bond formation so the reaction is called a condensation.
- The sequence of amino acids in a polypeptide is called the **primary structure** and is determined by the order of nucleotides in DNA and mRNA.





Links between amino acids



4. (a) What type of bond joins neighbouring amino acids together? _____

   (b) How is this bond formed? _____

   (c) Circle this bond in the dipeptide above:

   (d) How are di- and polypeptides broken down? _____

5. Use the diagram above to answer the following:

   (a) Name the different interactions that can shape the polypeptide: _____

   _____

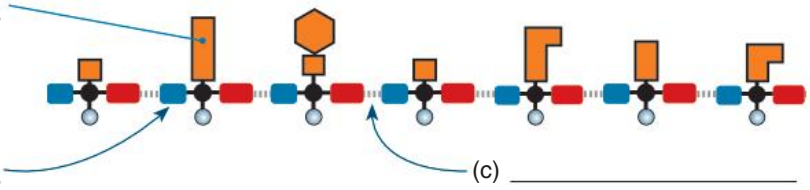
   (b) Which of the interactions would be the strongest: _____

6. In the diagram below identify following, *R group, amine group, peptide bond*:

   (a) _____

   (b) _____

   (c) _____



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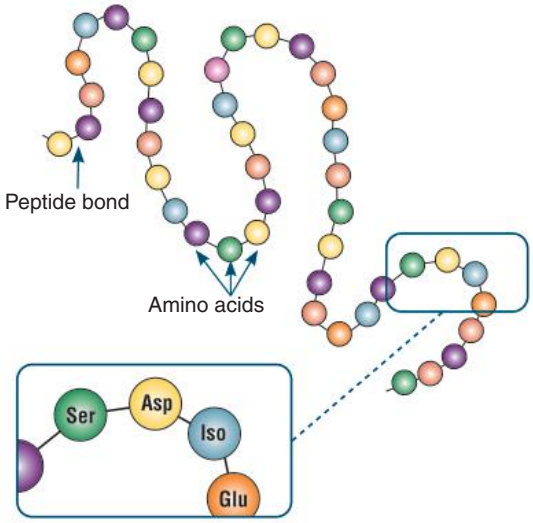
14 Protein Structure is Hierarchical

Key Idea: The sequence and type of amino acids in a protein determine its three-dimensional shape and function.

Proteins are large, complex **macromolecules**, built up from a linear sequence of repeating units called **amino acids**. Proteins account for more than 50% of the dry weight of most cells and are important in virtually every cellular process. The various properties of the amino acids, which are conferred by the different R groups, determine how the polypeptide chain folds up. This three dimensional **tertiary structure** gives a protein its specific chemical properties. If a protein loses this precise structure (through **denaturation**), it is usually unable to carry out its biological function.

Primary (1°) structure

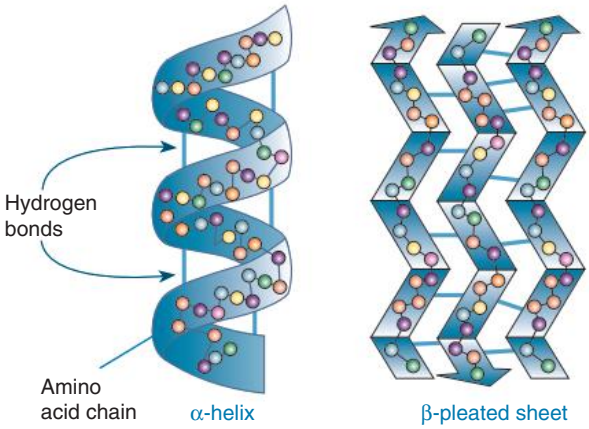
The amino acid sequence



Hundreds of amino acids are linked by peptide bonds to form polypeptide chains. The attractive and repulsive charges on the amino acids determine the higher levels of organisation in the protein and its biological function.

Secondary (2°) structure

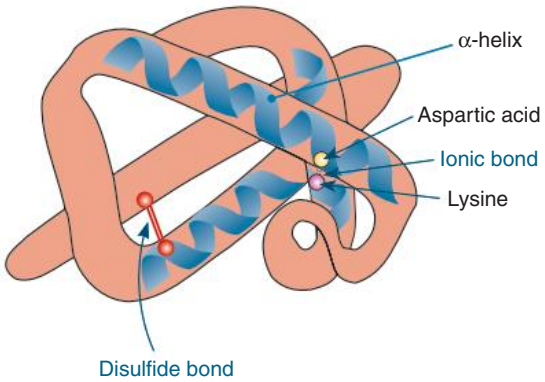
α-helices or β pleated sheets



Secondary (2°) structure is maintained by hydrogen bonds between neighbouring CO and NH groups. Hydrogen bonds are individually weak but collectively strong and determine how the [primary chain folds. The coiled α-helix and β-pleated sheet are common 2° structures. Most globular proteins contain regions of both 2° configurations.

Tertiary (3°) structure

Folding of the 2° structure

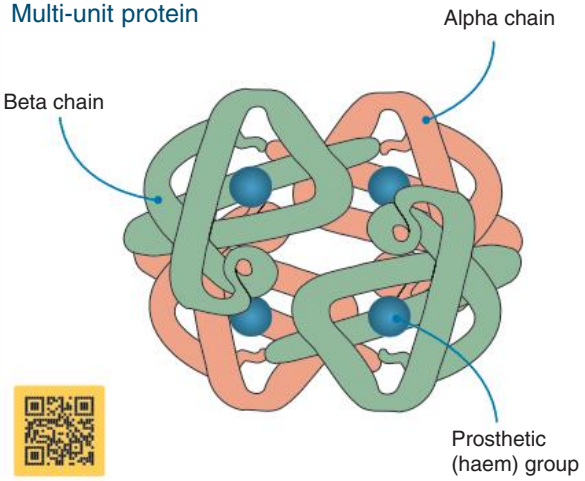


The tertiary (3°) structure is maintained by more distant interactions such as **disulfide bridges** between cysteine amino acids, ionic bonds, and hydrophobic interactions.

A protein's 3° structure is the three-dimensional shape formed when the 2° structure folds up and more distant parts of the polypeptide chains interact.

Quaternary (4°) structure

Multi-unit protein



Some complex proteins are only functional when as a group of polypeptide chains. Haemoglobin (above) has a 4° structure made up of two alpha and two beta polypeptide chains, each enclosing a complex iron-containing prosthetic (or haem) group.

A protein's 4° structure describes the arrangement and position of each of the subunits in a multiunit protein. The shape is maintained by the same sorts of interactions as those involved in 3° structure.

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1. Describe the main features in the formation of each part of a protein's structure:

   (a) Primary structure: _____

   _____

   (b) Secondary structure: _____

   _____

   (c) Tertiary structure: _____

   _____

   (d) Quaternary structure: _____

   _____

2. How are proteins built up into a functional structure? _____

   _____

   _____

   _____

3. Strong chemicals and extremes of temperature or pH can disrupt the bonds in proteins. What would this do to the protein's function and why?

   _____

   _____

   _____

Investigation 1.3 Modelling protein structure

See appendix for equipment list.

Work in pairs for this activity.

1. You will need pipe cleaners with four colours. We have used 2 white, 2 pink, 2 purple, and 4 blue but you can swap out for the colours you have. Each colour represents a different amino acid.
2. Twist a loop in the center of each pipe cleaner (Figure 1). The twist represents the amino acid's functional group.
3. Join the amino acids together (figure 2) by twisting their arms together in the following sequence:
   1) white 2) pink 3) blue 4) purple 5) blue 6) pink 7) blue 8) white 9) blue 10) purple.

   What level of protein organisation does the structure in Figure 2 represent?

   _____
4. Attach sticky tape to the loops of the purple pipe cleaners and to one arm of each of the blue pipe cleaners. These represent places where hydrogen bonding can occur.

Figure 1

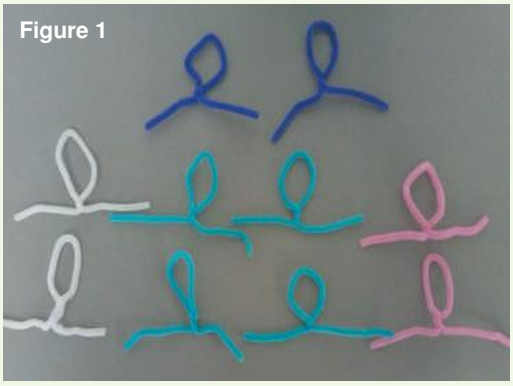
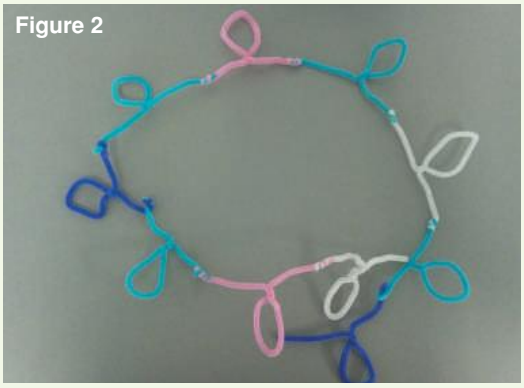


Figure 2



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5. Join the sticky tape together between amino acids 3 and 5 and also between amino acids 7 and 9 (Figure 3).

   Describe what happens to the shape of the model when you do this: _____

   _____

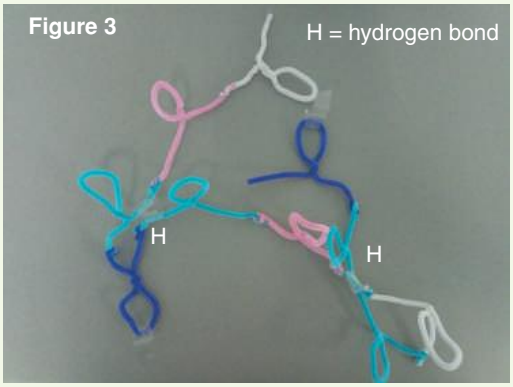
   _____

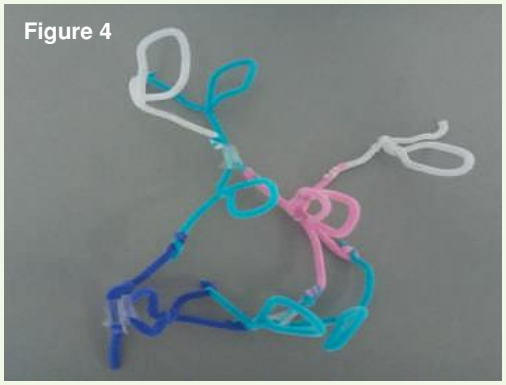
   What level of protein structure does this represent? _____

6. Attach binder clips or paper clips to the loops of the two pink amino acids and then use the clips to join the two pink amino acids together. The clips represent a disulfide bond.

7. Join the sticky tape together on the two purple amino acids (Figure 4). Your protein has now formed its fully functional structure.

   What level of protein structure does this represent? _____





4. (a) Label figure 4 to show the location of all of the hydrogen bonds (H) and the disulfide bond (S).

   (b) Based on the properties of your model and its components, which of these bonds is likely to be the strongest:

   _____

5. Break the hydrogen bonds between amino acids 3 and 5 and also between 7 and 9 in your molecule.

   (a) What happens to the shape of the protein? _____

   _____

   (b) What process does breaking these bonds represent? _____

   _____

   (c) What effect will this process have on the protein's ability to carry out its job? _____

   _____

   _____

   _____

6. How could you adapt your model to demonstrate quaternary structure? _____

   _____

   _____

   _____

   _____

   _____

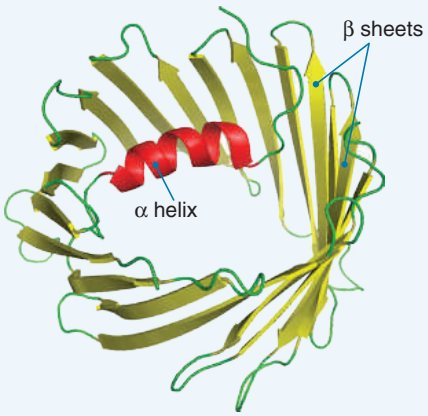
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15 Protein Shape is Related to Function

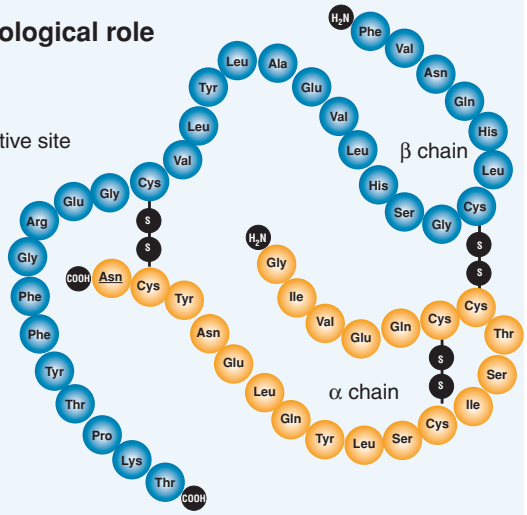
Key Idea: The three dimensional shape of a protein reflects its role. When a protein is denatured, it loses its functionality.

As we have seen, a protein may consist of one polypeptide chain, or several polypeptide chains linked together. Hydrogen bonds between amino acids cause the polypeptide chain to form its **secondary structure**, either an α-helix or a β-pleated sheet. The interaction between R groups causes a polypeptide to fold into its **tertiary structure**, a three dimensional shape held by ionic bonds and disulfide bridges (bonds formed between sulfur containing amino acids). If bonds are broken (through denaturation), the protein loses its tertiary structure, and its functionality.

The shape of a protein reflects its biological role







Channel proteins

Proteins that fold to form channels in the plasma membrane present non-polar R groups to the membrane and polar R groups to the inside of the channel. Hydrophilic molecules and ions are then able to pass through these channels into the interior of the cell. Ion channels are found in nearly all cells and many organelles.

Enzymes

Enzymes are globular proteins that catalyse specific reactions. Enzymes that are folded to present polar R groups at the active site will be specific for polar substances. Non-polar active sites will be specific for non-polar substances. Alteration of the active site by extremes of temperature or pH cause a loss of function.

Sub-unit proteins

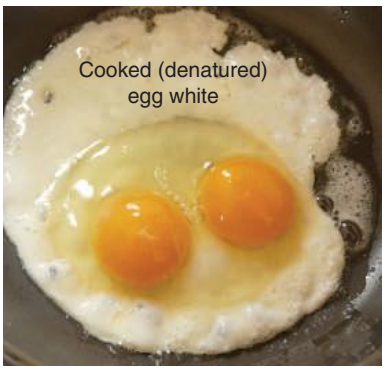
Many proteins, e.g. insulin and haemoglobin, consist of two or more sub-units in a complex quaternary structure, often in association with a metal ion. Active insulin is formed by two polypeptide chains stabilised by disulfide bridges between neighbouring cysteines. Insulin stimulates glucose uptake by cells.

Protein denaturation

When the chemical bonds holding a protein together become broken the protein can no longer hold its three dimensional shape. This process is called **denaturation**, and the protein usually loses its ability to carry out its biological function.

There are many causes of denaturation including exposure to heat or pH outside of the protein's optimum range. The main protein in egg white is albumin. It has a clear, thick fluid appearance in a raw egg (right). Heat (cooking) denatures the albumin protein and it becomes insoluble, clumping together to form a thick white substance (far right).





1. Using the example of insulin, explain how interactions between R groups stabilise the protein's functional structure:

2. Why do channel proteins often fold with non-polar R groups to the channel's exterior and polar R groups to its interior?

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16 Comparing Globular and Fibrous Proteins

Key Idea: The very different structure and properties of globular and fibrous proteins reflect their contrasting roles.

Proteins can be classified according to structure or function. **Globular proteins** are spherical and soluble in water (e.g. enzymes). **Fibrous proteins** have an elongated structure and are not water soluble. They provide stiffness and rigidity to the more fluid components of cells and tissues and have important structural and contractile roles.

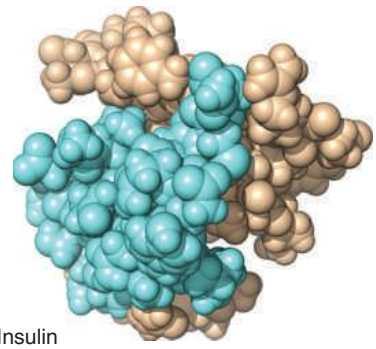
Globular proteins

Properties

- Easily water soluble
- Tertiary structure critical to function
- Polypeptide chains folded into a spherical shape

Functions

- Catalytic, *e.g. enzymes*
- Regulatory, *e.g. hormones (insulin)*
- Transport, *e.g. haemoglobin*
- Protective, *e.g. immunoglobulins (antibodies)*



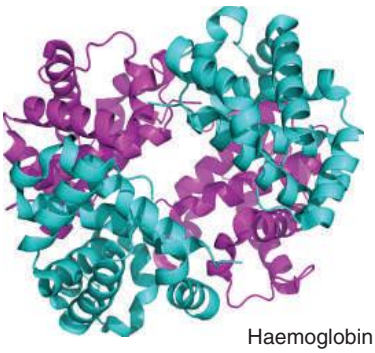
Insulin

Insulin is a peptide hormone involved in the regulation of blood glucose. Insulin is composed of two peptide chains linked together by two disulfide bonds.



RuBisCO

RuBisCo is a large multi-unit enzyme. It catalyses the first step of carbon fixation in photosynthesis. It consists of 8 large and 8 small subunits and is the most abundant protein on Earth.

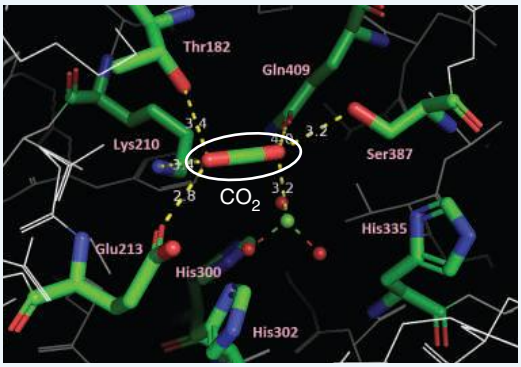


Haemoglobin

Haemoglobin is a multi-unit oxygen-transporting protein found in vertebrate red blood cells. One haemoglobin molecule consists of four polypeptide subunits. Each subunit contains an iron-containing haem group, which binds oxygen.

Proteins as catalysts

- Globular proteins act as catalysts for important biological reactions. In this capacity they are enzymes.
- The polypeptide chain folds up in such a way as to produce a site where the reaction occurs. This is called the active site.
- Substrates (chemicals that will react) are drawn into the active site, the reaction occurs, and the products are then released.
- The enzyme is not used up in the reaction but is free to react again. Enzymes are typically named with the suffix *-ase* after the substrate they work on. For example, RuBisCo's full name is *Ribulose-1,5-bisphosphate carboxylase-oxygenase.*



The active site of RuBisCo shown with CO_2 in position

1. What are the functional roles of globular proteins? _____

2. Why is RuBisCo one of the most important proteins on Earth? _____

3. How does the shape of a catalytic protein (enzyme) relate to its functional role? _____

4. What type of protein is haemoglobin? _____

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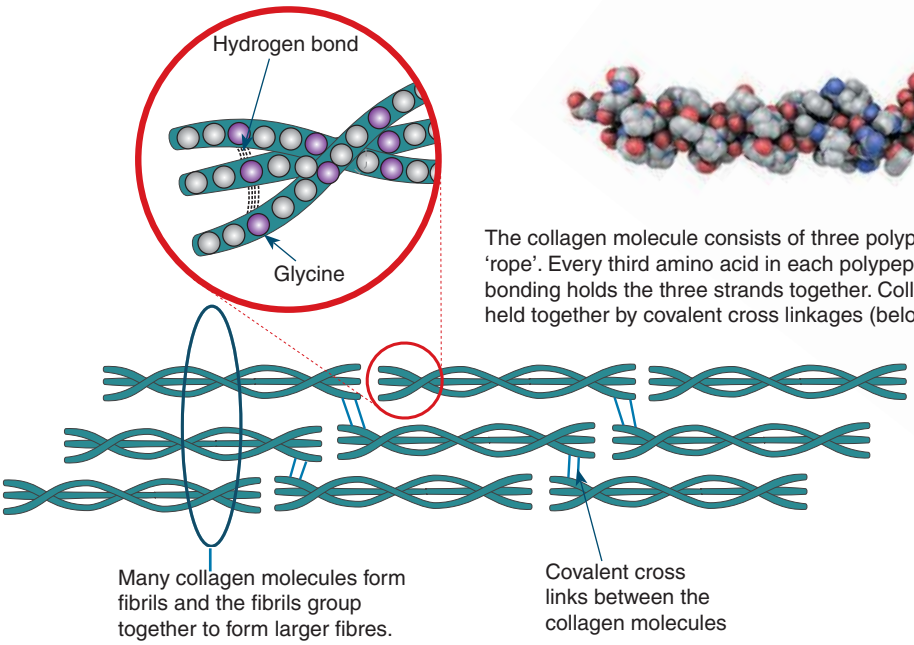
Fibrous proteins

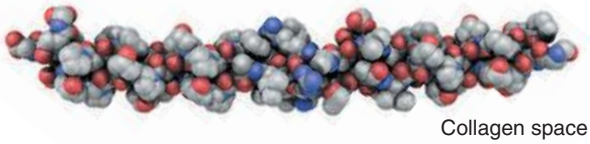
Properties

- Water insoluble
- Very tough physically; may be supple or stretchy
- Parallel polypeptide chains in long fibres or sheets

Functions

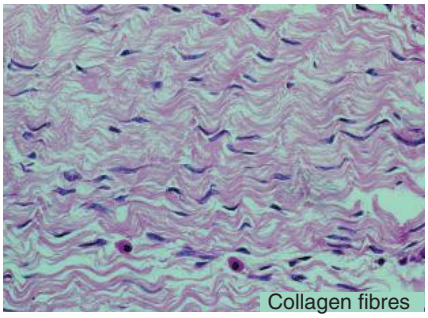
- Structural role in cells and organisms *e.g. collagen in connective tissues, skin, and blood vessel walls.*
- Contractile *e.g. myosin, actin (muscles)*





Collagen space filling model

The collagen molecule consists of three polypeptides wound together to form a helical 'rope'. Every third amino acid in each polypeptide is a glycine (Gly) where hydrogen bonding holds the three strands together. Collagen molecules self assemble into **fibrils** held together by covalent cross linkages (below). Bundles of fibrils form fibres.



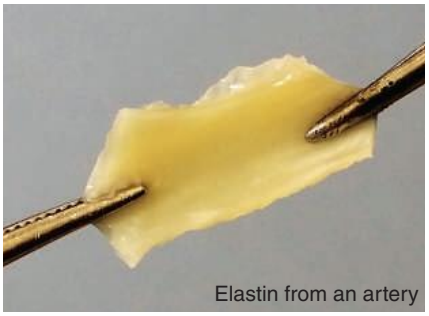
Collagen fibres

Collagen is the main component of connective tissue, and is mostly found in fibrous tissues (e.g. tendons, ligaments, and skin).



Rhinoceros horn is keratin

Keratin is found in hair, nails, horn, hooves, wool, feathers, and the outer layers of the skin. The polypeptide chains of keratin are arranged in parallel sheets held together by hydrogen bonding.



Elastin from an artery

Christian Schmelzer CC4.0

Elastin is found in the extracellular matrix and connective tissue. It allows tissue to stretch, contract, and reform. It an important component of skin, helping skin reform its shape as one moves.

5. (a) What is the role of proteins involved in structural tissues? Give examples to help illustrate your answer:

_____

_____

_____

_____

(b) How does the shape of a fibrous protein relate to its functional role? _____

_____

_____

6. What level of protein structure does collagen have? _____

7. Snake venom is a cocktail of proteins. Some snake venom is so toxic, a single bite can kill a human in less than a hour. Some of the proteins act to break down the victim's proteins. What functional group do these kinds of proteins fit into?

_____

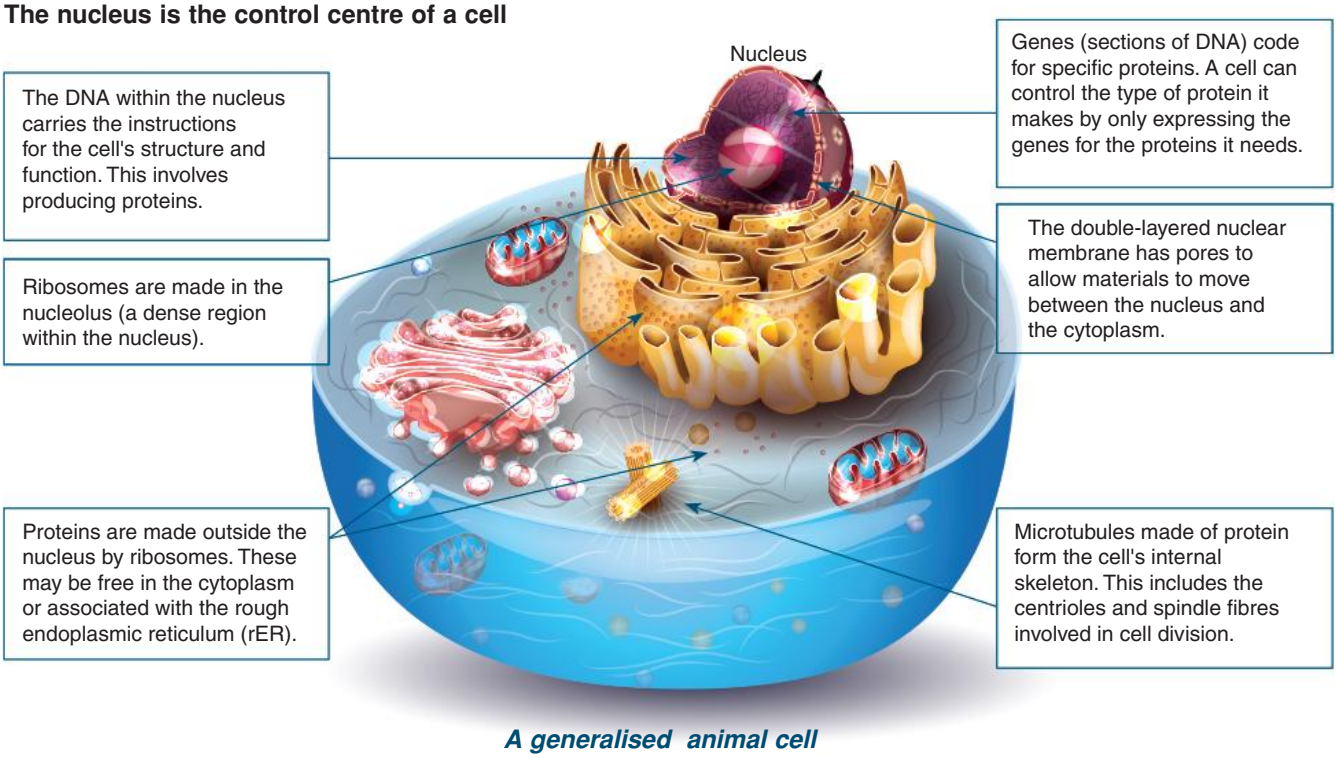
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17 Protein Functional Diversity

Key Idea: Proteins carry out the essential functions of life and have structural, catalytic, and regulatory roles.

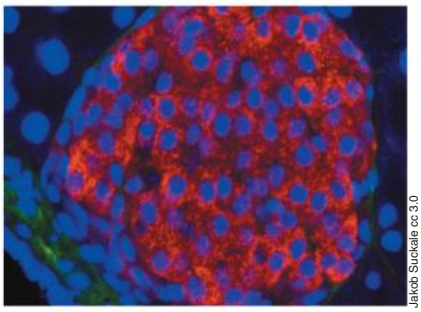
In eukaryotic cells, most of a cell's genetic information (DNA) is found in a large membrane-bound organelle called the nucleus. DNA provides the instructions that code for the formation of proteins and the nucleus directs all cellular activities by controlling the synthesis of proteins, which carry out most of a cell's work. A cell produces many different types proteins, each with a specific task. Proteins have roles in structure, function, and regulation of the body's cells, tissues, and organs. Without a full complement of functional proteins, a cell can not carry out its specialised role. All of the proteins encoded by an organism's DNA is called its **proteome**. The proteome is larger than the genome because, as you saw earlier, cells are able to produce many different proteins from one set of instructions.

The nucleus is the control centre of a cell

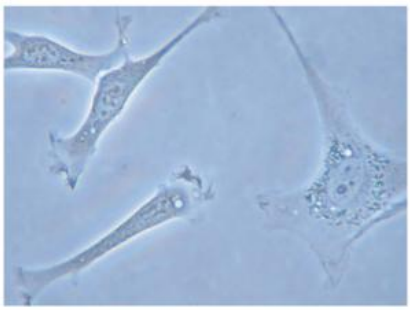


A generalised animal cell

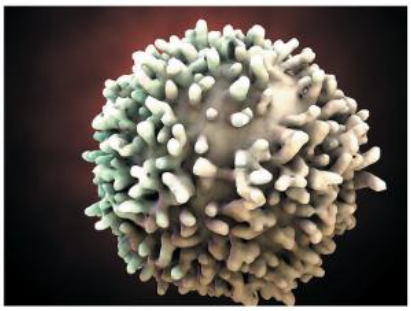
- While a generalised cell produces a range of proteins, some cells in the body are highly specialised to produce large amounts of a specific protein. This specialisation defines their functional role. Three examples are pictured below.



Cells within specialised regions of the pancreas produce and release the protein hormone insulin. Insulin (red in photo) helps to regulate blood glucose.



Fibroblasts are specialised cells that continuously produce and secrete the materials that form connective tissue, including the protein collagen.

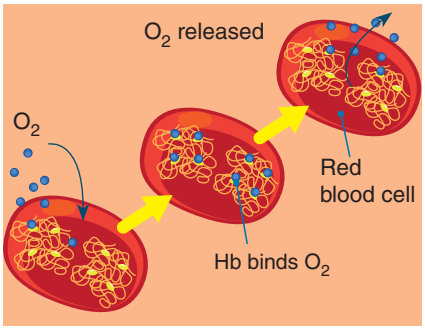
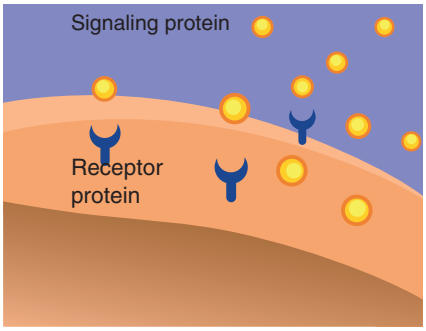
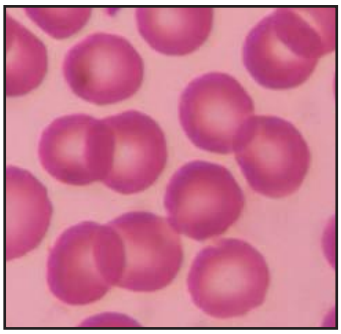
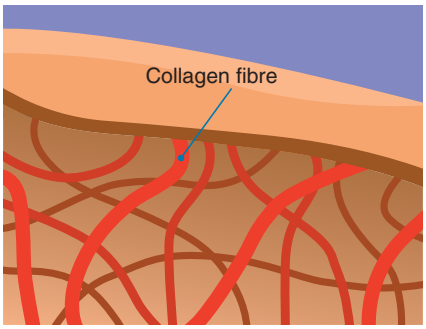
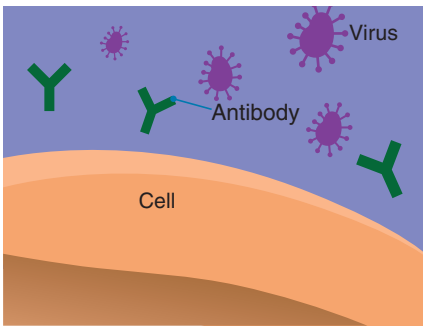
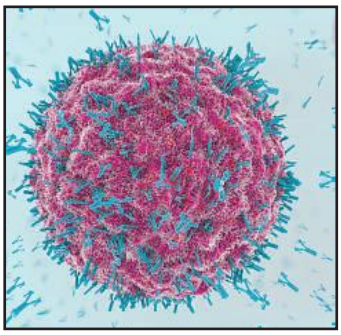
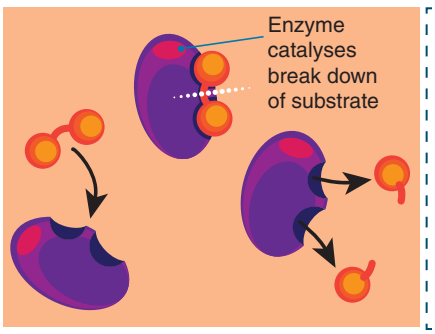
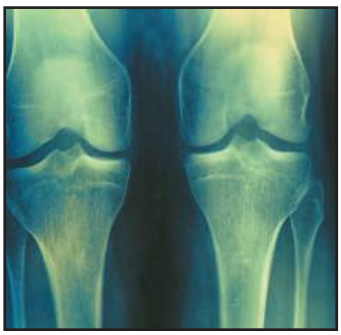
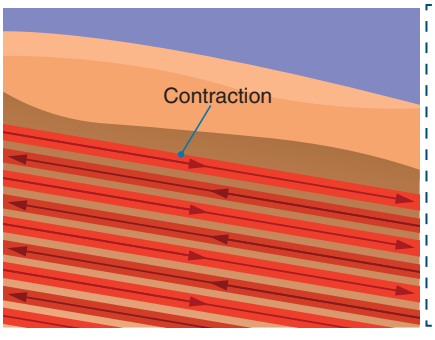


B lymphocytes (B cells) are white blood cells that are specialised to produce and secrete proteins called antibodies, which protect the body against diseases.

1. Suggest what might happen to a protein's functionality if it was incorrectly encoded by the DNA. Explain your answer:

2. The opposite page shows six pictograms of proteins in action, six protein functions, six protein examples, and six photographs. These are not in any matched order. Cut out the 24 boxes and paste or tape them into the grid on the next page so that each pictogram is matched with its correct function, example, and illustrative photograph.

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PICTOGRAM	FUNCTION	EXAMPLE	PHOTO EXAMPLE
	**Internal defence** Antibodies (also called immunoglobulins) are "Y" shaped proteins that protect the body by identifying and killing disease-causing organisms such as bacteria and viruses.	**Immunoglobulin A** IgA is found in the gut and airways. It destroys disease-causing organisms growing in these areas.	
	**Catalytic** Thousands of different chemical reactions take place in an organism every minute. Each chemical reaction is catalysed by enzymes. The word-ending "ase" indicates an enzyme.	**Actin & myosin** Two proteins that work together to bring about contraction (movement) in all the muscles of the body, including those that work without your awareness.	
	**Regulation** Regulatory proteins such as hormones act as signal molecules to control biological processes and coordinate responses in cells, tissues, and organs.	**Haemoglobin** A protein found in red blood cells. It binds oxygen and carries it through the blood, delivering it to cells.	
	**Movement** Contractile proteins are involved in movement of muscles and form the internal supporting structures of cells.	**Collagen** Found in the skin and connective tissues, including bones, tendons, and ligaments. It is the most abundant protein in the body.	
	**Transport** Proteins can carry substances across membranes or around the body. In the blood, they transport and store oxygen. In cell membranes they help molecules move into and out of cells.	**Oestrogen** A hormone that is critical for reproduction in females. Oestrogen levels increase during pregnancy to maintain a healthy pregnancy.	
	**Structural** Structural proteins provide physical support or protection. They are strong, fibrous (thread like) and stringy.	**Amylase** An enzyme that breaks down starch into sugars in the first stage of digestion.	

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PICTOGRAM	FUNCTION	EXAMPLE	PHOTO EXAMPLE

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18 How Are Proteins Modified?

Key Idea: The modification of proteins allows the cell to specify their use and final destination.

Proteins may be modified after they have been produced by **post translational modification**. Two important modifications involve adding carbohydrates or lipids to the protein. **Glycoproteins** are formed by adding carbohydrates to proteins as they pass through the rough endoplasmic reticulum (rER) and Golgi. The carbohydrates may help position and orientate the glycoprotein in the membrane, guide a protein to its final destination, or help in cell-to-cell recognition and cell signalling. Other proteins may have fatty acids added to them in the rER to form **lipoproteins**. These modified proteins transport lipids in the plasma between various organs in the body (e.g. gut, liver, and adipose tissue). Other common post-translational modifications include degradation, cleavage, and phosphorylation (below).

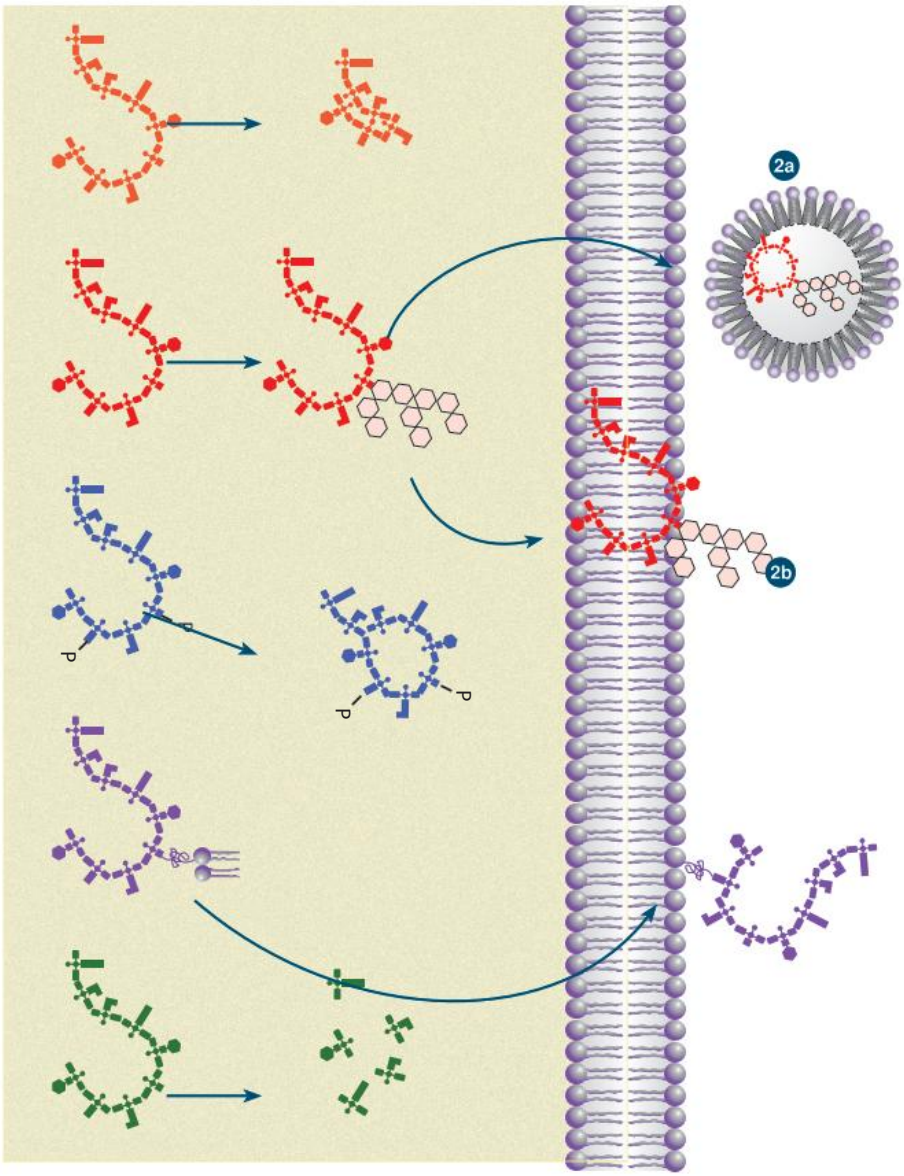
Cleaving: Polypeptide chains may be cleaved to give smaller chains, which then fold or join to give the functional protein. An example is human insulin which is transcribed as one long polypeptide chain before being cleaved in two places to form two shorter chains that form the functional protein.

Glycosylation (adding carbohydrate groups): This is used to add an ID tag to the protein that will allow the cell to recognise its use and where it is to be transported (2a). The resulting glycoprotein may be used in the cell membrane or secreted. The carbohydrate tag may help position the glycoprotein within the membrane (2b).

Phosphorylation (the addition of phosphate groups) takes place in the Golgi. It may contribute to the protein's three dimensional structure or help with cell signalling.

Lipid attachment: Proteins may have lipids attached to them which anchor the protein to the plasma membrane.

Degradation: Some polypeptide chains may be tagged for degradation when they are no longer useful and their amino acids reused in the formation of other proteins.



1. (a) Describe some of the modifications that polypeptide chains undergo before becoming functional proteins:

_____

_____

_____

(b) Why are these changes necessary? _____

_____

_____

2. Why might the orientation of a protein in the plasma membrane be important? _____

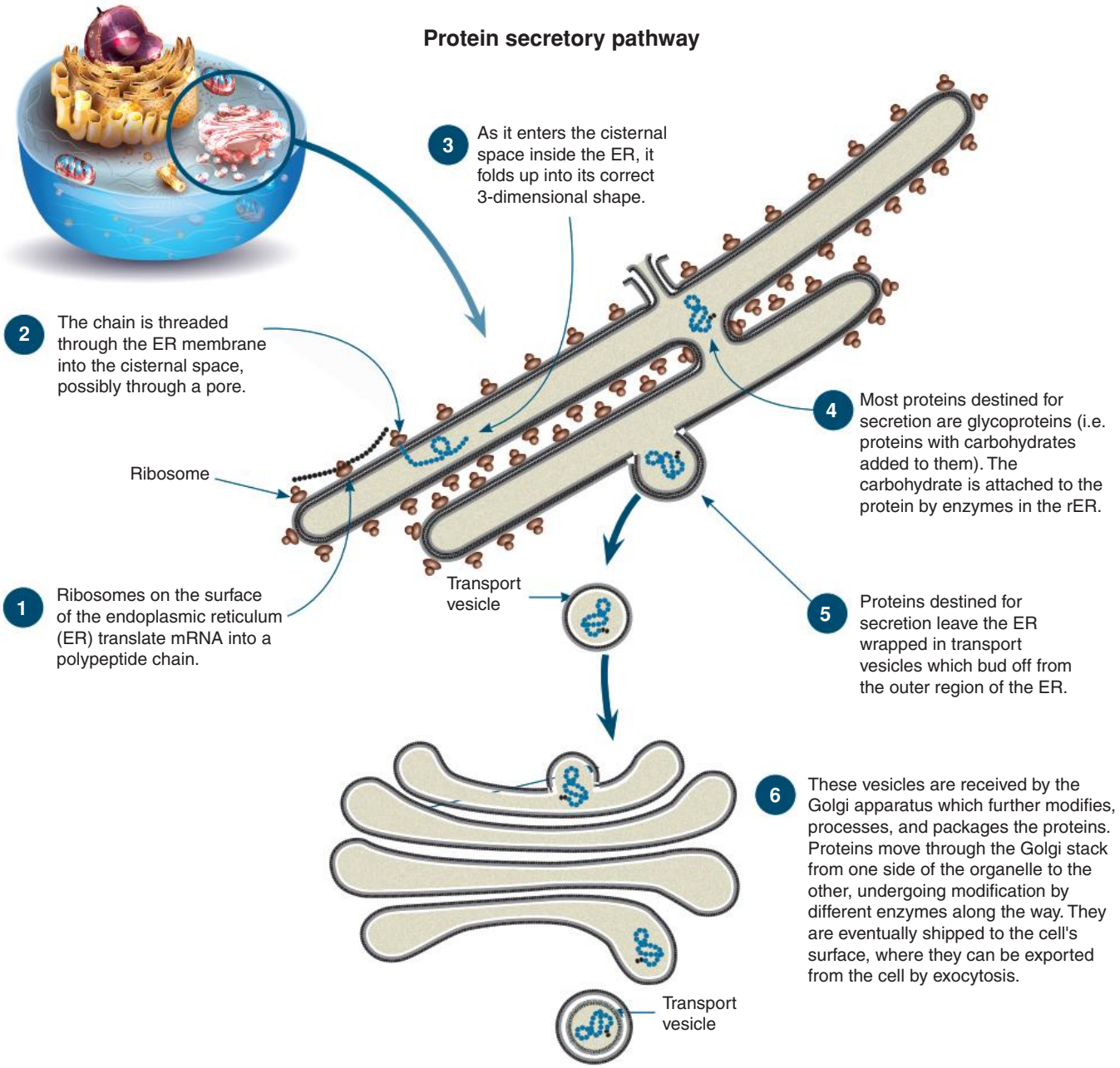
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19 Protein Export

Key Idea: The synthesis, packaging and movement of macromolecules inside the cell involves coordination between several membrane-bound organelles.

Many proteins need to be modified in order to become functional. This modification takes place in the rough endoplasmic reticulum (rER). From the rER, proteins are transported to the Golgi where the protein is further modified before packaged and shipped to its final destination.



1. Explain the role of each of the following organelles in the production and transport of proteins:

   (a) Ribosomes: _____

   _____

   (b) Endoplasmic reticulum: _____

   _____

   (c) Transport vesicles: _____

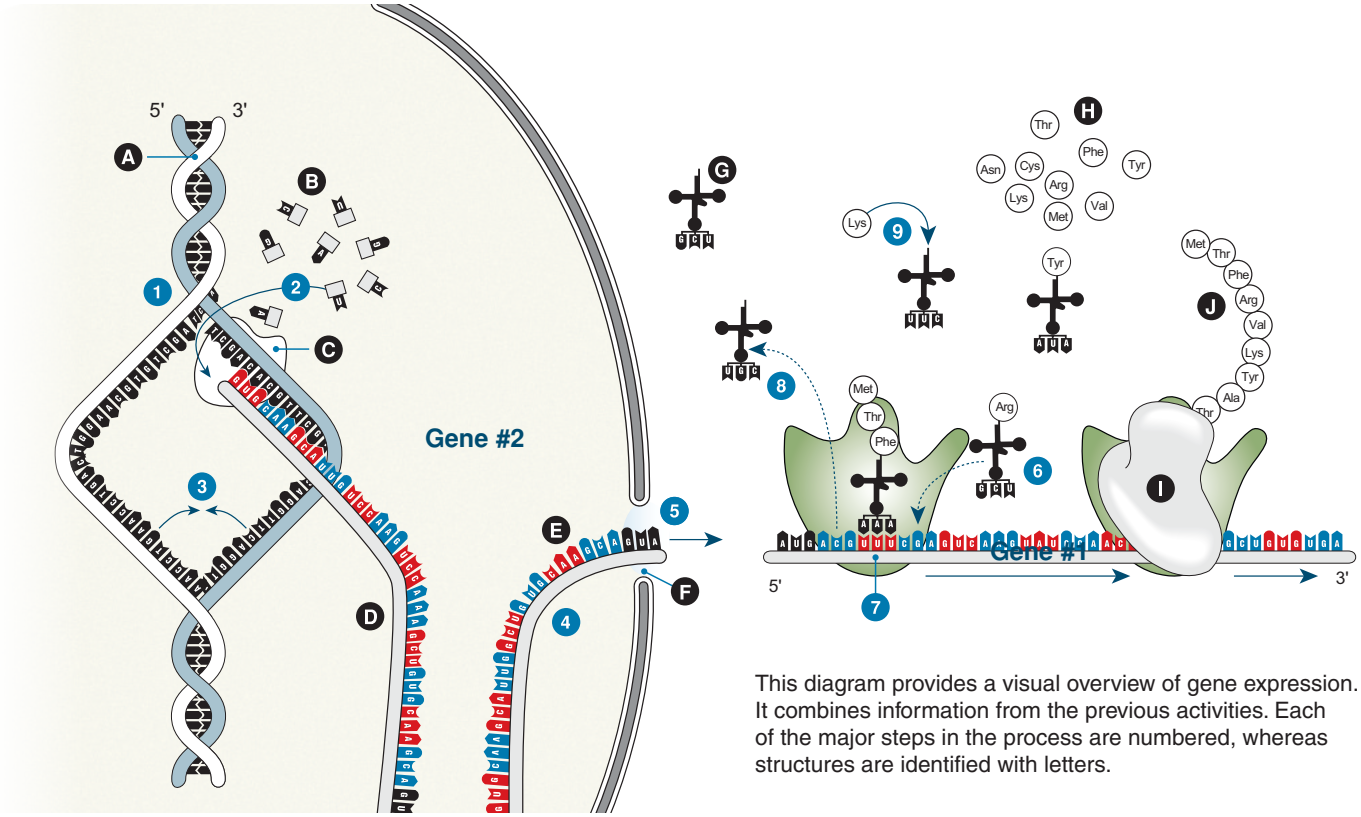
   _____

   (d) Golgi apparatus: _____

   _____

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20 Chapter Review: Did You Get It?



This diagram provides a visual overview of gene expression. It combines information from the previous activities. Each of the major steps in the process are numbered, whereas structures are identified with letters.

1. Briefly describe each of the numbered processes in the diagram above:

   (a) Process 1: _____

   (b) Process 2: _____

   (c) Process 3: _____

   (d) Process 4: _____

   (e) Process 5: _____

   (f) Process 6: _____

   (g) Process 7: _____

   (h) Process 8: _____

   (i) Process 9: _____

2. Identify each of the structures marked with a letter and write their names below in the spaces provided:

   (a) Structure A: _____ (f) Structure F: _____

   (b) Structure B: _____ (g) Structure G: _____

   (c) Structure C: _____ (h) Structure H: _____

   (d) Structure D: _____ (i) Structure I: _____

   (e) Structure E: _____ (j) Structure J: _____

3. Describe two factors that would determine whether or not a particular protein is produced in the cell:

   (a) _____

   _____

   (b) _____

   _____

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CHAPTER 2

DNA Manipulation

Key terms

annealing
CRISPR-Cas9 system
DNA (genetic) profiling
DNA amplification
DNA ligase
DNA ligation
DNA polymerase
forensics
gel electrophoresis
genetic modification
GMO
marker gene
microsatellite
plasmid
polymerase chain reaction (PCR)
primer
recognition site
recombinant DNA
recombinant plasmid
restriction enzyme
STR
transgenic organism
vector

Enzymes enable us to manipulate DNA

Key skills and knowledge

Activity number

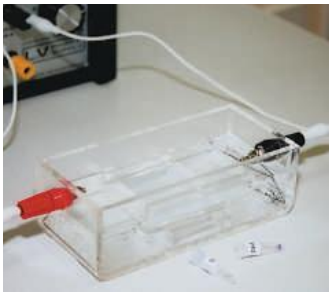
- ☐ 1 Describe how enzymes are used to manipulate DNA. Include reference to the principles and applications of endoncleases (restriction enzymes and the CRISPR-Cas9 system), ligases, and polymerases. — 21 23 24
- ☐ 2 Describe the function of CRISPR-Cas9 in bacteria. Explain how gene editing technology using CRISPR-Cas9 allows genomes to be manipulated more easily, more precisely, and at less cost than ever before. — 24

PCR amplifies DNA and gel electrophoresis sorts it

Key skills and knowledge

- ☐ 3 Describe DNA amplification using the polymerase chain reaction (PCR). Include reference to the role of primers and the thermally stable taq polymerase. — 22
- ☐ 4 Describe when DNA amplification might be useful or necessary. — 22
- ☐ 5 Explain the role of gel electrophoresis (of DNA) in DNA technologies. How does electrophoresis sort the fragments? — 25
- ☐ 6 Explain how the DNA fragments on a gel are made visible and the role of DNA markers in identifying fragments of different size. — 25
- ☐ 7 Describe the applications of DNA profiling. Interpret the results of gel runs for DNA profiling. What sort of information can be provided by the banding patterns? — 26
- ☐ 8 TEST Analyse and evaluate the success of DNA profiling in forensic analysis. — 27







Recombinant plasmids can transform bacterial cells

Key skills and knowledge

- ☐ 9 Recall what is meant by a recombinant plasmid. What sort of genes are included in a recombinant plasmid and why? — 23
- ☐ 10 Describe how recombinant plasmids are used to transform bacterial cells, as demonstrated by the production of human insulin. — 28 29
- ☐ 11 Describe the applications of transformed bacterial cells in industry, medicine, and agriculture. — 28 29 30

Genetically modified and transgenic organisms

Key skills and knowledge

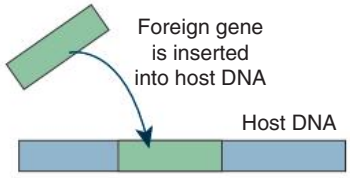
- ☐ 12 Distinguish between genetically modified and transgenic organisms, recognising one as a subset of the other. Use your knowledge of DNA to explain why it is possible to create transgenic organisms. Understand that transgenic organisms can include all types of species from bacteria to plants and animals. — 28
- ☐ 13 Describe the use of transgenic organisms in agriculture to change crop characteristics, increase crop production or yield, or confer resistance to pests or diseases. — 28 30

21 What is DNA Manipulation?

Key Idea: DNA manipulation alters an organism's DNA either by adding new DNA or editing the existing DNA.

DNA manipulation (also called genetic engineering) involves the direct manipulation of an organism's genome using biotechnology. This can be achieved by introducing new DNA into an organism or by editing its existing DNA. DNA manipulation aims to repair damage or to produce improved or novel organisms with specific traits. Genetic engineering has wide applications in food technology, industry, agriculture, environmental clean up, pharmaceutical production, and vaccine development. Organisms that have had their DNA altered are called genetically modified organisms (GMOs).

How are genetically modified organisms produced?

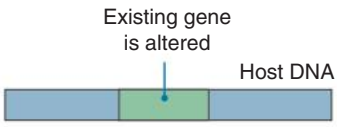


Add a foreign gene

A novel gene (foreign to the recipient) is inserted from another species. This will enable the GMO to express the trait encoded by the new gene. Organisms genetically altered in this way are referred to as **transgenic**.

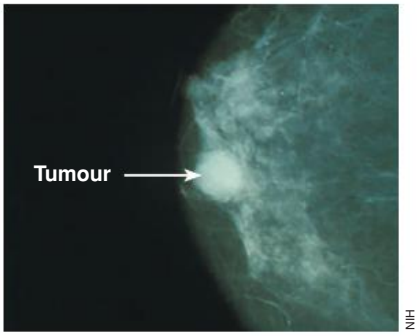


Human insulin, used to treat diabetic patients, is produced by inserting the insulin gene into bacteria or yeast.

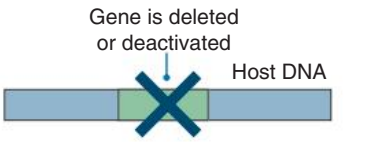


Alter an existing gene

An existing gene may be altered to make it express at a higher level (e.g. growth hormone) or in a different way (in tissue that would not normally express it). The technique may provide a way to fix a malfunctioning gene.



Gene editing technologies, such as CRISPR, are being explored to treat breast cancer (above) and sickle cell disease.



Delete or 'turn off' a gene

An existing gene may be deleted or deactivated (switched off) to prevent the expression of a trait (e.g. the deactivation of the ripening gene in tomatoes produced the Flavr-Savr tomato).



Manipulating gene action is one way in which to control processes such as ripening in fruit so it stays fresher longer.

1. (a) What is DNA manipulation? _____

(b) Using examples, discuss the ways in which an organism may be genetically modified: _____

2. Describe some of the applications of DNA manipulation: _____

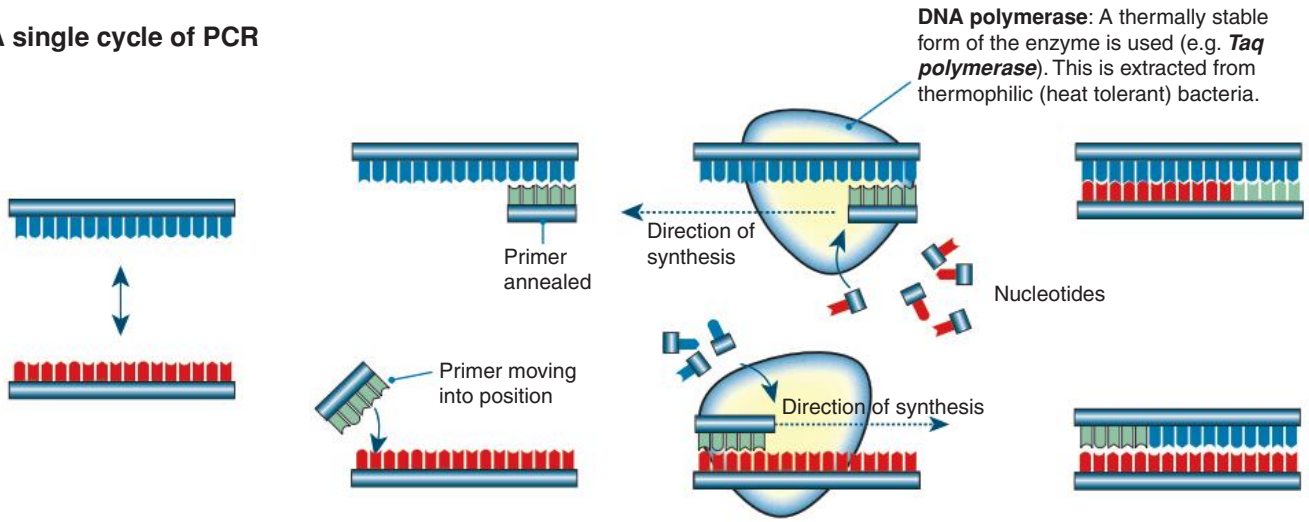
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22 DNA Amplification Using PCR

Key Idea: PCR uses a polymerase enzyme to copy a DNA sample, producing billions of copies in a few hours.

Often it is very hard to get enough DNA to analyse (e.g. DNA from a crime scene or from an extinct organism). Researchers need to increase the amount of DNA they have to work with, this is done using **polymerase chain reaction** (PCR). PCR can make billions of copies of a target DNA sequence of interest so that it can be analysed. The technique is carried out *in vitro* (e.g. in tubes) rather than in a living organism. An overview of PCR given below.

A single cycle of PCR



1. Denaturing
A DNA sample (called target DNA) is obtained. It is denatured (DNA strands are separated) by heating at 98°C for 5 minutes.

2. Annealing
The sample is cooled to 60°C. Primers are annealed (bonded) to each DNA strand. In PCR, the primers are short strands of DNA; they provide the starting sequence for DNA extension.

3. Extension/elongation
Free nucleotides and DNA polymerase are added. DNA polymerase binds to the primers and synthesises complementary strands of DNA, using the free nucleotides.

4. Completed strands
After one cycle, there are now two copies of the original DNA.

Repeat cycle of heating and cooling until enough copies of the target DNA have been produced

1. Outline the process of PCR: _____

2. (a) Explain the purpose of PCR: _____

   (b) Give two examples where PCR is needed to amplify DNA: _____

3. After only two cycles of replication, four copies of the double-stranded DNA exist. Calculate how much a DNA sample will have increased after:

   (a) 10 cycles: _____ (b) 25 cycles: _____

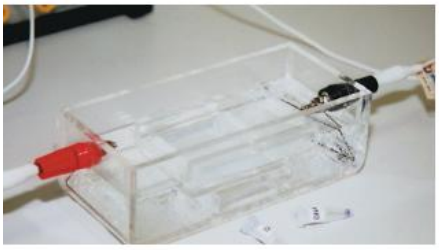
4. Researchers take great care to avoid DNA contamination during PCR preparation. Explain why: _____

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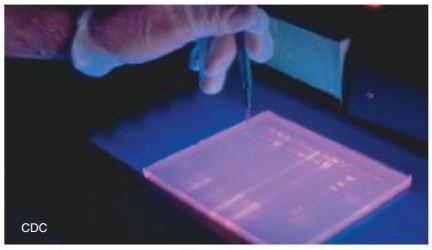
23 Making Recombinant DNA

Key Idea: Recombinant DNA (rDNA) is produced by first isolating (or synthesising) a DNA sequence, then inserting it into the genome of a different organism, e.g. a bacterium.
Recombinant DNA (rDNA) is produced by combining genetic material from two or more different sources. The production of rDNA is possible because the DNA of every organism is made of the same building blocks (nucleotides). rDNA allows a gene from one organism to be moved into, and expressed in, a different organism. Two important tools are used to create rDNA. **Endonucleases** (such as restriction enzymes or the CRISPR-Cas9 system) cut the DNA and the enzyme **DNA ligase** is used to join the sections of DNA together.

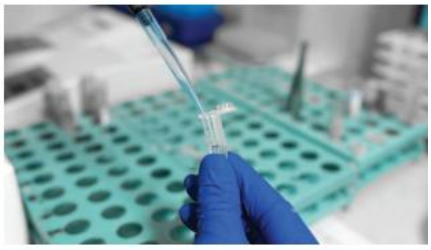
Overview: How is recombinant DNA made?



The target DNA is amplified by PCR. It is then cut with restriction enzymes (below). The plasmid into which it will be inserted is cut with the same enzymes. The DNA and plasmid are then run on an electrophoresis gel to separate them from other DNA fragments.



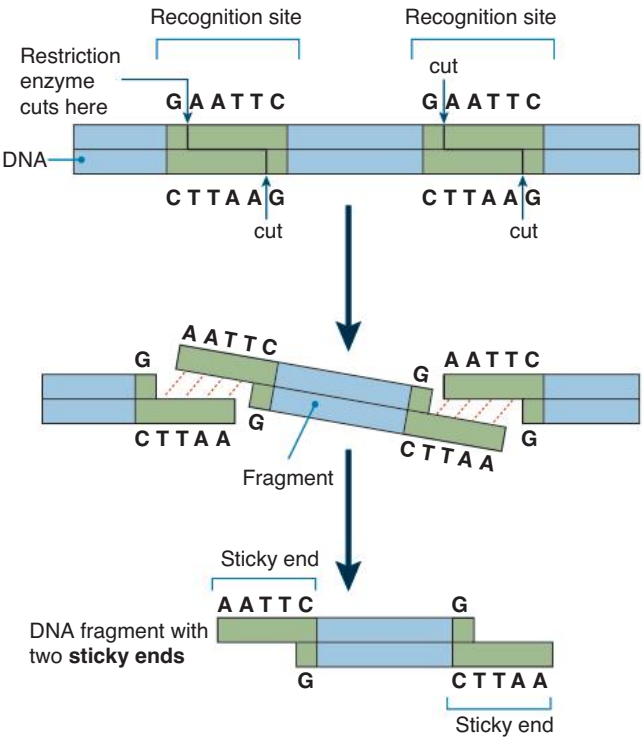
Once the DNA fragments are separated, the gel is placed on a UV viewing platform. The areas of the gel containing the target DNA and plasmid are cut out and placed in a solution that dissolves the gel. This releases the DNA into the solution.



The target DNA and the plasmid into which it will be inserted are mixed together. They were cut with the same restriction enzymes so the cut sites will have matching DNA overhangs and bond together to produce a recombinant plasmid (below and opposite).

What are restriction enzymes?

- A **restriction enzyme** is an enzyme that cuts a double-stranded DNA molecule at a specific **recognition site** (a specific DNA sequence). There are many different types of restriction enzymes, each has a unique recognition site.
- Some restriction enzymes produce DNA fragments with two **sticky ends** (right). A sticky end has exposed nucleotide bases at each end. DNA cut in such a way is able to be joined to other DNA with matching sticky ends. Such joins are specific to their recognition sites.
- Some restriction enzymes produce a DNA fragment with two blunt ends (ends with no exposed nucleotide bases). The piece it is removed from is also left with blunt ends. DNA cut in such a way can be joined to any other blunt end fragment. Unlike sticky ends, blunt end joins are non-specific because there are no sticky ends to act as specific recognition sites.



1. What is the purpose of restriction enzymes in making recombinant DNA? _____

2. What is the difference between sticky end and blunt end fragments? _____

3. Why is it useful to have many different kinds of restriction enzymes? _____

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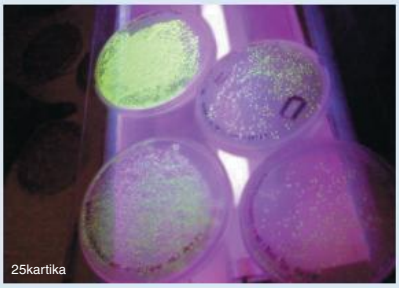
Creating a recombinant DNA plasmid

1. Two pieces of DNA are cut by the same restriction enzyme (they will produce fragments with matching sticky ends).

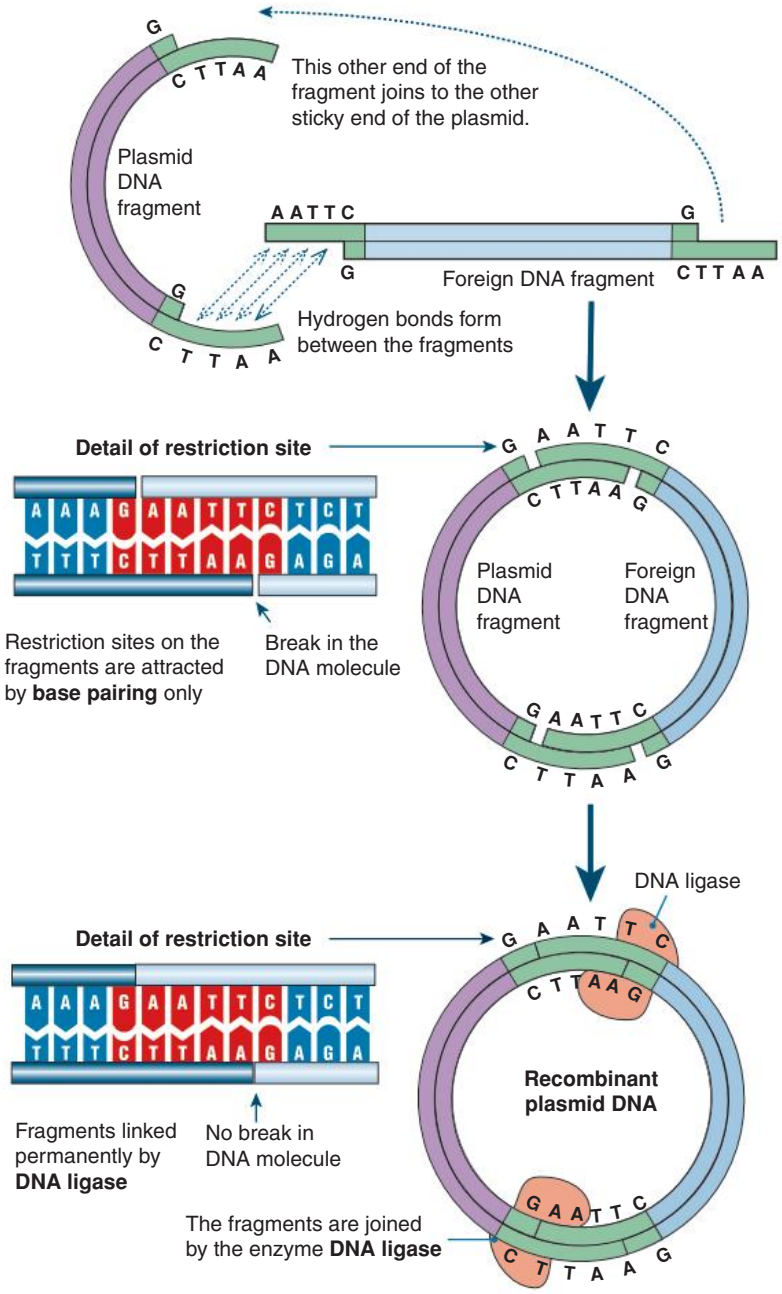
2. Fragments with matching sticky ends can be joined by base-pairing. This process is called **annealing.** This allows DNA fragments from different sources to be joined.

   The DNA fragments are joined by the enzyme **DNA ligase**, which catalyses the formation of a phosphodiester bond. This produces a molecule of recombinant DNA.

3. The joined fragments will usually form either a linear or a circular molecule, as shown here (right) as recombinant plasmid DNA.



pGLO is a plasmid engineered to contain Green Fluorescent Protein (*gfp*). pGLO has been used to create fluorescent organisms, including the bacteria above (bright green patches on agar plates).



4. Explain in your own words the two main steps in the process of joining two DNA fragments together:

   (a) Annealing: _____

   _____

   (b) DNA ligase: _____

   _____

5. Explain why ligation can be considered the reverse of the restriction digestion process: _____

   _____

6. Why can recombinant DNA be expressed in any kind of organism, even if it contains DNA from another species?

   _____

   _____

   _____

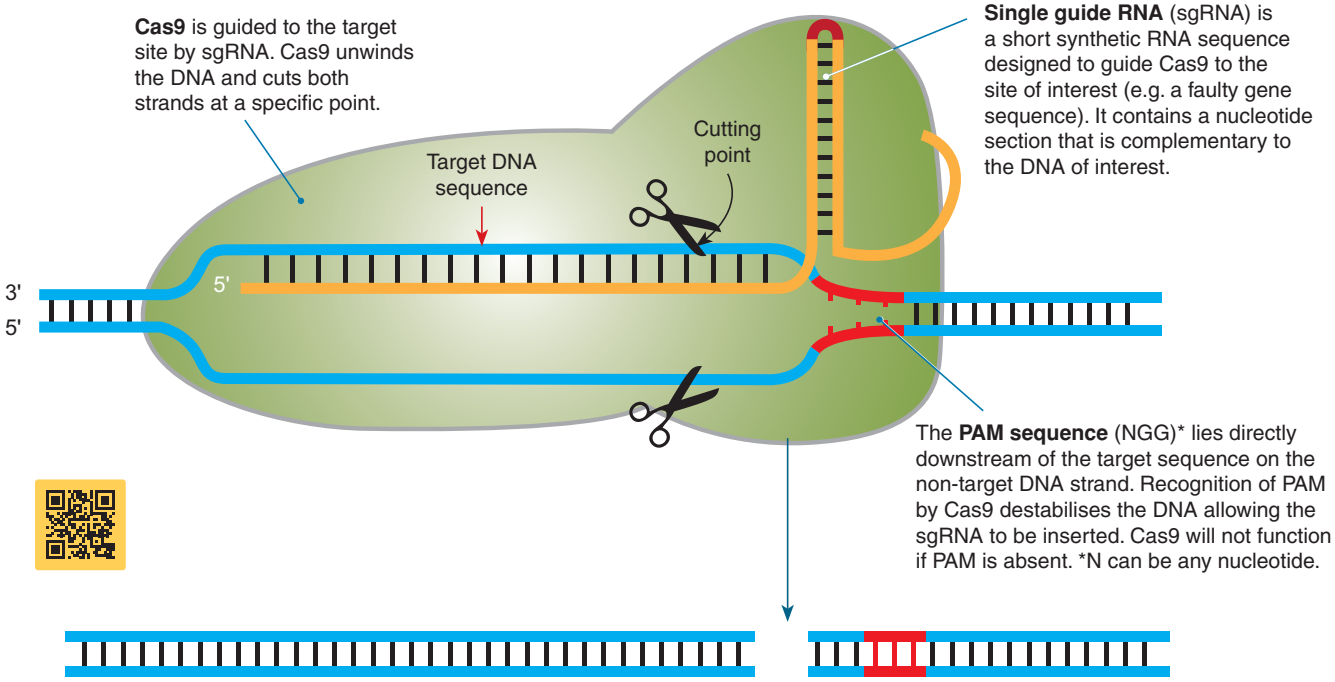
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24 New Tools: Gene Editing with CRISPR

Key Idea: CRISPR is a complex made up of Cas9 endonuclease and sgRNA. The CRISPR complex cuts DNA at very specific sequences and can be used to edit genes.

CRISPR-Cas9 (shortened to CRISPR and pronounced crisper) is an endonuclease complex occurring naturally in bacteria, which use it to edit the DNA of invading viruses. CRISPR is able to target specific stretches of DNA and edit it at very precise locations. Two key components are required for CRISPR to work: an RNA guide that locates and binds to the target piece of DNA and the Cas9 endonuclease that unwinds and cuts the DNA. The technology has potential applications in correcting mutations responsible for disease, switching faulty genes off, adding new genes to an organism, or studying the effect of specific genes. It represents a major advance because it allows more precise and efficient gene editing at much lower cost than ever before.



The cut DNA can be repaired using one of the following methods:

Gene knock in "gene editing"

A new DNA sequence is inserted into the DNA break. For example allows a faulty gene sequence can be replaced with the correct sequence to restore normal gene function.

Gene knock out "gene silencing"

Errors occur as the cell's normal repair mechanisms mend the broken DNA, causing the insertion or deletion of bases. The resulting frame-shift mutation changes the way the nucleotide sequence is read, either disabling gene function or producing a STOP signal. This technique can be used to silence a faulty gene.



1. What are the roles of the following in CRISPR gene editing:

   (a) Cas9: _____

   (b) sgRNA: _____

2. Outline two ways CRISPR can be used to edit genes: _____

   _____

   _____

   _____

3. What benefits are offered by CRISPR technology? _____

   _____

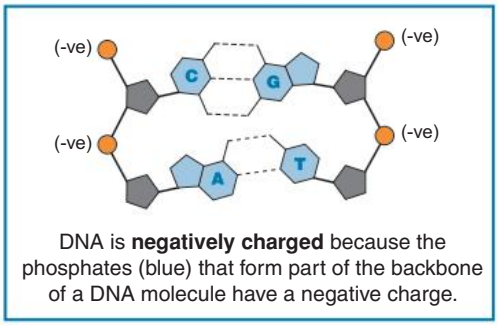
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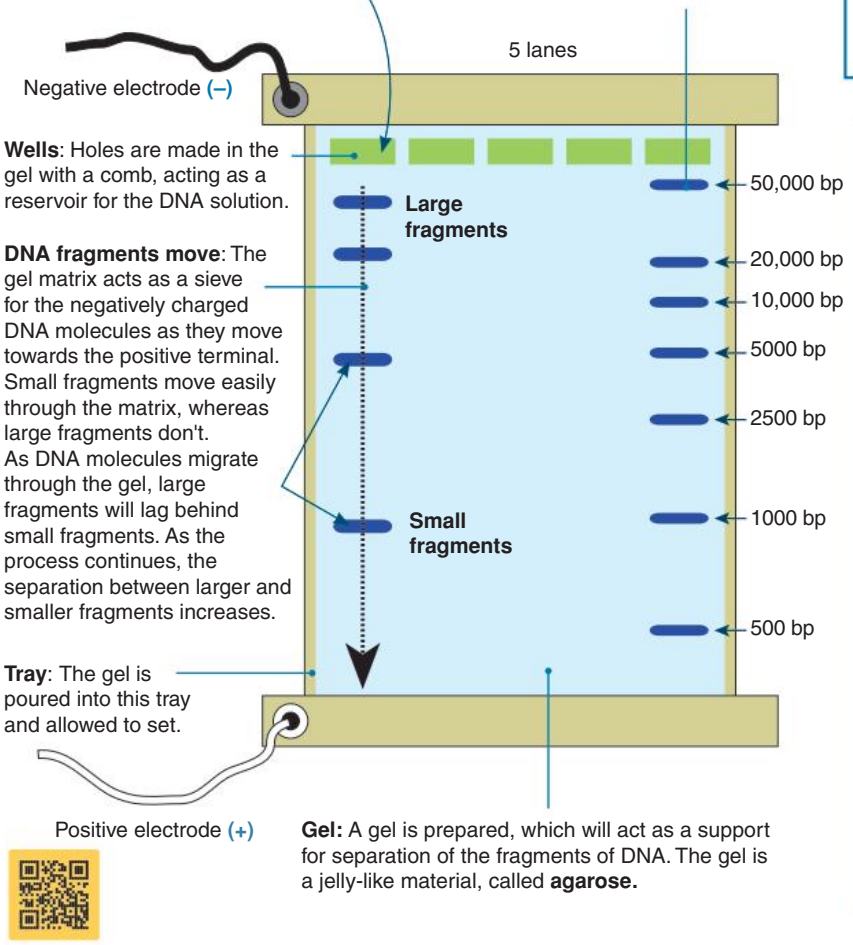
25 Gel Electrophoresis

Key Idea: Gel electrophoresis is used to separate DNA fragments on the basis of size.

Gel electrophoresis is a tool used to isolate DNA of interest for further study. It is also used for DNA profiling (comparing individuals based on their unique DNA banding profiles). DNA has an overall negative charge, so when an electrical current is run through a gel, the DNA moves towards the positive electrode. The rate at which the DNA molecules move through the gel depends primarily on their size and the strength of the electric field. The gel they move through is full of pores (holes). Smaller DNA molecules move through the pores more quickly than larger ones. At the end of the process, the DNA molecules can be stained and visualised as a series of bands. Each band contains DNA molecules of a particular size. The bands furthest from the start of the gel contain the smallest DNA fragments.



DNA is **negatively charged** because the phosphates (blue) that form part of the backbone of a DNA molecule have a negative charge.



Steps in the process of gel electrophoresis of DNA

1. The gel is placed in an electrophoresis chamber and the chamber is filled with buffer, covering the gel. This allows the electric current from electrodes at either end of the gel to flow through the gel.
2. DNA samples are mixed with a "loading dye" to make the DNA sample visible. The dye also contains glycerol or sucrose to make the DNA sample heavy so that it will sink to the bottom of the well.
3. The gel is covered, electrodes are attached to a power supply and turned on.
4. When the dye marker has moved through the gel, the current is turned off and the gel is removed from the tray.
5. DNA molecules are made visible by staining the gel with methylene blue or ethidium bromide which binds to DNA and will fluoresce in UV light.
6. The band or bands of interest are cut from the gel and dissolved in chemicals to release the DNA. This DNA can then be studied in more detail (e.g. its nucleotide sequence can be determined).

1. What is the purpose of gel electrophoresis? _____

_____

_____

2. Describe the two forces that control the speed at which fragments pass through the gel:

   (a) _____

   (b) _____

3. Why do the smallest fragments travel through the gel the fastest? _____

_____

_____

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Recognition sites for selected restriction enzymes

Enzyme	Source	Recognition sites
***Eco*RI**	*Escherichia coli* RY13	G A A T T C
***Hae*III**	*Haemophilus aegyptius*	G G C C
***Hind*III**	*Haemophilus influenzae* Rd	A A G C T T
***Hpa*I**	*Haemophilus parainfluenzae*	G T T A A C
***Hpa*II**	*Haemophilus parainfluenzae*	C C G G
***Mbo*I**	*Moraxella bovis*	G A T C
***Taq*I**	*Thermus aquaticus*	T C G A

DNA fragments for gel electrophoresis are produced by restriction digestion of DNA using restriction enzymes. Restriction enzymes are produced by bacteria as a method of eliminating foreign DNA. About 3000 different restriction enzymes have been isolated. Around 600 are commonly used in laboratories.

Restriction enzymes are named according to the species they were first isolated from, followed by a number to distinguish different enzymes isolated from the same organism.

4. (a) A scientist uses *Hpa*II to cut a length of DNA. State the recognition site for *Hpa*II: _____

   (b) Circle where on the DNA sequence below *Hpa*II would cut the following DNA sequence:

   GTTAGGCCCGGCTAGCTTGACCAGTCCCGGGTCACAGTCTCTGACCCGGCTTTAGACACACTCCGGTTACTACCG

5. In 1988, the disease BLAD (Bovine Leukocyte Adhesion Deficiency) specific to Holstein cattle was affecting the US dairy industry. The disease is recessive and two alleles are needed for its expression. The disease is caused by two mutations of the CD18 gene. One of the affected regions is shown below. The DNA of three individuals is shown: an unaffected animal, a carrier, and an affected animal. Only a single strand of DNA from each chromosome is shown to save space.

Unaffected	GTGACCTTCCGGAGGGCCAAGGGCTACCCCATCGGCCTGTACTACCTGATGGACCTCT	Allele 1
	GTGACCTTCCGGAGGGCCAAGGGCTACCCCATCGGCCTGTACTACCTGATGGACCTCT	Allele 2
Carrier	GTGACCTTCCGGAGGGCCAAGGGCTACCCCATCGACCTGTACTACCTGATGGACCTCT	Allele 1
	GTGACCTTCCGGAGGGCCAAGGGCTACCCCATCGGCCTGTACTACCTGATGGACCTCT	Allele 2
Affected	GTGACCTTCCGGAGGGCCAAGGGCTACCCCATCGACCTGTACTACCTGATGGACCTCT	Allele 1
	GTGACCTTCCGGAGGGCCAAGGGCTACCCCATCGACCTGTACTACCTGATGGACCTCT	Allele 2

--

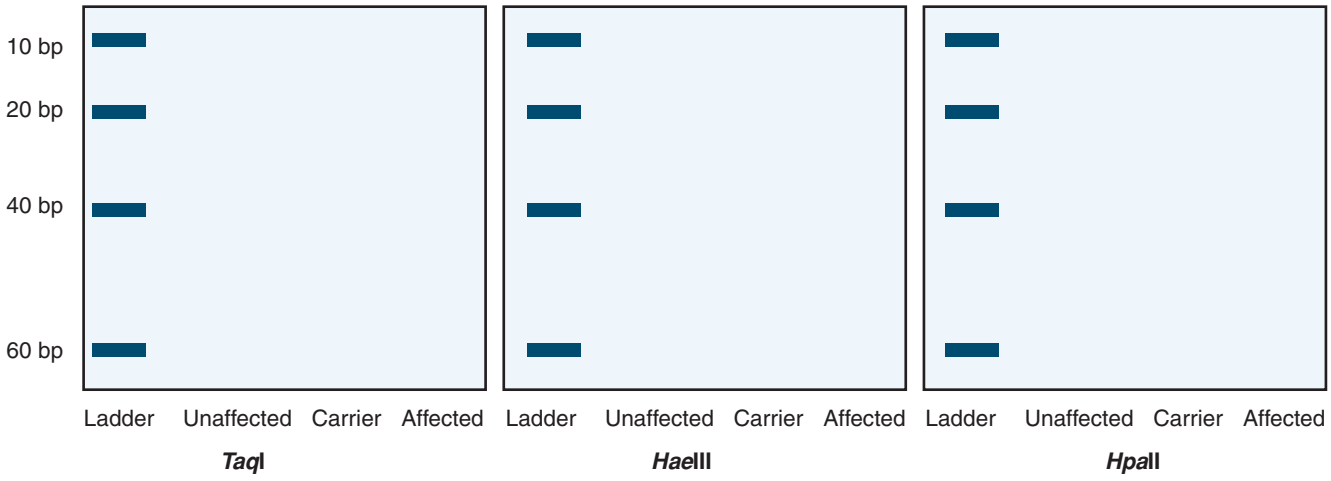
   (a) Use the restriction enzymes *Taq*I, *Hae*III, and *Hpa*II to "cut" the sequences. Write the length of the segments (in bases) produced in the spaces below. **U** = unaffected; **C** = carrier; **A** = affected.
   NOTE: *Taq*I cuts between T and C. *Hae*III cuts between G and C. *Hpa*II cuts between C and C:

   *Taq*1: **U**: _____ **C**: _____ **A**: _____

   *Hae*III: **U**: _____ **C**: _____ **A**: _____

   *Hpa*II: **U**: _____ **C** _____ **A**: _____

   (b) On the gels below draw in the bands that would be seen for each individual for each restriction enzyme:



   (c) Decide which restriction enzyme(s) would be useful for identifying carriers of BLAD: _____

_____

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26 Applications of DNA Profiling

Key Idea: DNA profiling has many forensic applications, from identifying criminal offenders to saving endangered species.

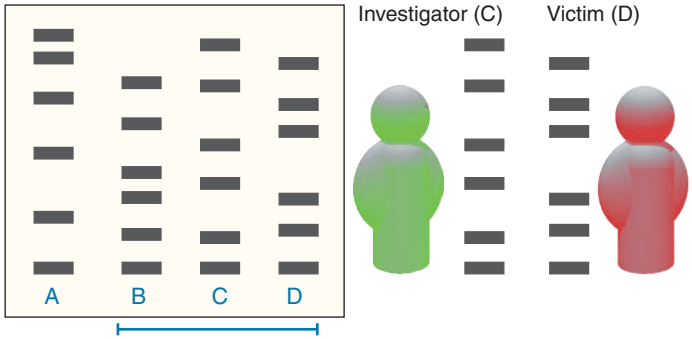
The use of DNA as a tool for solving crimes such as homicide is well known, but it can also has several other applications. DNA evidence has been used to identify body parts, solve cases of industrial sabotage and contamination, for paternity testing, and even in identifying animal products illegally made from endangered species.

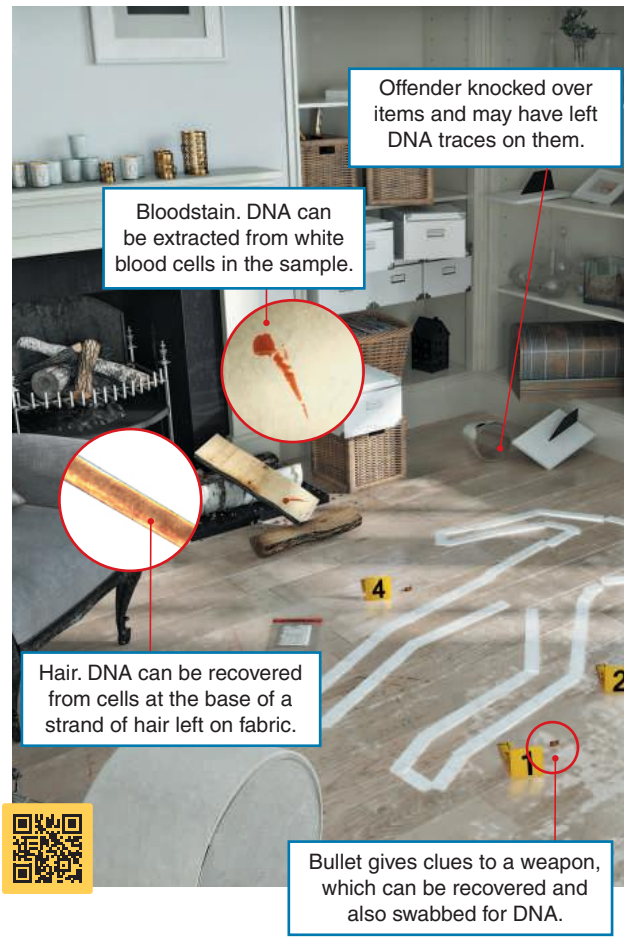
Using DNA to solve crimes

Although it does not make a complete case on it own, DNA profiling (in conjunction with other evidence) is one of the most powerful tools in identifying offenders or unknown tissues.

A lot of DNA is found at crime scenes and the information collected can be used to help identify the criminal. However, not all of the DNA collected will be from the criminal. Other DNA could belong to the victim, people who came to their aid (e.g. paramedics) or the police investigators (if they have not taken correct precautions).

In the example (right) the criminal who broke into this home has left behind several samples of their DNA. Samples of material that may contain DNA are taken for analysis. At a crime scene, this may include blood and body fluids as well as samples of clothing or objects that the offender might have touched. Samples from the victim and the investigator are also taken to eliminate them as a possible source of contamination (below). In this example the DNA of the people who live in the house will also be collected so their profiles can be eliminated. A calibration or standard is run so the technician knows the profile has run correctly.

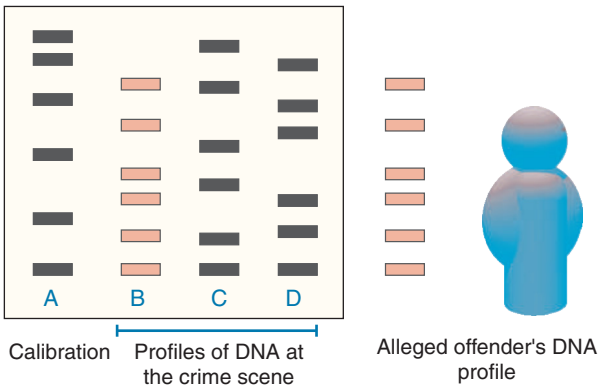




There are two different ways an offender can be identified through DNA profiling.

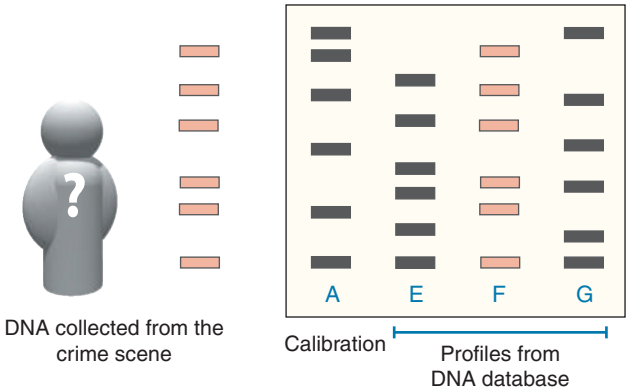
1. If a person is suspected of a crime, a sample of their DNA can be taken (e.g. blood sample) and compared to DNA evidence collected at the crime scene. A match indicates they are the offender. If there is no match, the person can be cleared as a suspect.
2. In cases where the suspect is unknown, biological evidence from the crime scene is analysed and the profile is compared to known offender profiles in DNA databases. The profile may match that of a known offender.

1 A person is suspected of the crime



Match! The alleged offender's profile matches the DNA collected at the crime scene.

2 The offender is unknown



Match! The DNA collected from the crime scene matches the profile of a known offender in the database.

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Paternity testing

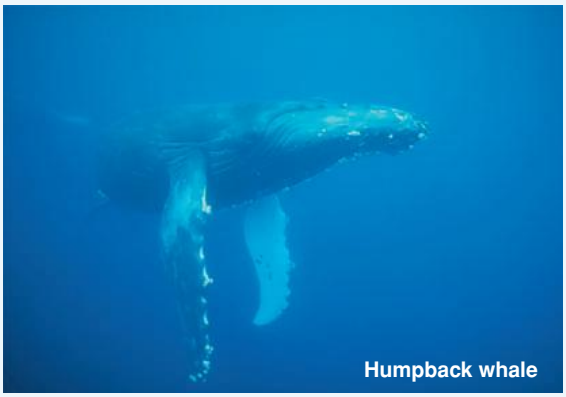
DNA profiling can be used to determine paternity (and maternity) by looking for matches in alleles between parents and children. It is used in cases such as child support or inheritance. DNA profiling can establish the certainty of paternity (and maternity) to a 99.99% probability of parentage.

Every STR allele is given the number of its repeats as its name, e.g. 8 or 9. In a paternity case, the mother may be 11, 12 and the father may be 8, 13 for a particular STR. The child will have a combination of these. The table below illustrates this:

DNA marker	Mother's alleles	Child's alleles	Father's alleles
CSF1PO	7, 8	8, 9	9, 12
D10S1248	14, 15	11, 14	10, 11
D12S391	16, 17	17, 17	17, 18
D13S317	10, 11	9, 10	8, 9

The frequency of the each allele occurring in the population is important when determining paternity (or maternity). For example, DNA marker CSF1PO allele 9 has a frequency of 0.0294 making the match between father and child very significant (whereas allele 12 has a frequency of 0.3446, making a match less significant). For each allele, a paternity index (PI) is calculated. These indicate the significance of the match. The PIs are combined to produce a probability of parentage. 10-13 different STRs are used to identify paternity. Mismatches of two STRs between the male and child is enough to exclude the male as the biological father.

Whale DNA: tracking illegal slaughter


Humpback whale

Under International Whaling Commission regulations, some species of whales can be captured for scientific research and their meat can be sold legally. Most whales, including humpback and blue whales, are fully protected and to capture or kill them is illegal.

Between 1999 and 2003, researchers used DNA profiling to investigate whale meat sold in markets in Japan and South Korea. They found 10% of the samples tested were from fully protected whales including western grey whales and humpbacks. They also found that many more whales were being killed than were being officially reported.

1. Why are DNA profiles obtained for both the victim and investigator? _____

_____

_____

2. Study the profile on the right.

   (a) Is the alleged offender innocent or guilty? _____

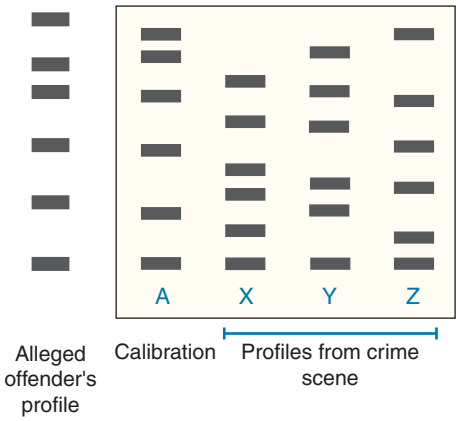
   (b) Explain your decision: _____

_____

_____

_____

_____



3. For the STR D10S1248 in the example above, what possible allele combinations could the child have?

_____

4 A paternity test was carried out and the abbreviated results are shown right:

DNA marker	Mother's alleles	Child's alleles	Man's alleles
CSF1PO	7, 8	8, 9	9, 12
D10S1248	14, 15	11, 14	10, 11
D19S433	9, 10	10,15	14, 16
D13S317	10, 11	9, 10	8, 9
D2S441	7, 15	7, 9	14, 17

   (a) Could the man be the biological father? _____

   (b) Explain your answer: _____

_____

_____

_____

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27 A Case Study in DNA Profiling Success

Assessment Task, Outcome 1: *Analysis and evaluation of a selected biological case study*

DNA profiling to identify an offender at a crime scene is an important aspect of forensic investigations. To cope with the large number of samples analysed in Australia, investigators are continually improving their processes to increase throughput but still maintain the accuracy and reliability of the data produced.

Throughput cannot be increased at the expense of reliability. Identifying the wrong person could mean they are convicted for a crime they didn't commit, while the person who committed the crime goes unpunished (and may offend again). Several factors increase the chances of obtaining a successful profile. One factor is the type of sample used to obtain the DNA (below).





How does sample type affect profiling success?

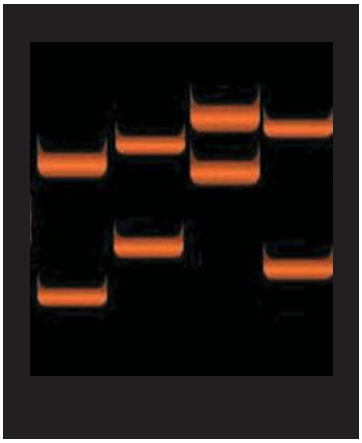
Between 2012 and 2013, new DNA analysis kits were introduced into forensic laboratories in Australia. The new kits analysed more DNA markers than the old kits. A project was carried out to compare the results from the old kits with those from the new kits.

One of the study areas examined how sample type affected the success rate. These results are shown in Table 1. The criterion for a successful match was matching more than 6 alleles. When fewer than 6 alleles matches were obtained, the match was recorded as unsuccessful. DNA analysis was carried out using blood, saliva, and trace DNA samples. Trace DNA is any sample that falls below the recommended thresholds for the analysis, and cannot be defined by a precise picogram amount.

Table 1. DNA profile success rates from different samples using two different types of DNA analysis kits, the older Profiler Plus and newer Powerplex 21.

	Sample type	Profiler plus kit		Powerplex 21 kit	
		Number of items	Successful profiles (%)	Number of items	Successful profiles (%)
Swab	Blood	43	92.6	20	90.4
Clothing	Blood	3	100.0	6	100.0
Cigarette butt	Saliva	53	71.1	9	66.6
Mouth/rim bottle	Saliva	54	68.5	1	100.0
Clothing	Trace	50	70.0	57	50.8
Items: Probable friction	Trace	16	37.5	41	48.7
Items: No probable friction	Trace	27	55.5	133	46.6

Data: DNA Profiling success rates on volume crime cases to determine the optimal number and type of samples that should be analysed per case.
Linzi Wilson-Wilde, Mojca Kegolvic, and Simon Walsh. Presented to the 25th Congress of the International Society for Forensic Genetics, 2 – 7 September 2013.



Palewhalegail CC 3.0

1. Explain why it is important that a fast analysis time is balanced with high accuracy rate: _____

2. Table 1 shows the successful rates of DNA profiles obtained from a number of different sample types.

   (a) Identify the sample type with the highest successful profiles: _____

   (b) Identify which sample type is the least likely to produce a successful profile: _____

   (c) Explain why the sample you named in (b) is the less likely to produce a successful profile: _____

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3. 252 trace samples of DNA were taken from various surfaces at a crime scene, including entry points and touched items. The data below show the average quantity of DNA recovered from those surfaces and the success at producing profiles.

Fig. 1 Average DNA quantity recovered by sample type

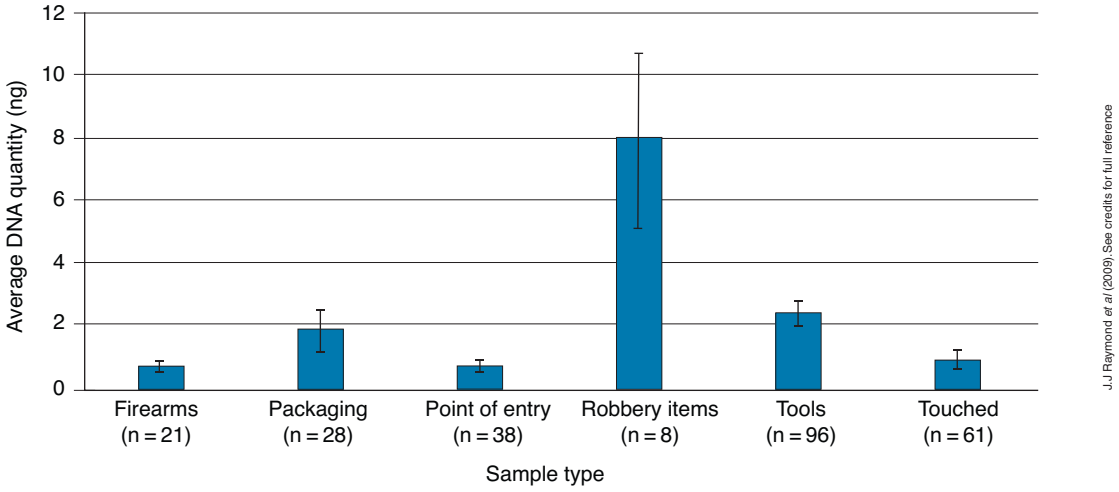
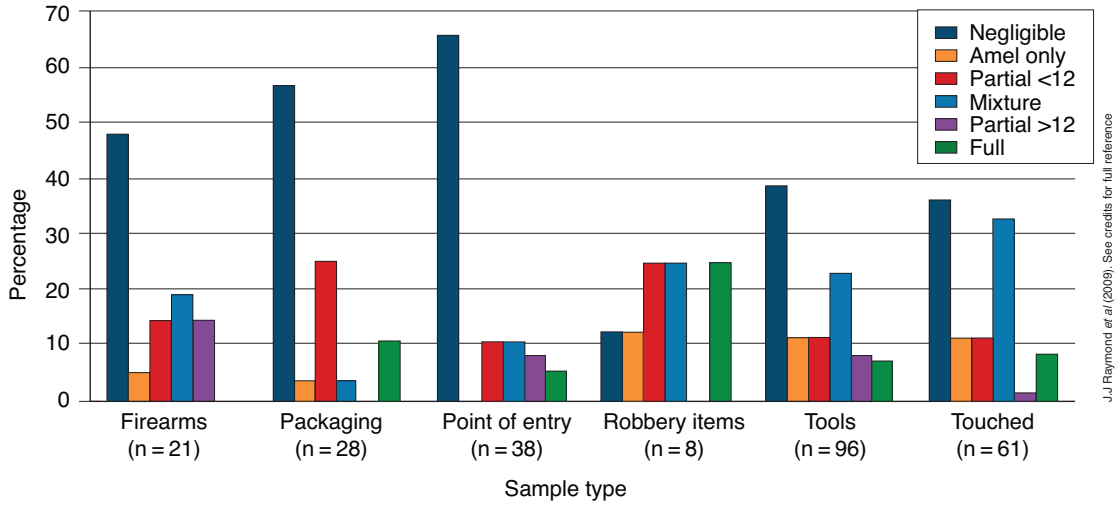


Fig. 2 Completeness of profiles recovered by sample type



(a) Which surface produced the most amount of DNA? _____

(b) Why might this be? _____

_____

_____

(c) Which surface produced the most full DNA profiles? _____

(d) Of the 252 samples, how many produce full DNA profiles? _____

_____

_____

(e) Of the 252 samples, how many produced negligible DNA profiles? _____

_____

_____

(f) How useful is using trace DNA at producing DNA profiles? _____

_____

_____

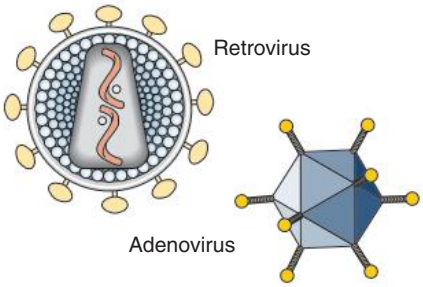
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28 What is a Transgenic Organism?

Key Idea: Transgenesis involves inserting a gene from one species into another, so its protein product is expressed in the second species, the transgenic. Transgenesis has applications in agriculture, and food and medical technologies. Transgenesis refers to the specific genetic engineering technique of inserting a gene from one species into another that does not normally contain the gene. It allows direct modification of a genome so that novel traits can be introduced to an organism. Organisms that have undergone transgenesis are called transgenic organisms. The genes are inserted using **vectors** or by direct insertion of the DNA. The most commonly used vectors are shown below

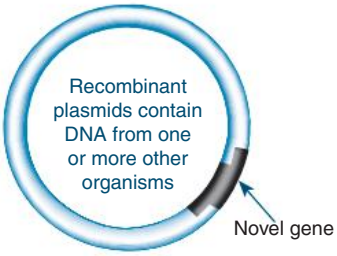
Examples of vectors for gene transfer

Viruses



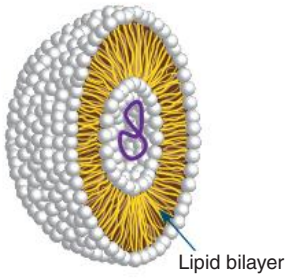
Viruses are able to insert DNA into a host cell. This ability makes them a powerful tool in transgenesais. Different types of viruses integrate their DNA into the host in different ways, so scientists can choose vectors to meet their specific needs. However, the length of DNA that can be transferred is small (<8 kb). Also, integration of the DNA into the host DNA can cause unexpected side effects depending on where in the host's chromosome the DNA inserts itself.

Plasmids



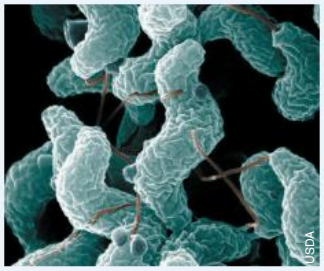
Plasmids are circular lengths of DNA that can be up to 1000 kb long (1 kb = 1000 bp). Recombinant plasmids are frequently used to produce transgenic organisms, especially bacteria. The bacteria maybe the final target for the recombinant DNA (e.g. transgenic *E. coli* producing insulin) or it can be used as a vector to transfer the DNA to a different host (e.g. *Agrobacterium tumefaciens* is used to transfer the *Ti* plasmid to plants).

Liposomes

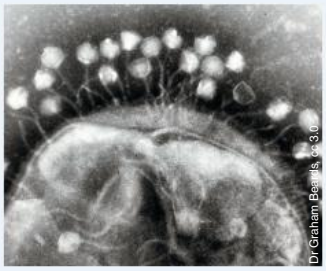


Liposomes are spherical bodies of lipid bilayer. They can be quite large and targeted to specific types of cell by placing specific receptors on their surfaces. Because of their size, liposomes can carry plasmids 20 kb or more, but are less efficient than viruses at transferring the plasmid into a target cell. Liposomes are sometimes used to transfer new DNA into sperm cells, which will then transfer their DNA to the zygote. However it is not particularly efficient.

Transferring the DNA



Transformation is the direct uptake of foreign DNA and is common in bacteria. Recombinant DNA plasmids are mixed with bacteria and the bacteria that take up the DNA are used.



Transduction is the transfer of DNA into a bacterium by a virus. Bacteriophages (viruses that infect bacteria) are commonly used to integrate recombinant DNA into a target bacterium.



Transfection is the deliberate, often non-viral, introduction of foreign DNA into a cell. There are numerous methods including electroporation and the use of the gene gun (above).



Electroporation is a method in which an electric field is applied to cells, causing the plasma membrane to become more permeable. This allows DNA to cross the plasma membrane.

1. Explain what is meant by a transgenic organism and describe the methods by which the transgenesis is achieved:

2. Suggest why different vectors and methods of transfer are used in making different types of transgenic organisms:

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Extending shelf life: Shelf life in fresh produce (e.g. tomatoes) can be extended by switching off the genes for specific enzymes involved in fruit ripening (e.g. the enzymes involved in softening of the fruit wall or controlling ethylene production).



USDA

Pest or herbicide resistance: Plants can be engineered to carry and express genes for insect toxins or herbicide resistance. Pest resistant crops do not require spraying and herbicide resistance allows weed control without damage to the crop.



Diabetic injecting insulin

Biofactories: Transgenic bacteria are widely used to produce desirable commodities, such as hormones or proteins. Large quantities of a product can be produced using bioreactors. One example is injectable human insulin (above) produced by recombinant bacteria or yeast.



Covid vaccine development

Vaccine development: Vaccines can be made by isolating the genes encoding antigenic components (e.g. RNA or protein). These are inserted into a bacterial cell, which then expresses the genes. The purified gene product generates an immune response without the risk of disease.



Golden rice

IRRI CC 2.0

Crop improvement: Gene technology is now an integral part of the development of new crop varieties. Crops can be engineered to produce higher protein or vitamin levels (e.g. golden rice) or to grow in inhospitable conditions (e.g. salty or arid land).



Coal mine

Environmental clean-up: Bacteria can be engineered to thrive on waste products, such as liquefied newspaper pulp or oil. They degrade pollutants and wastes, and minerals (e.g. mercury) may also be recovered from the bacteria after the remediation.



Wool production



Milk production

Livestock improvement and production of pharmaceuticals: Transgenic sheep have been used to enhance wool production. The keratin protein of wool is largely made of a single amino acid, cysteine. Injecting developing sheep with the genes for the enzymes that generate cysteine produces woollier sheep. Transgenic sheep carrying the human gene for a protein, α-1-antitrypsin, produce the protein in their milk. The antitrypsin is extracted from the milk and can be used to treat hereditary emphysema.

3. Suggest one economic advantage of extending shelf life in fresh produce:

4. Suggest one disadvantage of engineering crop plants to be herbicide resistant:

5. What is the advantage of using GE bacteria to produce a human hormone like insulin?

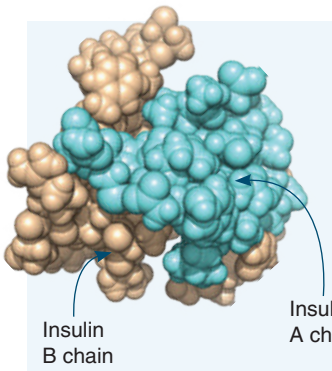
6. Why might it be safer to produce a vaccine using gene technology, rather than the pathogen itself?

7. What advantages could be gained by developing a GE crop that produces more protein?

8. Using animals as live biofactories to produce a valuable product (e.g. a human protein) is controversial. What welfare issues could be associated with such practices?

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29 Using Recombinant Plasmids in Medicine



Key Idea: By using microorganisms to make human insulin, problematic issues of cost, allergic reactions, and ethics have been addressed.

The Issue

- **Type I diabetes mellitus** is a metabolic disease caused by a lack of **insulin**. Around 25 people in every 100,000 suffer from type I diabetes.
- It is treatable only with injections of insulin.
- In the past, insulin was taken from the pancreatic tissue of cows and pigs and purified for human use. The method was expensive and some patients had severe allergic reactions to the foreign insulin or its contaminants.

Concept 1

DNA can be cut at specific sites using **restriction enzymes** and joined together using **DNA ligase**. Genes can be inserted into self-replicating bacterial **plasmids** at the point where the cuts are made.

Concept 2

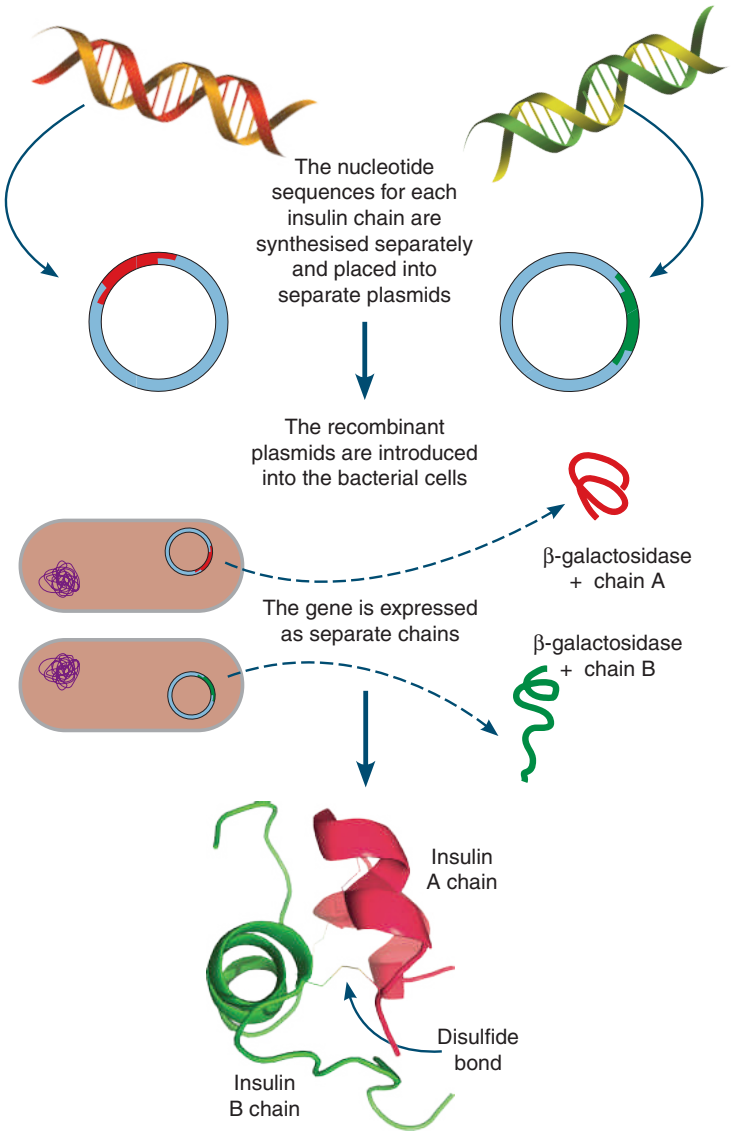
Plasmids are small, circular pieces of DNA found in some bacteria. They usually carry genes useful to the bacterium. *E. coli* plasmids can carry promoters required for the transcription of genes.

Concept 3

Under certain conditions, Bacteria are able to lose or pick up plasmids from their environment. Bacteria can be readily grown in vat cultures at little expense.

Concept 4

The DNA sequences coding for the production of the two polypeptide chains (A and B) that form human insulin can be isolated from the human genome.



Techniques

The gene is chemically synthesised as two DNA sequences, one for the insulin A chain and one for the insulin B chain. The two sequences are small enough to be inserted into a plasmid.

Plasmids are extracted from *Escherichia coli*. The gene for the bacterial enzyme β-galactosidase is located on the plasmid. To make the bacteria produce insulin, the insulin gene must be linked to the β-galactosidase gene, which carries a promoter for transcription.

Restriction enzymes are used to cut plasmids at the appropriate site and the A and B insulin sequences are inserted. The sequences are joined with the plasmid DNA using DNA ligase.

The recombinant plasmids are inserted back into the bacteria by placing them together in a culture that favours plasmid uptake by bacteria.

The **transgenic bacteria** are then grown and multiplied in vats under carefully controlled growth conditions.

Outcomes

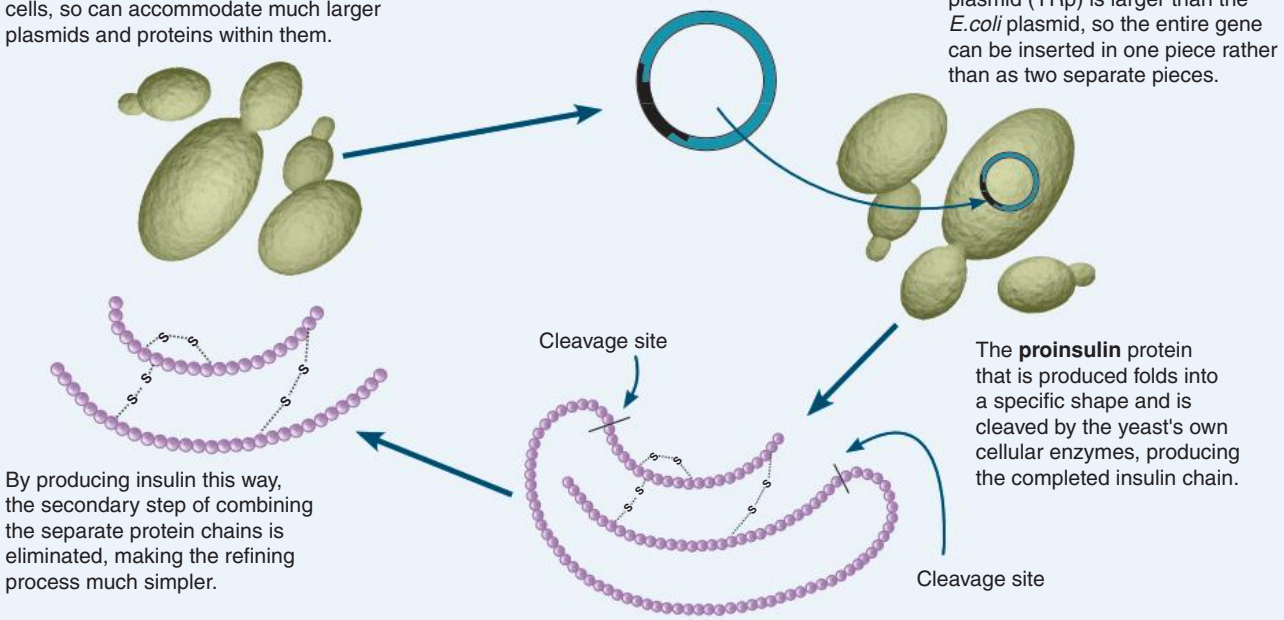
The product consists partly of β-galactosidase, joined with either the A or B chain of insulin. The chains are extracted, purified, and mixed together. The A and B insulin chains connect via disulfide cross linkages to form the functional insulin protein. The insulin can then be made ready for injection in various formulations.

Further Applications

The techniques used to produce human insulin from genetically modified bacteria can be applied to a range of human proteins and hormones. Proteins currently being produced include human growth hormone, interferon, and factor VIII.

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Insulin production in *Saccharomyces*



1. Describe the three major problems associated with the traditional method of obtaining insulin to treat diabetes:

   (a) _____

   (b) _____

   (c) _____

2. Explain the reasoning behind using *E. coli* to produce insulin and the benefits that GM technology has brought to diabetics:

   _____

   _____

   _____

3. Explain why, when using *E. coli*, the insulin gene is synthesised as two separate A and B chain nucleotide sequences:

   _____

   _____

4. Why are the synthetic nucleotide sequences (‘genes’) 'tied' to the β-galactosidase gene? _____

   _____

   _____

5. Yeast (*Saccharomyces cerevisiae*) is also used in the production of human insulin. Discuss the differences in the production of insulin using yeast and *E. coli* with respect to:

   (a) Insertion of the gene into the plasmid: _____

   _____

   _____

   (b) Secretion and purification of the protein product: _____

   _____

   _____

   _____

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30 Using Recombinant Plasmids in Agriculture

Key Idea: Up to one fifth of the world's crops are lost due to insects each year. Losses can be reduced through the use of genetic engineering to introduce the Bt gene into crop plants.

A key goal in horticulture is the reduction of insect crop damage. Normally this is done using sprays. However this requires a lot of effort and leaves potentially harmful chemical residues on the food and in the environment. Using genetic engineering to produce crop plants with their own in-built insect deterrents can result in greater crop yields and reduced chemical use.

Bt toxin

Bacillus thuringiensis is a soil living bacterium. It also occurs naturally in the gut of caterpillars and on leaf surfaces. The bacteria form spores that are associated with crystalline proteins called δ-endotoxins. These are lethal to lepidopteran (butterfly and moth) larvae but do not affect other insects such as beetles or bees (or any other animal). For this reason the Bt toxin has been used as a targeted insecticide since the 1960s.

In 1996 the seed company Monsanto released its first versions of Bt corn. This corn had been genetically modified to contain the gene that produces the Bt protein. The target insect pest for Bt corn is the larval stage of the European corn borer, which causes hundreds of millions of dollars worth of damage to crops annually.

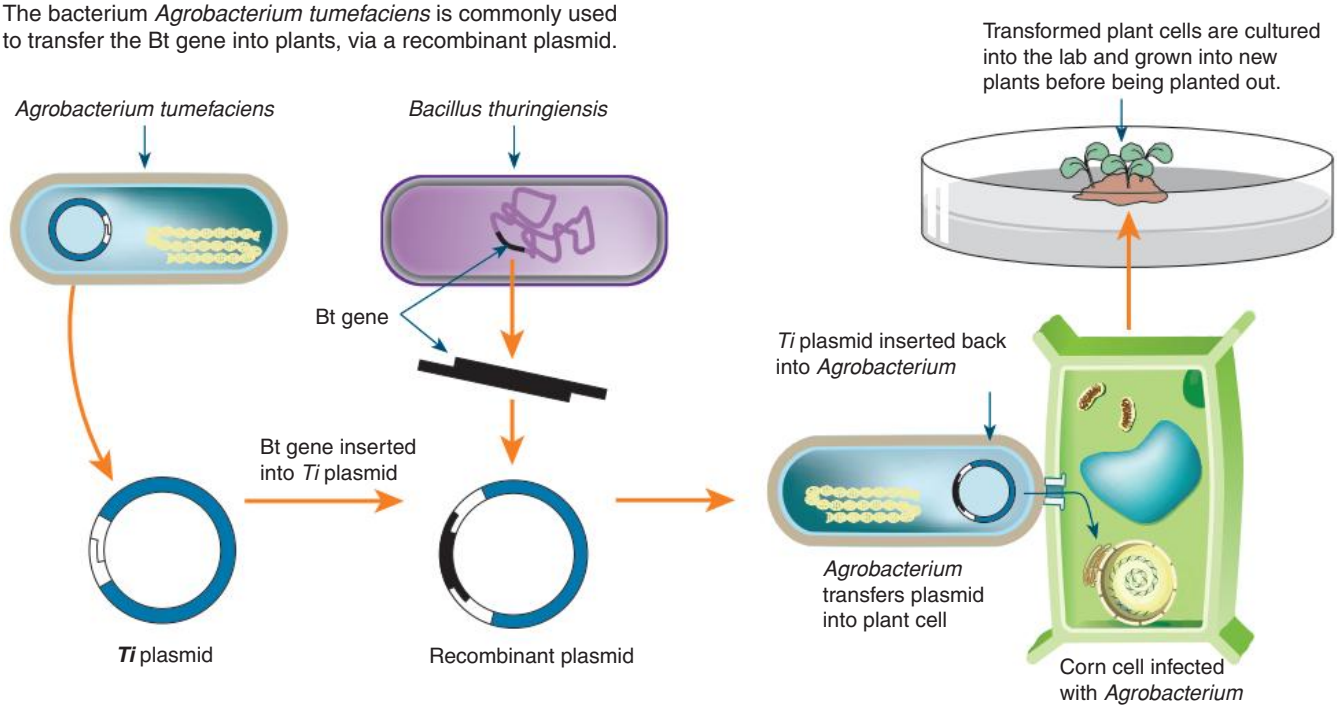


Both photos USDA

The effects of the Bt toxin on insect deterrence. The plant on the right has been treated with Bt toxin before being exposed to caterpillars. The plant on the left had not been treated with Bt toxin.

Producing a Bt plant

Genetic engineering has been used to produce transgenic cotton, corn, and potato varieties that produce the Bt toxin. The bacterium *Agrobacterium tumefaciens* is commonly used to transfer the Bt gene into plants, via a recombinant plasmid.



1. Name the bacteria that produces Bt toxin: _____

2. Why is Bt toxin a useful insecticide? _____

_____

3. What is the primary target of the Bt toxin in Bt corn? _____

_____

4. Explain how Bt corn is produced using *Agrobacterium tumefaciens*: _____

_____

_____

_____

_____

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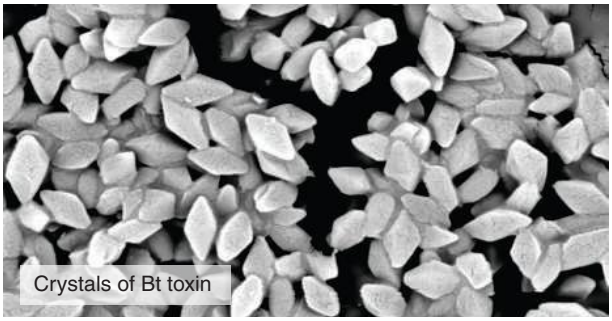
What's killing the monarchs? It's not the corn

Bt corn was developed by the company Monsanto and sales began in 1996. There are many different types of Bt corn, each one engineered to produce the toxin in slightly different ways. One of the first produced was Bt 176.

By 1999 monarch butterfly populations in the American Midwest began declining. During that year, Cornell University published a paper showing that the Bt toxin could be dispersed to other plants by the corn's pollen. Pollen landing on milkweed near corn crops could potentially kill the monarch caterpillars that fed exclusively on the milkweed. This resulted in a backlash against Bt corn by environmental activists. However, in 2001 a study was released that argued the toxin in pollen was not causing monarch decline. The toxicity in pollen was due mainly to the Bt 176 variety which was used in less than 2% of the corn grown and was in the process of being phased out. Other Bt corn varieties did not develop enough toxin, or their pollen density was too low to affect monarch caterpillars.

It now appears that there is a related but quite different reason for the Monarch butterfly decline. In 1996, Monsanto also began selling "Roundup Ready" corn, engineered to withstand glyphosate herbicide. Corn crops could be sprayed with herbicide and while the weeds die the corn would keep on growing, allowing less targeted spraying applications. As a result milkweed, which often grew in or near corn crops, was also killed, leaving no food for monarch caterpillars.

So... What's killing the monarchs?



Crystals of Bt toxin

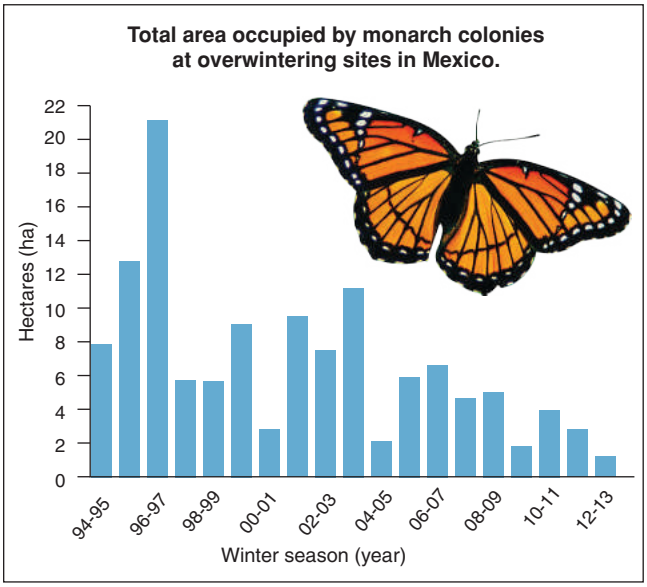


David R. Tribble



Scott McDougall

Above: North American populations of monarchs migrate (above) to overwintering sites in Mexico and California.
Right: Monarch caterpillars feed exclusively on milkweed.



5. As a group, discuss the ethical issues surrounding GM corn and monarch declines. Who is to blame for the decline of monarchs and what can be done to help the population recover? Summarize the main points of your discussion below:

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31 Chapter Review: Did You Get It?

1. Match each term to its definition, as identified by its preceding letter code.

CRISPR/cas9 system

gel electrophoresis

plasmid

polymerase chain reaction

recombinant DNA

restriction enzyme

A A reaction that is used to amplify fragments of DNA using cycles of heating and cooling.

B A small circular piece of DNA commonly found in bacteria and often used as a vector in genetic modification.

D A process used to separate different lengths of DNA by placing them in a gel matrix placed in a buffered solution through which an electric current is passed.

E DNA that has had a foreign sequence added so that the original sequence has been changed.

F An enzyme that is able to cut a length of DNA at a specific sequence or site.

G A tool in genetic modification that uses a prokaryotic sequence of DNA and its associated protein to edit the base pairs of a gene.

2. The electrophoresis gel (below, right) shows four profiles containing five STR sites: the mother (A) her daughter (B) and two possible fathers (C and D). Which of the possible fathers is the biological father?

(a) The biological father is: _____

(b) Why do profiles B and D only have 9 bands?

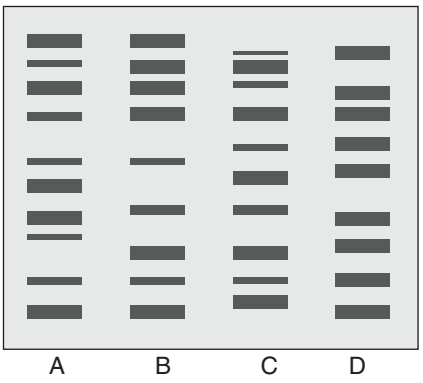
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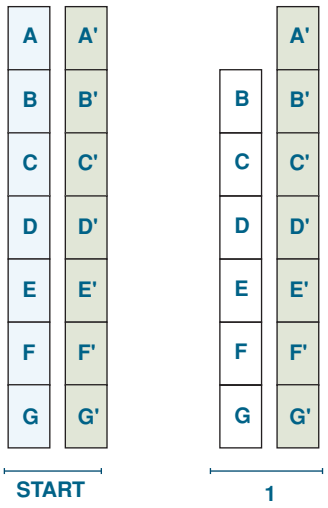
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3. Below is a DNA sequence of sections A, B, C, D, E, F and G and A', B', C', D', E', F' and G'. You want to isolate sections B, C, D and E as a continuous group by PCR. Primers are E' and B. Complete the diagram **using just the right hand A' strand** to show how the daughter lengths of DNA change over successive PCR cycles to produce the isolated section. The first cycles are shown:



4. Write a brief description of how a to produce a genetically modified plasmid for insertion into a bacteria:

_____

_____

_____

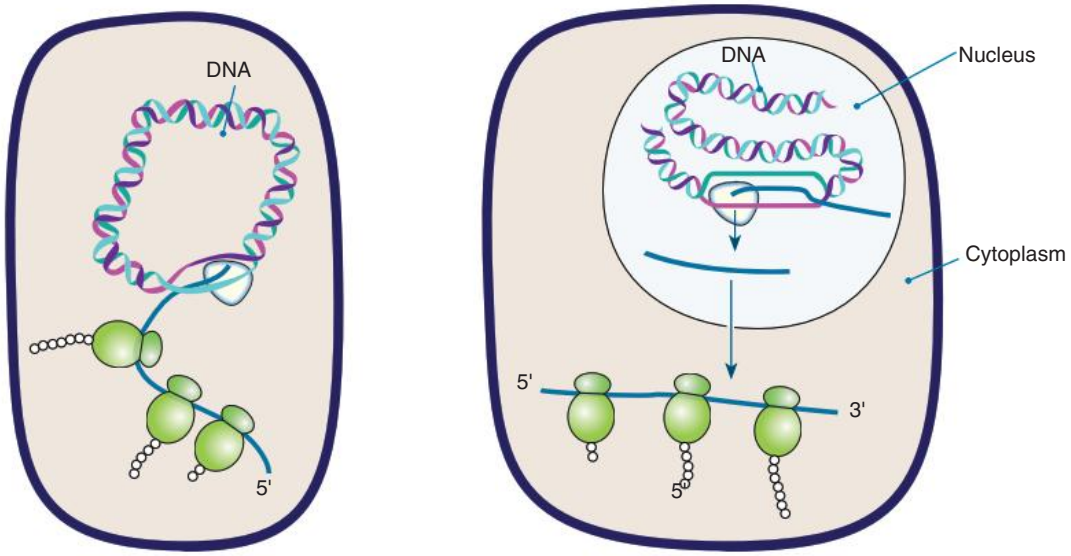
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32 Synoptic Questions: Unit 3, Area of Study 1

1. Describe the differences in the structure and function of mRNA, tRNA, and rRNA:

2. Study the diagram below and use it to describe the main differences between gene expression in prokaryotes and eukaryotes:



Prokaryote **Eukaryote**

3. Describe the differences between RNA and DNA:

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4. The diagrams below depict the *trp* operon in *E. coli*. The first structural gene is identified for reference.

(a) Label i - iiiv below with the labels: *operator, regulator, trpB, trpC, trpD, trpE, promoter*



(i) _____ (ii) _____ (iii) _____

(iv) _____ (v) _____ (vi) _____

(vii) _____

(b) Describe the boundaries of the operon: _____

_____

_____

(c) Which gene produces the repressor molecule? _____



(d) Which letters Identify where the RNA polymerase attaches when tryptophan and the repressor is absent: _____

(e) Identify the structural genes transcribed: _____



(f) Which letters Identify where the RNA polymerase attaches when tryptophan and the repressor is absent: _____

(g) Are the structural genes transcribed? _____

5. A restriction enzyme binds to a DNA strand at the location CAGAT and cuts the DNA strand between GA. The DNA sequence is shown below:

GTGTACCGATGATCAGATCACATGATACAGATACATGACATAGAGACCACAGATGATTACATTACATTACA

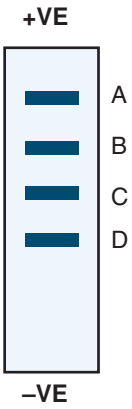
(a) Identify where the restriction cuts. Write down the resulting lengths of DNA that the cuts produce:

_____

_____

(b) The cut DNA is run on a electrophoresis DNA (right). Which of the letters represent the longest and shortest lengths of DNA?

_____



6. Describe how insulin is made synthetically by genetically modifying bacteria:

_____

_____

_____

_____

_____

_____

_____

_____

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03

Area of Study 2

How are biochemical pathways regulated?



You will find out about:

- The regulation of biochemical pathways in photosynthesis and cellular respiration
- Photosynthesis, factors affecting photosynthetic rates, and the adaptations of C_3, C_4, and CAM plants to maximising photosynthesis.
- The inputs, outputs, and stages in cellular respiration and factors affecting respiration rate. Fermentation pathways in animals and yeasts.
- Biotechnological applications of biochemical pathways

CHAPTER 3

Regulation of Biochemical Pathways

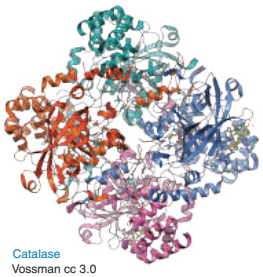
Key terms
activation energy
active site
anabolic reaction
catabolic reaction
catalyst
cellular respiration
coenzyme
competitive inhibition
denaturation
enzyme
enzyme inhibition
extracellular
feedback inhibition
induced fit model
intracellular
metabolic pathway
metabolism
non-competitive inhibition
optimum (for enzyme)
photosynthesis

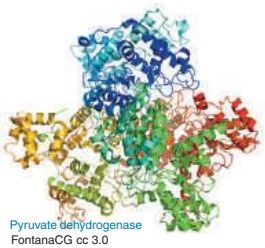
Enzymes are protein catalysts

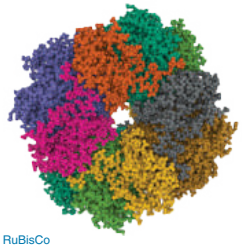
Key skills and knowledge

Activity number

- ☐ 1 Outline the general structure of the biochemical pathways in photosynthesis and cellular respiration, from initial reactants to final products. Explain how these metabolic pathways are linked in biological systems and show these linkages schematically. **33**
- ☐ 2 Outline the role of enzymes in catalysing both intracellular and extracellular biochemical reactions. Describe the structure and role of the active site, explaining its importance to the specificity of the enzyme to its substrate(s). **34**
- ☐ 3 Describe the induced fit model for enzyme function, with reference to the enzyme-substrate complex, enzyme-product complex, and product formation. **35**
- ☐ 4 Explain how enzymes catalyse reactions by lowering the activation energy and identify this on a plot of the progress of the reaction against the free energy. Know that all the biochemical reactions in living things constitute metabolism. Distinguish between anabolic and catabolic reactions and categorise cellular respiration and photosynthesis using these terms. **36**
- ☐ 5 Outline the general role of enzymes and coenzymes in facilitating the steps in photosynthesis and cellular respiration. **35 37 40**


Catalase
Vossman cc 3.0


Pyruvate dehydrogenase
FontanaCG cc 3.0


RuBisCo

Factors affecting enzyme activity

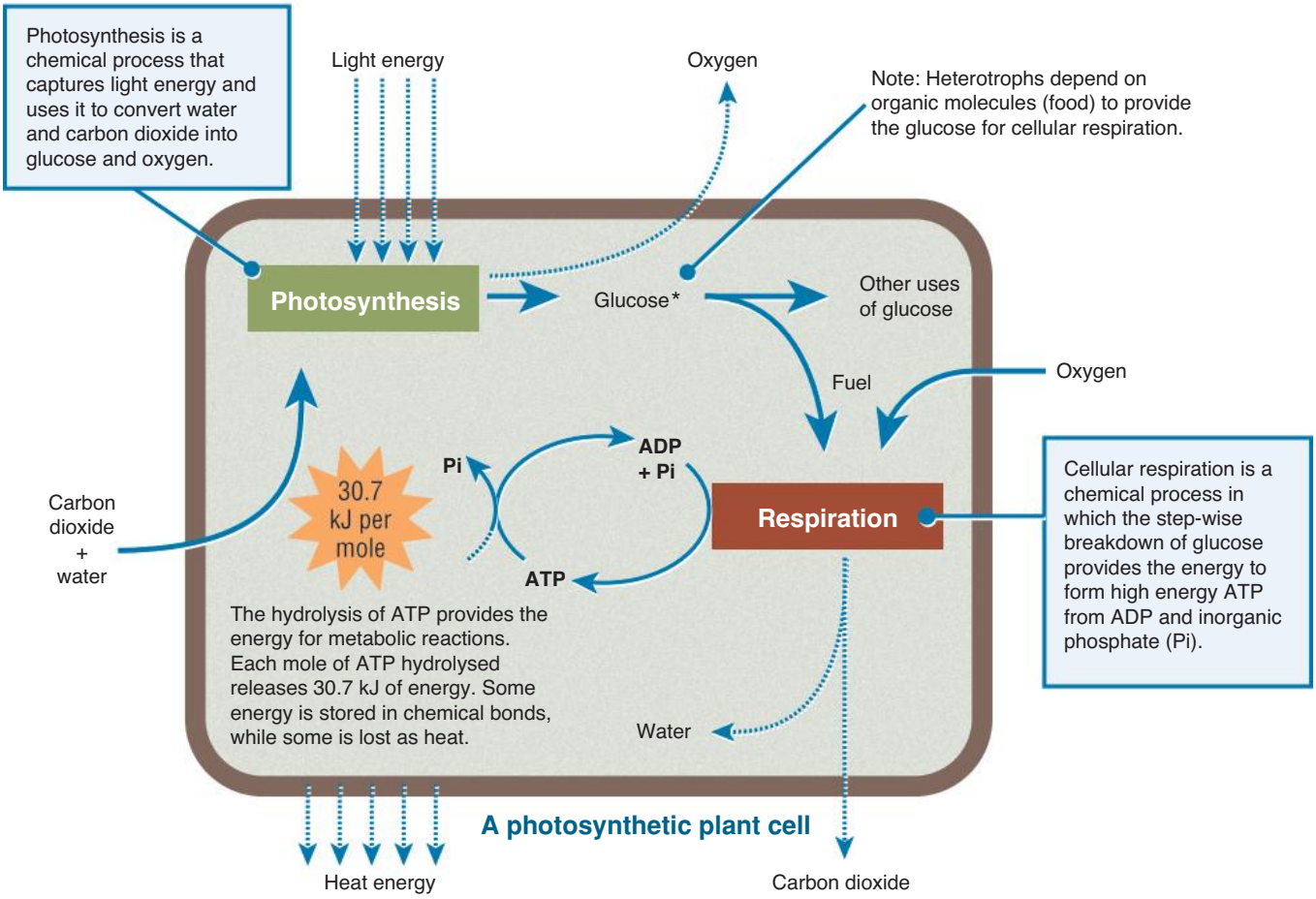
Key skills and knowledge

- ☐ 6 In relation to photosynthesis and cellular respiration, explain how enzyme reaction rates can be affected by pH, temperature, and the concentrations of reactants (substrates) and products. Recognise that enzymes, like all proteins, can be denatured by high temperatures or extremes of pH and relate this to the loss of their functional tertiary structure. **37**
- ☐ 7 PRAC Investigate the effects of pH, temperature, substrate concentration, or enzyme concentration on enzyme activity. **38**
- ☐ 8 Using examples, describe the effects of inhibitors on the rate of enzyme-controlled reactions. Include reference to competitive and non-competitive inhibition and identify these on graphs of reaction rate vs substrate concentration. **39**
- ☐ 9 Recall the general role of coenzymes in biochemical pathways. Describe the cycling of the coenzymes ATP, NADH, and NADPH as loaded and unloaded forms move energy, protons, and electrons between cellular reactions. **40**
- ☐ 10 With reference to cellular respiration or photosynthesis, explain the role of reversible end-product inhibition in regulating biochemical pathways. Using an example, explain how the compartmentalised structure of cells and their organelles contributes to the efficiency of metabolic processes. **41**

33 General Metabolic Pathways

Key Idea: The energy from sunlight is captured and stored as glucose, which powers the production of ATP in the process of cellular respiration. Hydrolysis of ATP provides the energy for the chemical reactions in living systems.

Energy flow in the cell of an autotroph (a plant) is shown below. Note that ATP has a central role in acting as an energy carrier to power metabolic reactions. Some of the energy is lost as heat during these reactions.





DID YOU KNOW?

It takes energy to break bonds, so how does the hydrolysis of ATP provide energy for metabolic reactions?
The hydrolysis of ATP is linked to the formation of a reactive intermediate, which can be used to do work. The reactions that make the energy in ATP available occur virtually simultaneously, so the reaction is simplified to omit the intermediates:



1. How does ATP act as a supplier of energy to power metabolic reactions? _____

_____

_____

2. (a) Identify the ultimate source of energy for most autotrophs: _____

   (b) Identify a group of autotrophic organisms that do not use this source of energy: _____

_____

3. Identify the ultimate source of energy for most heterotrophs: _____

4. In what way are the processes pictured above (photosynthesis and cellular respiration) connected? _____

_____

_____

_____

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34 Enzymes

Key Idea: Enzymes are biological catalysts. The active site is critical to this functional role.

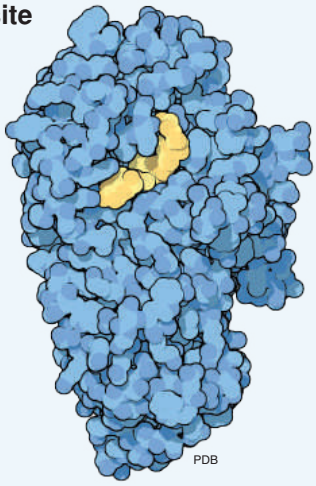
Most enzymes are globular proteins. Enzymes are biological catalysts because they speed up biochemical reactions, but the enzyme itself remains unchanged. The substrate in a reaction binds to a region of the enzyme called the active site, which is formed by the precise folding of the enzyme's amino acid chain. Enzymes control metabolic pathways. One enzyme will act on a substance to produce the next reactant in a pathway, which will be acted on by a different enzyme.

The active site

An enzyme acts on a specific chemical called a substrate. The substrate binds to a specific part of the enzyme called the **active site**.

The shape and chemistry of the active site is specific to an enzyme and is a function of the polypeptide's tertiary structure (the way the protein folds up). The amylase shown here breaks starch (a large molecule made of repeating glucose units) into smaller pieces with 2-3 glucose units.

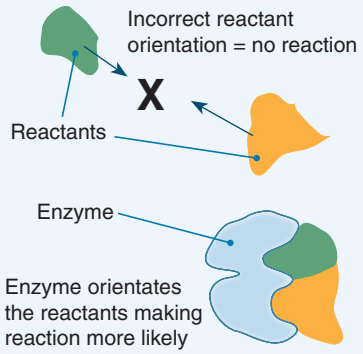
Extremes of temperature or pH can alter the enzyme's active site and lead to loss of function. This process is called **denaturation**.



Amylase (blue) with bound glucose (yellow) in the active site

Substrates collide with an enzyme's active site

For a reaction to occur, reactants must collide with sufficient speed and with the correct orientation. Enzymes enhance reaction rates by providing a site for reactants to come together in such a way that a reaction will occur. They do this by orientating the reactants so that the reactive regions are brought together. They may also destabilise the bonds within the reactants making it easier for a reaction to occur.



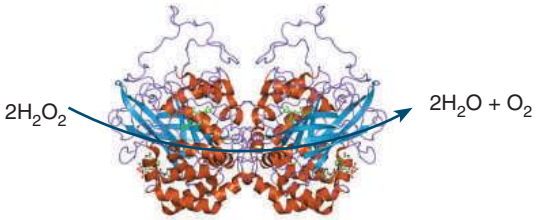
Intracellular and extracellular enzymes

Enzymes can be defined based on where they are produced relative to where they are active.

An I**ntracellular enzyme** performs its functions within the cell that produces it. Most enzymes are intracellular enzymes, e.g. respiratory enzymes.

Example: Catalase (a type of peroxidase).

Many metabolic processes produce hydrogen peroxide, which is harmful to cells. Catalase converts hydrogen peroxide into water and oxygen gas (below) to prevent damage to cells and tissues.



Catalase

An **extracellular enzyme** is an enzyme that functions outside the cell from which it originates (i.e. it is produced in one location but active in another).

Examples: Trypsin and α-amylase.

Trypsin is a protein-digesting enzyme and hydrolyses the peptide bond immediately after a basic residue (e.g. arginine). It is produced in an inactive form (called trypsinogen) and secreted into the small intestine by the pancreas. It is activated in the intestine by the enzyme enteropeptidase to form trypsin. Active trypsin can convert more trypsinogen to trypsin.

Alpha amylase is a digestive enzyme produced in the salivary glands and pancreas in humans. It acts in the mouth and small intestine respectively to hydrolyse starch by cleaving the α-1,4 glycosidic bond. The end-products are sugars (maltose, dextrin).

1. (a) What is meant by the active site of an enzyme and relate it to the enzyme's tertiary structure: _____

   _____

   _____

   _____

   (b) Why are enzymes specific to one substrate (or group of closely related substrates)? _____

   _____

   _____

2. How do substrate molecules come into contact with an enzyme's active site? _____

   _____

3. (a) Suggest why digestion (the breakdown of large macromolecules) is largely performed by extracellular enzymes:

   _____

   (b) Why would an extracellular enzyme be produced and secreted in an inactive form? _____

   _____

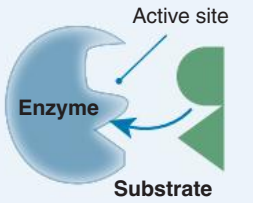
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35 Models of Enzyme Activity

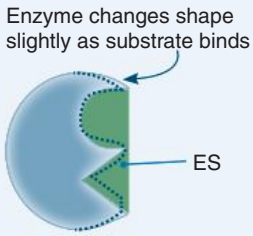
Key Idea: Enzymes catalyse reactions by providing a reaction site for a substrate. The model that describes the behaviour of enzymes the best is the induced fit model.

The initial model of enzyme activity was the lock and key model proposed by Emil Fischer in the 1890s. Fischer proposed that enzymes were rigid structures, similar to a lock, and the substrate was the key. Although some aspects of this model were correct, it was not supported by empirical evidence. The model has been modified as techniques to study molecular structures improved. The current 'induced-fit' model of enzyme function (below) is supported by several lines of structural and functional evidence.

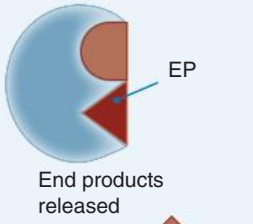
The induced fit model of enzyme action



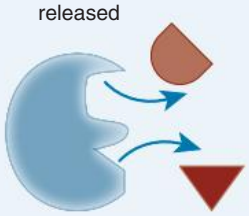
1. A substrate molecule is drawn into the enzyme's active site by its particular properties (resulting from its amino acid side chains). The active site is like a cleft into which the substrate molecule(s) fit.



2. The enzyme changes shape as the substrate binds, forming an enzyme-substrate (ES) complex. Chemical and electrostatic interactions are important in forming the ES complex. The shape change makes a change in the substrate more likely. In this way, the enzyme's interaction with its substrate is an induced fit.

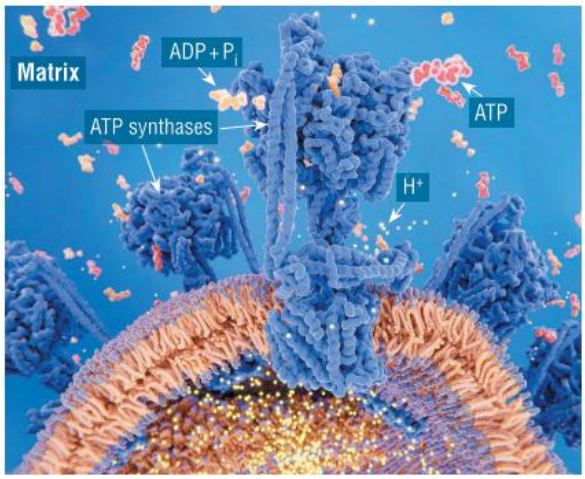


3. The ES interaction results in an intermediate enzyme-product (EP) complex. The substrate becomes bound to the enzyme by weak chemical bonds, straining bonds in the substrate and allowing the reaction to proceed more readily.

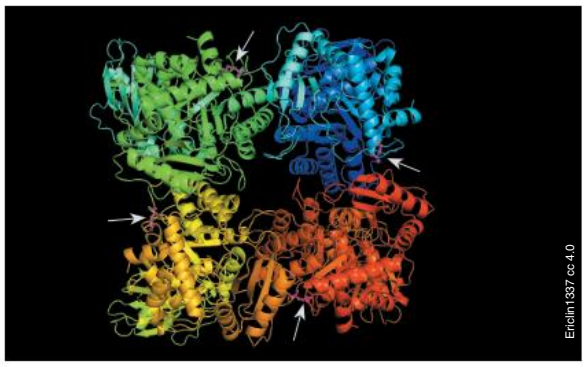


4. The end products are released and the enzyme returns to its previous shape.

Once the substrate enters the active site, the shape of the active site changes to form an active complex. The formation of an ES complex strains substrate bonds and lowers the energy required to reach the transition state, which allows the reaction to proceed. The **induced-fit model** is supported by X-ray crystallography, chemical analysis, and studies of enzyme inhibitors, which show that enzymes are flexible and change shape when interacting with the substrate.



ATP synthase is a transmembrane enzyme that catalyses the synthesis of ATP from ADP and inorganic phosphate, driven by a proton gradient generated by electron transfer. The image shows ATP synthase in the membrane of a mitochondrion, but it is also found in the membranes of chloroplasts, where ATP is generated in the light dependent reactions of photosynthesis. ATP synthase is classified as a type of ligase because the catalysis involves the formation of P-O (phosphodiester) bond. It has three active sites.



RuBisCo is an enzyme involved in the first main step of carbon fixation in plants and other photosynthetic organisms. It catalyses the attachment of CO_2 to a 5-C sugar derivative called RuBP. The active sites are indicated by arrows.

1. Describe the key features of the 'induced' fit model of enzyme action: _____

2. In the 1960s, it was proposed that ATP synthesis depended on a shape change in ATP synthase generated by rotation of membrane-bound subunit. This rotation has now been demonstrated experimentally. How does this provide evidence for the induced fit model of enzyme function?

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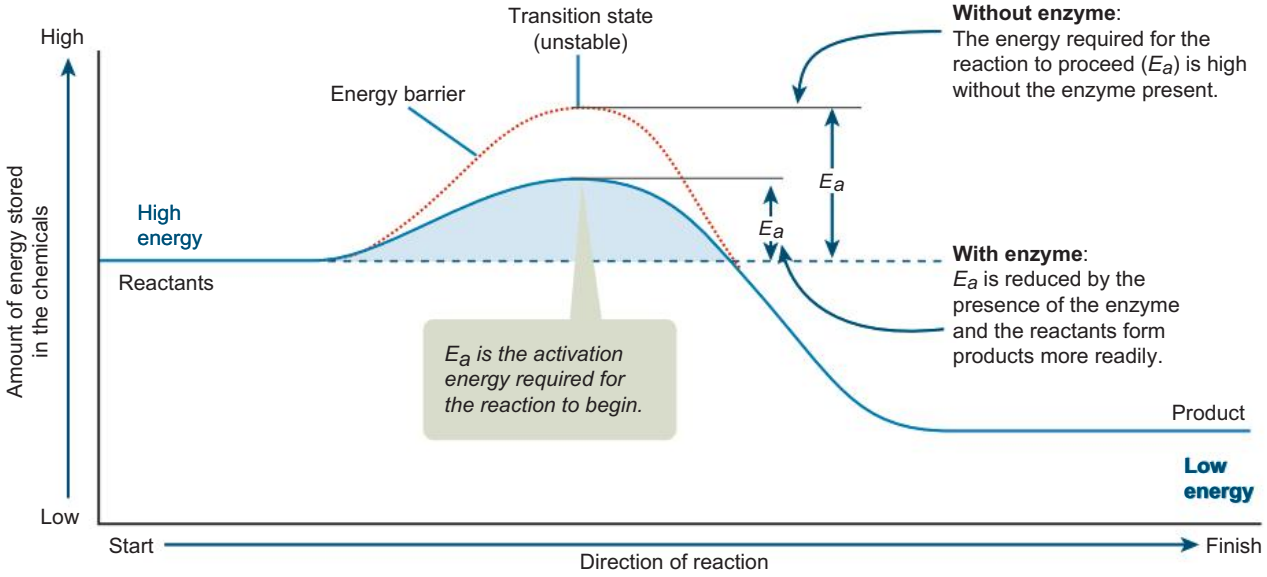
36 How Enzymes Work

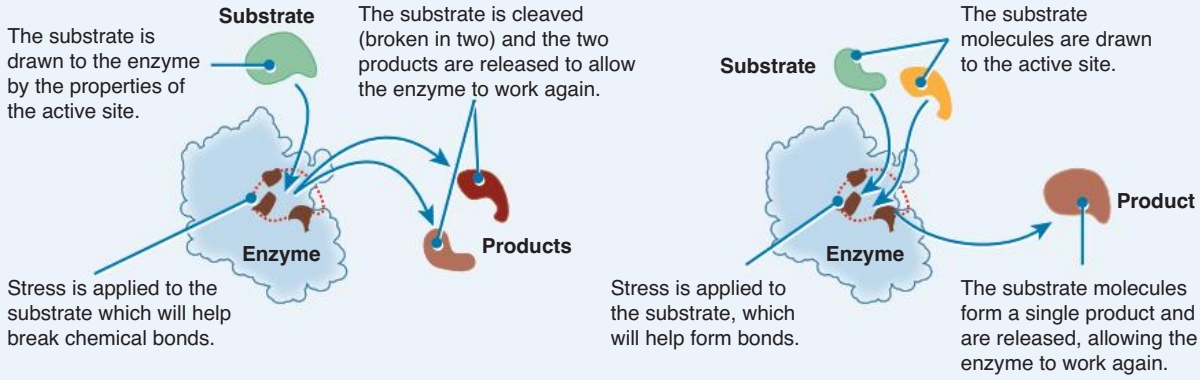
Key Idea: Enzymes increase the rate of biological reactions by lowering the reaction's activation energy.

Chemical reactions in cells are accompanied by energy changes. The amount of energy released or taken up is directly related to the tendency of a reaction to run to completion (for all the reactants to form products). Any reaction needs to raise the energy of the substrate to an unstable transition state before the reaction will proceed (below). The amount of energy needed to do this is the **activation energy** (E_a). Enzymes lower the E_a by destabilising bonds in the substrate so that it is more reactive. Enzyme reactions can break down a single substrate molecule into simpler substances (catabolic reactions), or join two or more substrate molecules together (anabolic reactions).

Lowering the activation energy

The presence of an enzyme simply makes it easier for a reaction to take place. All catalysts speed up reactions by influencing the stability of bonds in the reactants. They may also provide an alternative reaction pathway, thus lowering the activation energy (E_a) needed for a reaction to take place (see the graph below).





Enzymes can catalyse the breakdown of molecules

The properties of an enzyme's active site can draw in a single substrate molecule. Chemical bonds are broken, cleaving the substrate molecule to form two separate molecules. Reactions that break down complex molecules into simpler ones are called **catabolic reactions** and involve a net release of energy (they are **exergonic**).
Examples: *cellular respiration, digestion*.

Enzymes can catalyse the building of molecules

The properties of an enzyme's active site can draw in two substrate molecules. The two substrate molecules form bonds and become a single molecule. Reactions that build more complex molecules and structures from simpler ones are called **anabolic reactions** and involve a net use of energy (they are **endergonic**).
Examples: *photosynthesis, protein synthesis.*

1. Why do reactants need energy added to them in order for them to react? _____

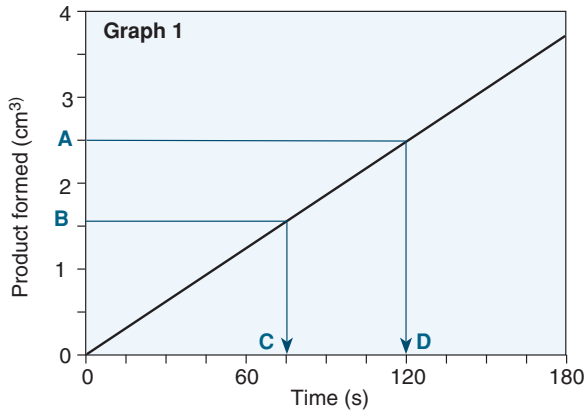
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37 Factors Affecting Enzyme Activity

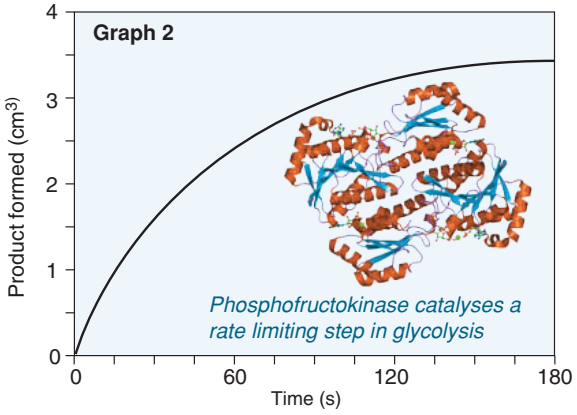
Key Idea: Enzymes operate most effectively within a narrow range of conditions. The rate of enzyme-catalysed reactions is influenced by both enzyme and substrate concentration.

Enzymes usually have an optimum set of conditions (e.g. of pH and temperature) under which their activity is greatest. Many plant and animal enzymes show little activity at low temperatures. Enzyme activity increases with increasing temperature, but falls off after the optimum temperature is exceeded and the enzyme is denatured. Extremes in pH can also cause denaturation. Within their normal operating conditions, enzyme reaction rates are influenced by enzyme and substrate concentration in a predictable way.

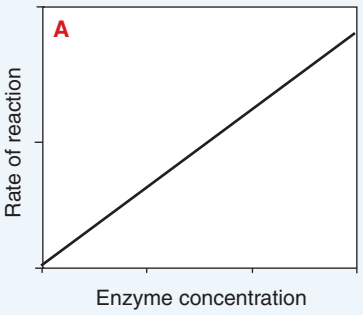
Interpreting an enzyme catalysed reaction



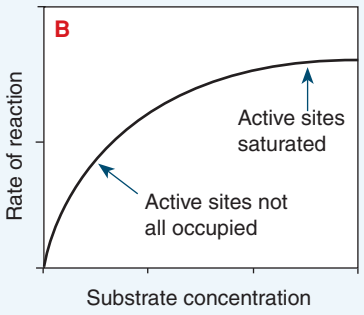
If you plot the amount of product formed during a reaction against time, the rate of a reaction can be calculated from the amount of product made during a given time period. For a reaction in which the rate does not vary (above) the reaction rate calculated at any one point in time will be the same. For example: B ÷ C = A ÷ D.



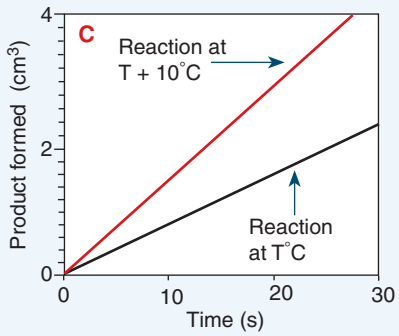
In most biological systems, substrates are limiting, so the reaction rate often levels off over time (above). The enzyme forms product at an initial rate that is roughly linear for a short period after the start of the reaction. As the reaction proceeds and substrate is consumed, the rate continuously slows.



Given an unlimited amount of substrate, the rate of reaction will continue to increase as enzyme concentration increases. More enzyme means more reactions between substrates can be catalysed in any given time (graph A).



If there is unlimited substrate but the enzyme is limited, the reaction rate will increase until the enzyme is saturated, at which point the rate will remain static (graph B).



The effect of temperature on a reaction rate is expressed as the temperature coefficient, usually given as the Q_{10}, which expresses the increase in reaction rate for every rise of 10°C. It is a useful way to express the temperature dependence of a process. For most biological systems, its value is ~2 to 3.

1. Use A ÷ D to calculate the reaction rate in the graph top left: _____

2. (a) What must be happening to the reaction mix in graph 1 to produce the straight line (constant reaction rate)?

_____

_____

(b) Explain why the reaction rate in graph 2 changes over time: _____

_____

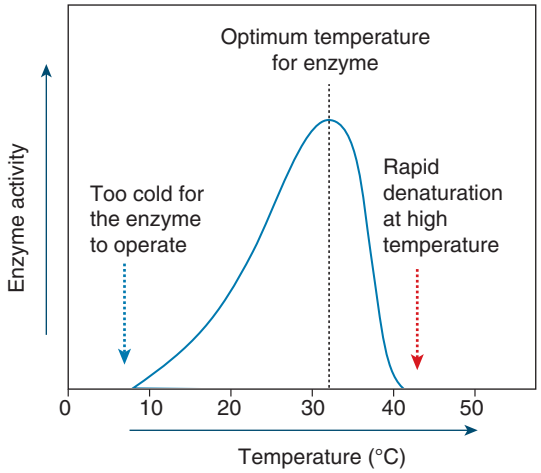
3. Explain why a reaction rate might drop off as the enzyme-catalysed reaction proceeds over time:

_____

_____

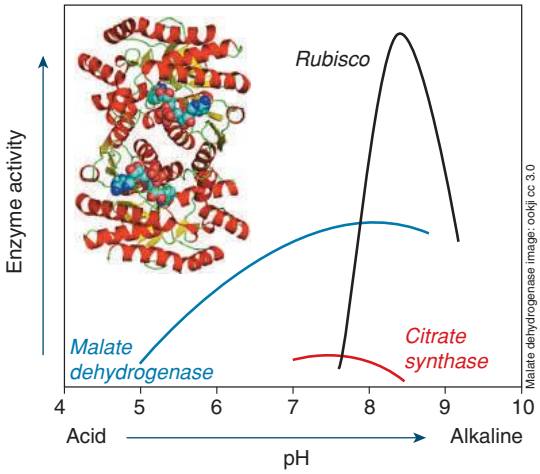
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Temperature and enzyme function



Higher temperatures speed up all reactions, but few enzymes can tolerate temperatures higher than 50–60°C. The rate at which enzymes are **denatured** (change their shape and become inactive) increases with higher temperatures. The temperature at which an enzyme works at its maximum rate is called the **optimum temperature**.

Acidity and enzyme function



Like all proteins, enzymes are **denatured** by extremes of pH. Within these extremes, each enzyme has a pH range for optimum activity. For example, RuBisCo, the enzyme involved in fixing carbon in photosynthesis, has a relatively high optimal pH as it works in an environment where H^+ ions have been removed to create a proton gradient. Image shows malate dehydrogenase with bound sugars.

4. (a) Describe the change in reaction rate when the enzyme concentration is increased and the substrate is not limiting:

(b) Suggest how a cell may vary the amount of enzyme present:

5. Describe the change in reaction rate when the substrate concentration is increased (with a fixed amount of enzyme):

6. (a) Describe what is meant by an optimum temperature for enzyme activity:

(b) Explain why most enzymes perform poorly at low temperatures:

(c) For graph C on the previous page, calculate the Q_{10} for the reaction:

7. (a) State the optimum pH for each of the enzymes:

Malate dehydrogenase: _____ Citrate synthase: _____ RuBisCo: _____

(b) Why does RuBisCo have a high optimal pH?

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38 Investigating Peroxidase Activity

Key Idea: The factors affecting peroxidase activity can be measured using the indicator guaiacol.

Enzymes control all the metabolic activities required to sustain life. Changes to environmental conditions (e.g. pH or temperature) may alter an enzyme's shape and functionality. This may result in a reduction or loss of activity. In this exercise you will use the information provided and your own understanding of enzymes to investigate the effect of pH on enzyme activity and then design an experiment to investigate the effect of inhibitors on enzyme function.

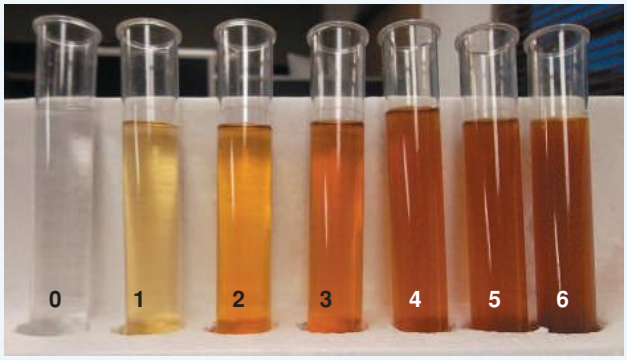
Background

Hydrogen peroxide (H_2O_2) is a toxic by-product of respiration and must be broken down in order to avoid cellular damage. **Peroxidase** acts in the presence of naturally occurring organic reducing agents (electron donors) to catalyse the breakdown of H_2O_2 into water and oxidised organic substrates.

$$2H_2O_2 + 2AH_2 \xrightarrow{Peroxidase} 4H_2O + A_2$$

Like all enzymes, the activity of peroxidase is highest within specific ranges of pH and temperature, and activity drops off or is halted altogether when the conditions fall outside of the optimal range. The conversion of H_2O_2 is also influenced by other factors such as the levels of substrate and enzyme.

The effect of peroxidase on H_2O_2 breakdown can be studied using a common reducing agent called guaiacol. Oxidation of guaiacol (as in the equation above) forms tetraguaiacol, which is a dark orange colour. The rate of the reaction can be followed by measuring the intensity of the orange colour as a function of time.



Increasing levels of oxygen production over time (minutes)

A time-colour palette is shown above. You can use it as a reference against which to compare your own results from the investigation below. The palette was produced by adding a set amount of peroxidase to a solution containing hydrogen peroxide and water. The colour change was recorded at set time points (0-6 minutes).

Investigation 3.1 Investigating peroxidase activity

See appendix for equipment list.

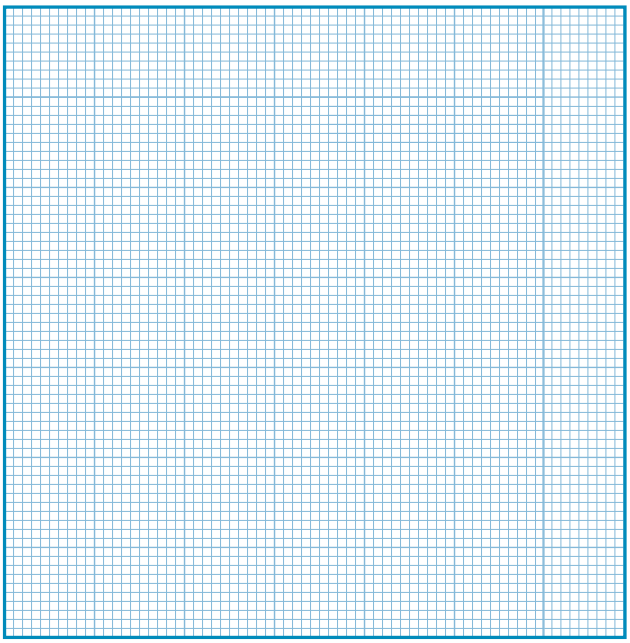
1. Prepare six substrate tubes by adding to a boiling tube 7 mL of distilled water, 0.3 mL of 0.1% H_2O_2 solution, and 0.2 mL of prepared guaiacol solution. Cover the tubes with parafilm and mix.
2. Prepare six enzyme tubes by adding 6.0 mL of prepared buffered pH solution (one of pH 3, 5, 6, 7, 8, and 10) and 1.5 mL of prepared turnip peroxidase solution. Cover the tubes with parafilm and mix.
3. Combine the contents of substrate and enzyme tubes and cover with parafilm. Mix and place back on the rack.
4. Begin timing immediately. Record the colour change every minute (1-6 based on the colour palette above).
5. You can take photos with your phones or keep a written record of the colour changes.

	Colour reference number						
	0 min	1 min	2 min	3 min	4 min	5 min	6 min
pH 3							
pH 5							
pH 6							
pH 7							
pH 8							
pH 10							

1. The colour palette (above) shows the relative amounts of tetraguaiacol formed when guaiacol is oxidised. How can this be used to determine enzyme activity?

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2. Graph your results on the grid (right).



3. (a) Describe the effect of pH on peroxidase activity:

(b) Was there a colour change at pH 10? Explain the result at this pH and relate it to the enzyme's structure and the way it interacts with its substrate:

4. In your experiment, the rate of enzyme activity is measured by comparing against a ranked colour palette. How could you have measured the results more quantitatively?

5. How might the results be affected if you did not begin timing immediately after mixing the contents of the enzyme and substrate tubes together?

6. Why is peroxidase written above the arrow in the equation for enzymatic breakdown of H_2O_2?

7. Based on the information provided and your answer to question 4, design an experiment to investigate the effect of lead nitrate (an enzyme inhibitor) on the activity of turnip peroxidase. Summarise your method as step by step instructions below. Note how you will record and display the data and calculate the reaction rate. Include reference to any limitations or sources of potential error in your design:

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39 Enzyme Inhibition

Key Idea: Enzyme activity can be reduced or stopped by inhibitors. These may be competitive or non-competitive.

Enzyme activity can be stopped, temporarily or permanently, by chemicals called enzyme inhibitors. Competitive inhibitors compete directly with the substrate for the active site and their effect can be overcome by increasing the concentration of available substrate. A non-competitive inhibitor does not occupy the active site, but distorts it so that the substrate and enzyme can no longer interact. Adding more substrate will not overcome the effect of non-competitive inhibition.

Competitive inhibition

Competitive inhibitors compete with the normal substrate for the enzyme's active site.

Competitive inhibitors compete directly with the substrate for the active site, and their effect can be overcome by increasing the substrate concentration.

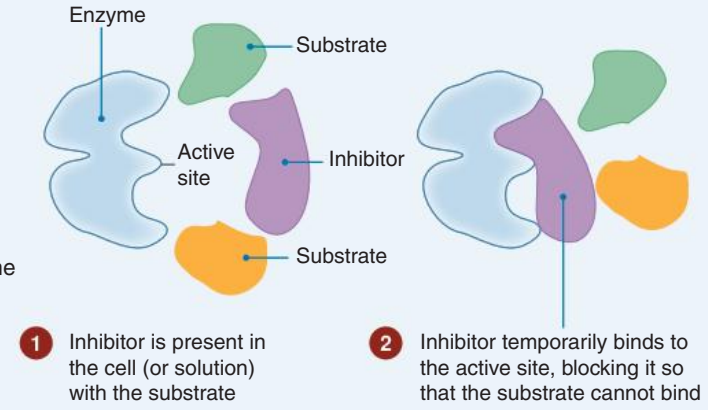
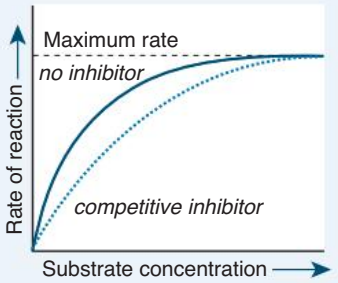


Fig.1: Effect of competitive inhibition on enzyme reaction rate at different substrate concentration



Non-competitive inhibition

Non-competitive inhibitors bind with the enzyme at a site other than the active site.

They inactivate the enzyme by altering its shape so that the substrate and enzyme can no longer interact.

Non-competitive inhibition cannot be overcome by increasing the substrate concentration.

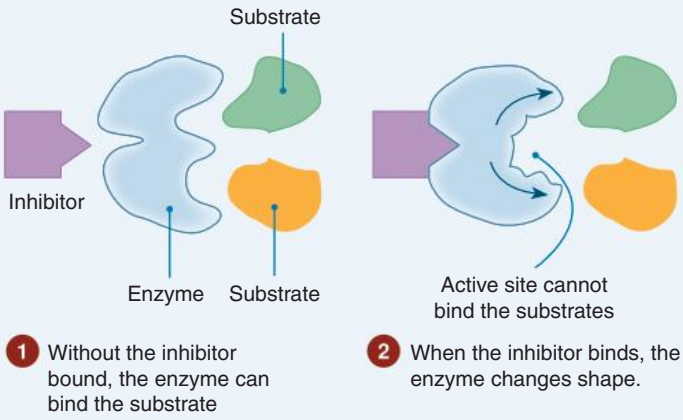
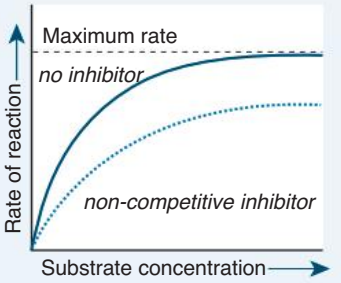


Fig. 2: Effect of non-competitive inhibition on enzyme reaction rate at different substrate concentration



1. Distinguish between competitive and non-competitive inhibition: _____

2. (a) Compare and contrast the effect of competitive and non-competitive inhibition on the relationship between the substrate concentration and the rate of an enzyme controlled reaction (figures 1 and 2 above):

(b) Suggest how you could distinguish between competitive and non-competitive inhibition in an isolated system:

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40 Coenzymes

Key Idea: Coenzymes are needed for the function of some enzymes. They play important roles in the movement of energy, electrons, and protons about the cell.

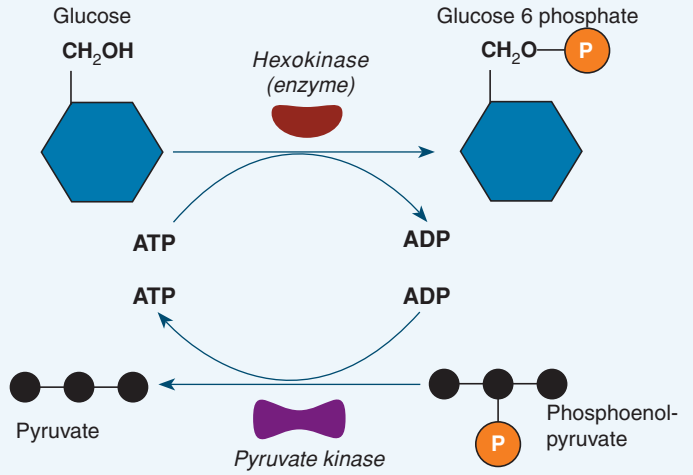
Many enzymes only function when working with a non-protein organic component called a **coenzyme**. The coenzyme can be attached to the enzyme to make it functional, e.g. by helping the enzyme recognise the substrate. Alternatively, it may be unbound and participate to enable the reaction, e.g. ATP provides the energy for many biological reactions. ATP, NADH, and NADPH are coenzymes with important roles in moving energy, protons, and electrons around the cell, as occurs in photosynthesis and cellular respiration.

Cycling coenzymes

Many coenzymes work by transferring chemical groups between enzyme controlled reactions. After the chemical group has been transferred, the coenzyme must be regenerated in a separate reaction. In this way, a cycle is formed between the loaded coenzyme (ready for the reaction) and the unloaded coenzyme (needing to be regenerated).

ATP is important in transferring energy around the cell. During an enzyme-controlled reaction, both ATP and the reactants dock with the enzyme. A phosphate group is transferred to the reactant from ATP, forming ADP and a phosphorylated reactant. The phosphorylated reactant now has the energy to react with another reactant. ADP is regenerated to ATP during cellular respiration. Thus ATP cycles as a loaded form (ATP) and an unloaded form (ADP).

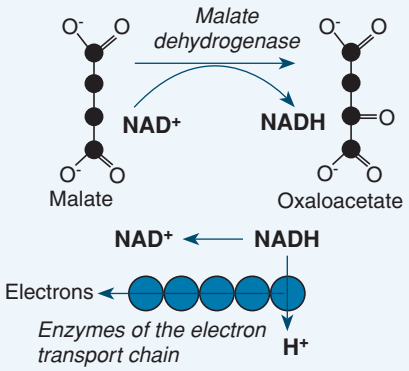
Example of a coenzyme: ATP



In the first step of glycolysis, the enzyme hexokinase catalyses the transfer of a phosphate group from ATP to glucose, producing the more reactive glucose-6-phosphate. In the last step of glycolysis, ATP is regenerated.

NADH

NADH functions as a coenzyme in redox reactions. It is found in two forms in the cell, NAD^+ and NADH. During cellular respiration (right) NAD^+ accepts electrons and protons from reactants in the citric acid cycle and transfers them as NADH to the electron transport chain where they are used to provide the energy for the production of ATP.



NADPH

NADPH is chemically similar to NADH but has an extra phosphate group. Unlike NADH it is not involved in ATP synthesis. In plants, it acts as an electron carrier in photosynthesis. Like NADH, it is found in two forms: NADPH and $NADP^+$. Light is used to add electrons (and hydrogen) to $NADP^+$ to form NADPH. NADPH then carries the electron to a second set of reactions where it is used to fix carbon.

1. (a) What is a coenzyme? _____

   _____

   (b) How does a coenzyme work? _____

   _____

2. Explain how ATP functions as a coenzyme and is able to provide energy for cellular reactions: _____

   _____

   _____

3. Explain how NADH is able to couple redox reactions in a cell: _____

   _____

   _____

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41 Achieving Metabolic Efficiency

Key Idea: Metabolic pathways are linked biochemical reactions that occur within organisms to maintain life.

Metabolic reactions often occur in as a linked series in which each step in the pathway relies on the completion of a previous step and each step is controlled by specific enzymes. The end product of one enzyme-controlled step provides the substrate for the next step, so failure of one step causes failure of all later steps. Metabolic pathways are tightly controlled to prevent energy being wasted. This energy conservation is termed metabolic efficiency. Metabolic reactions are often localised within specific organelles so that all the components of the pathway are kept together.

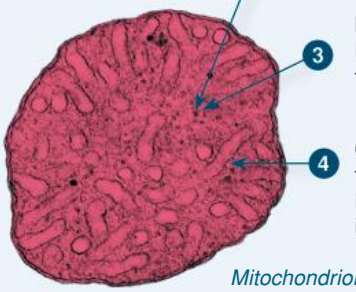
Cellular compartments assist efficiency

To increase metabolic efficiency, regions within a cell or an organelle are **compartmentalised** (separated) by membranes. Particular metabolic reactions are restricted to certain regions where all the necessary metabolic components are located. Having compartments within the cell and within organelles prevents interference between different reaction pathways and enables radically different reaction environments to be accommodated within different organelles.

Example: cellular respiration in the mitochondrion

The membrane system of the mitochondrion divides it into several regions. Glycolysis takes place outside of the mitochondrion, in the cell's cytoplasm, but the remaining steps take place in different specialised regions of the mitochondrion. This helps to regulate movement of substrates and end-products and therefore reaction rates, increasing the efficiency of the process (below).

1. Cytoplasm (outside the mitochondrion): Glycolysis
2. **Matrix**: Link reaction. Link reaction enzymes (e.g. pyruvate dehydrogenase complex) are in the matrix.
3. **Matrix**: Krebs cycle. Krebs cycle enzymes (e.g. fumarase) are in the matrix.
4. **Cristae**: Electron transport chain. Membrane-bound enzymes include ATP synthase



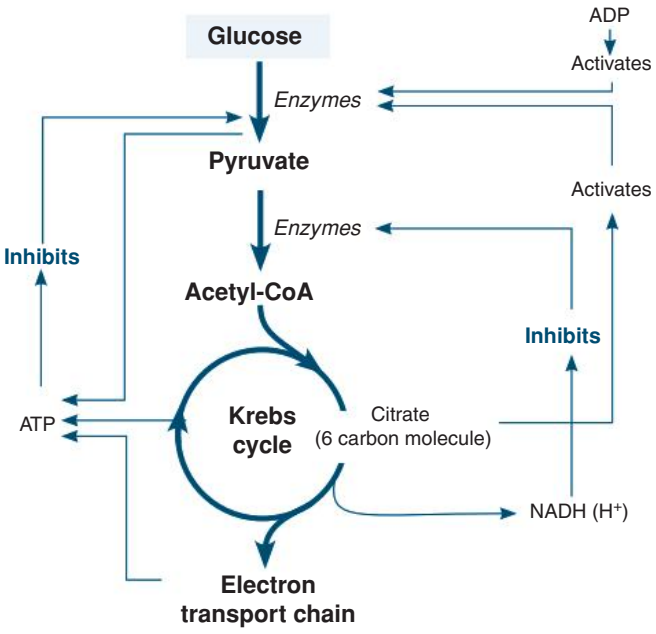
Mitochondrion (transverse section)

WMU

Achieving efficiency by inhibition

Many metabolic pathways are controlled by **feedback inhibition** (negative feedback loop). The pathway is stopped when there is a build-up of end product (or certain intermediate products). The build-up stops the enzymes in the pathway from working and allows the cell to shut down a pathway when it is not needed. This conserves the cell's energy, so it is not manufacturing products it does not need.

Both linear pathways (e.g. glycolysis), and cyclic pathways (e.g. the Krebs cycle) and can be regulated this way (below).



1. What does metabolic efficiency mean? _____

2. Describe how cells achieve metabolic efficiency through:

   (a) Compartmentalising: _____

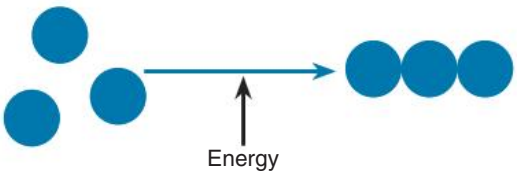
   (b) Feedback inhibition: _____

3. What would happen if cells could not regulate their metabolic pathways? _____

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42 Chapter Review: Did You Get It?

1. (a) What type of metabolic reaction is taking place in the diagram right?



(b) What is occurring during this reaction? _____

(c) Give an example of this type of metabolic reaction: _____

2. Identify the following statements as true of false (circle the correct answer)

(a) Enzymes are biological catalysts. They lower the activation energy of a reaction. True / False

(b) Competitive inhibition is when an inhibitor binds to a site other than the active site. True / False

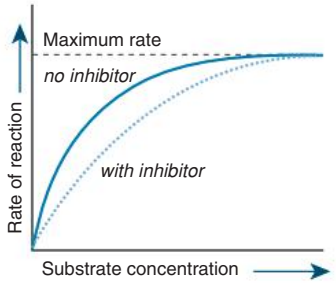
(c) The induced fit model states that the enzyme changes shape when a substrate fits into the active site. True / False

(d) End product inhibition causes a feedback loop that escalates the outcome of the loop. True / False

3. The graph (right) shows the effect of an enzyme inhibitor in enzyme reaction rate.

(a) It show competitive inhibition/non-competitive inhibition (delete incorrect answer).

(b) Circle the diagram below that illustrates your choice in (a):

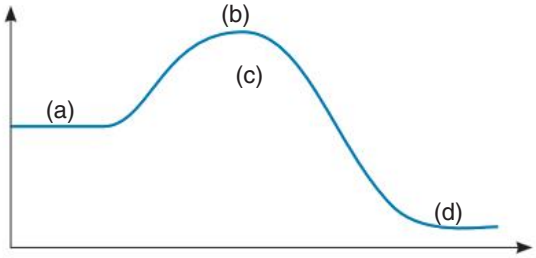




4. Identify the labels (a - d) on the graph, right, using the following labels: *Reactants, products, activation energy, transition state*.

(a) _____ (b) _____

(c) _____ (d) _____



5. Match each term to its definition, as identified by its preceding letter code.

activation energy

ATP

biological catalyst

competitive inhibition

enzyme

induced fit model

non-competitive inhibition

substrate

A Coenzyme that acts as the cell's energy carrier, transferring phosphate groups from itself to target molecules.

B A type of enzyme inhibition in which the substrate and inhibitor compete to bind to the active site.

C The currently accepted model for enzyme function.

D A globular protein that acts as a catalyst to speed up a specific biological reaction.

E A substance or molecule that lowers the activation energy of a reaction but is itself not used up during the reaction. In biological systems this function is carried out by enzymes.

F The energy required for a reactant to reach an unstable transition state in which it can react with another reactant.

G The compound on which an enzyme acts.

H A type of enzyme inhibition in which the inhibitor does not occupy the active site but binds to some other part of the enzyme.

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CHAPTER 4

Photosynthesis

Key terms

ATP
C_3 plant
C_4 plant
Calvin cycle
CAM metabolism
chloroplast
chlorophyll
grana
light dependent phase
light independent phase
limiting factor
NADP/NADPH
photolysis
photosynthesis
photosystem
producer
RuBisCo
stroma
stroma lamellae
thylakoid discs
triose phosphate

The role of photosynthesis

Key skills and knowledge

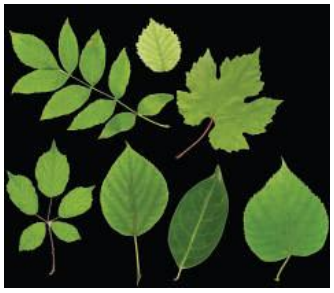
Activity number

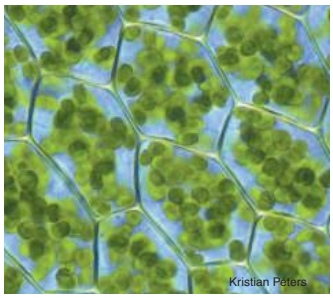
- ☐ 1 Recall the role of photosynthesis in supporting the majority of life on Earth. **43**
- ☐ 2 Write the overall equation for photosynthesis in words and symbols. What is the role of light in the process? **45**

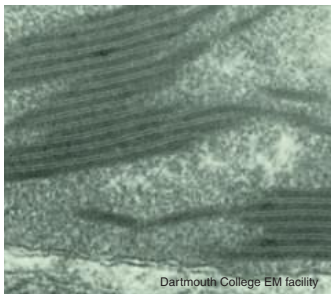
Chloroplasts are the site of photosynthesis

Key skills and knowledge

- ☐ 3 Describe the structure and role of chloroplasts, including the thylakoid membranes and liquid stroma. Explain the role of pigments, particular chlorophylls in light capture. Which wavelengths of light are most effective in driving photosynthesis and why? **44**
- ☐ 4 Describe the inputs and outputs of the light dependent stage of photosynthesis. **45**
- ☐ 5 Outline the events in the light dependent reactions of photosynthesis, including absorption of light, transfer of excited electrons between carriers in the thylakoid membranes, generation of ATP and NADPH, and the photolysis of water. **45**
- ☐ 6 Describe the inputs and outputs of the light independent stage of photosynthesis (the Calvin cycle), including the role of the enzyme RuBisCo. **45**
- ☐ 7 Outline the events in the Calvin cycle, including the fixation of carbon and the production of triose phosphate using reduced NADPH and ATP. **45**







RuBisCo and adaptations for maximising photosynthesis

Key skills and knowledge

- ☐ 8 Describe the role of the enzyme RuBisCo in photosynthesis. Explain why it is a relatively inefficient enzyme and explain the consequences of this. **46**
- ☐ 9 Describe the adaptations of biochemical pathways for photosynthesis in C_3, C_4, and CAM plants. Explain how the adaptations of C_4 and CAM plants account for their distribution, productivity in different environments, and tolerance to water stress. **46**

Factors affecting photosynthesis

Key skills and knowledge

- ☐ 10 Describe and explain limiting factors for photosynthesis (to include carbon dioxide concentration, light intensity, temperature, and availability of water). **48**
- ☐ 11 PRAC Investigate the effect of environmental factors, e.g. light intensity, on the rate of photosynthesis. **47**
- ☐ 12 Outline methods used to overcome limiting factors for photosynthesis, including the use of controlled environments. **49**

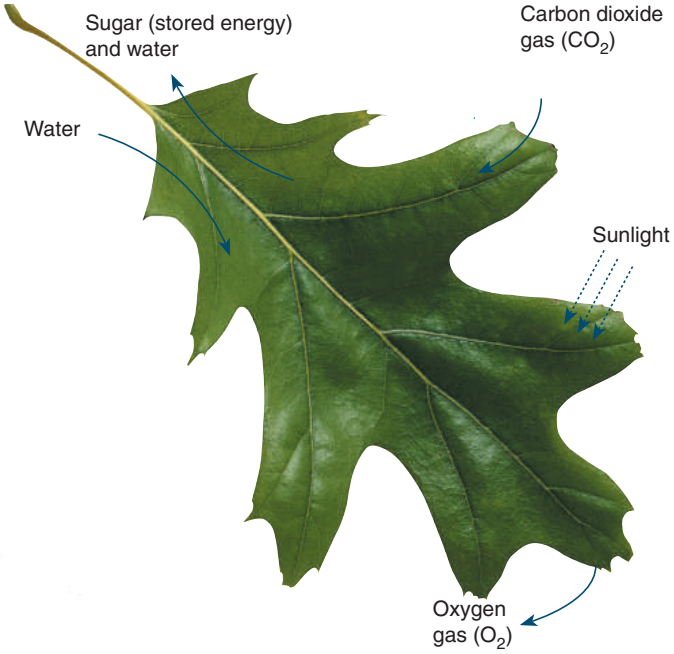
43 The Role of Photosynthesis

Key Idea: Photosynthesis is the chemical process in which autotrophs use sunlight to produce carbohydrates.

Photosynthesis is the process by which plants capture light energy and use it to fix (convert) the carbon in CO_2 into carbohydrates (e.g. glucose). The carbohydrate is used by the plant to power ATP production and build its body. Plants (and other photosynthetic organisms) carry out this process without input from other organisms, so they are called **producers** (as opposed to consumers, which depend on energy and carbon from other organisms).

Photosynthesis and producers

- A **producer** (or autotroph) is an organism that can make its own food.
- Plants, algae, and some bacteria are producers.
- Most producers use the energy in sunlight to make their food. The process by which they do this is called **photosynthesis**. Photosynthesis transforms sunlight energy into chemical energy.
- The chemical energy is stored as glucose, and the energy is released when the glucose undergoes further metabolic processes.
- The inputs and outputs of photosynthesis are shown on the leaf diagram (right).





DID YOU KNOW?

The evolution of oxygenic photosynthesis, in which water is split to provide the hydrogens to drive the process, was responsible for our current oxygen rich atmosphere. Using water (rather than hydrogen sulfide) to supply hydrogens provided far more energy for ATP production and produced oxygen gas as a waste product.



Photosynthesis by marine algae provides oxygen and absorbs carbon dioxide. Most algae are microscopic but some, like this kelp, are large.



Depending on the plant, 0.1% to 8% of the light intercepted is used in photosynthesis. Typically crop plants use about 1%-2%.



Producers, such as grasses, make their own food, and are also the ultimate source of food and energy for consumers, such as this cow.

1. (a) What is a producer? _____

   _____

   (b) Name some organisms that are producers: _____

   _____

2. Where do producers get their energy from? _____

   _____

3. Why are producers so important in an ecosystem? _____

   _____

   _____

   _____

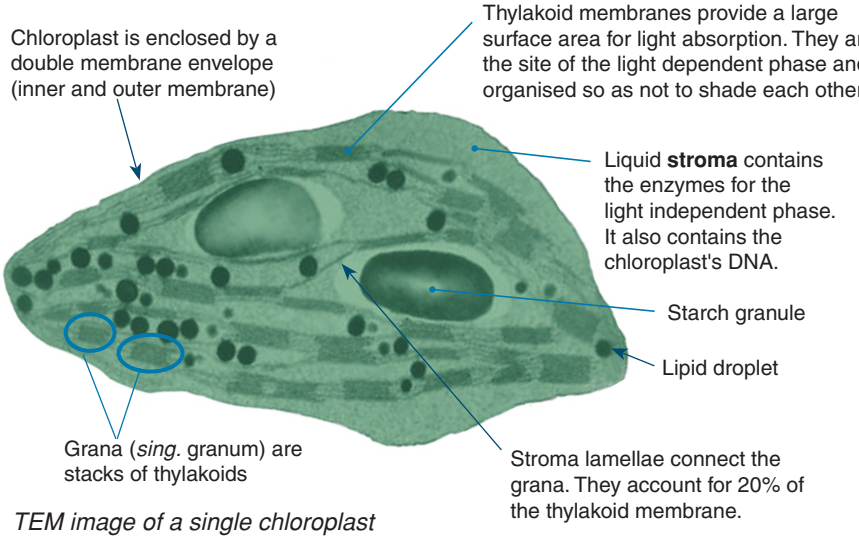
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44 Chloroplasts

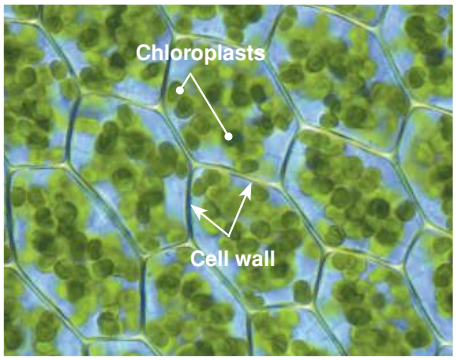
Key Idea: Chloroplasts have a complex internal membrane structure. They are the site of photosynthesis in plant cells.

Chloroplasts are the specialised plastids in which photosynthesis occurs. A mesophyll (photosynthetic) leaf cell contains between 50-100 chloroplasts. The chloroplasts are generally aligned so that their broad surface runs parallel to the cell wall to maximise the surface area available for light absorption. Chloroplasts have an internal structure characterised by a system of membranous structures called **thylakoids** arranged into stacks called **grana**. Special pigments, called **chlorophylls** and **carotenoids**, are bound to the membranes as part of light-capturing photosystems. Chlorophylls absorb light of specific wavelengths (blue and orange-red light) and thereby capture the light energy.

The structure of a chloroplast



TEM image of a single chloroplast



Kristian Peters

Chloroplasts visible in plant cells

1. Label the transmission electron microscope image of a chloroplast below:

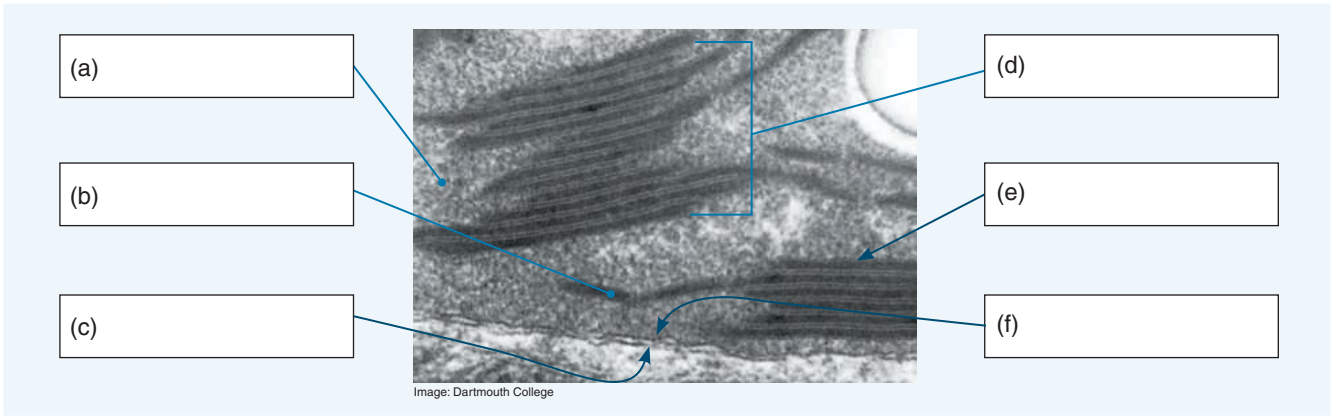


Image: Dartmouth College

2. (a) Where is chlorophyll found in a chloroplast? _____

_____

(b) Why is chlorophyll found there? _____

_____

_____

3. Explain how the internal structure of chloroplasts helps absorb the maximum amount of light: _____

_____

_____

_____

4. Explain why plant leaves appear green: _____

_____

_____

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45 Photosynthesis: Inputs and Outputs

Key Idea: Photosynthesis is the process by which light energy is used to convert CO_2 and water into glucose and oxygen.

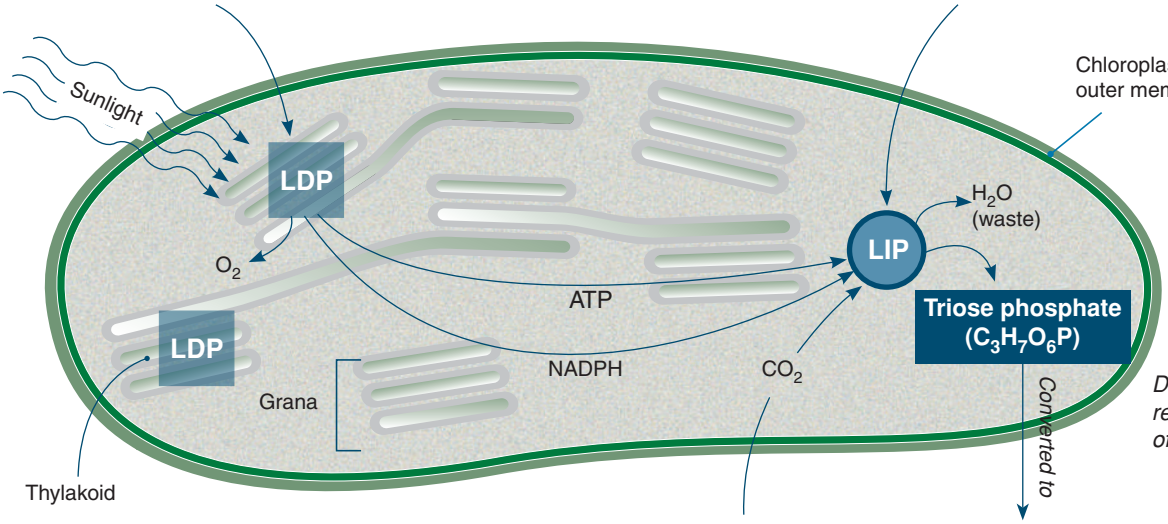
Photosynthesis is of fundamental importance to living things because it transforms sunlight energy into chemical energy stored in molecules, releases free oxygen gas, and absorbs carbon dioxide (a waste product of cellular metabolism). Photosynthesis has two sets of reactions, the light dependent phase and the light independent phase. In the light dependent phase, light energy is converted to chemical energy (ATP and NADPH). This phase occurs in the thylakoid membranes of chloroplasts. In the light independent phase, the ATP and NADPH are used to synthesise carbohydrate. This phase occurs in the stroma of chloroplasts. In photosynthesis, water is split and electrons are transferred together with hydrogen ions from water to CO_2, reducing it to triose phosphates (then converted to sugars).

Light dependent phase (LDP):

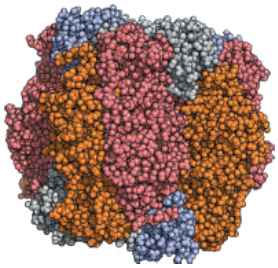
In the first phase of photosynthesis, chlorophyll captures light energy, which is used to split water, producing O_2 gas (waste). Electrons and H^+ ions are transferred to the molecule NADPH. ATP is also produced. The light dependent phase occurs in the thylakoid membranes of the grana.

Light independent phase (LIP):

The second phase of photosynthesis occurs in the stroma and uses the NADPH and the ATP to drive a series of enzyme-controlled reactions (the **Calvin cycle**) that fix carbon dioxide to produce triose phosphate. This phase does not need light to proceed.



Diagrammatic representation of a chloroplast



RuBisCo (left) is the central enzyme in the LIP of photosynthesis (carbon fixation) catalysing the first step in the Calvin cycle. However it is remarkably inefficient, processing just three reactions a second. To compensate, RuBisCo makes up almost half the protein content of chloroplasts.

The general equation for photosynthesis

$$6CO_2 + 6H_2O \xrightarrow[\text{Chlorophyll}]{\text{Light}} C_6H_{12}O_6 + 6O_2$$

1. Identify the two phases of photosynthesis and their location in the cell:

   (a) _____

   (b) _____

2. (a) What is the role of the enzyme RuBisCo? _____

   (b) RuBisCo is the most abundant protein on Earth. Suggest a reason for this: _____

   _____

   _____

3. State the origin and fate of the following molecules involved in photosynthesis:

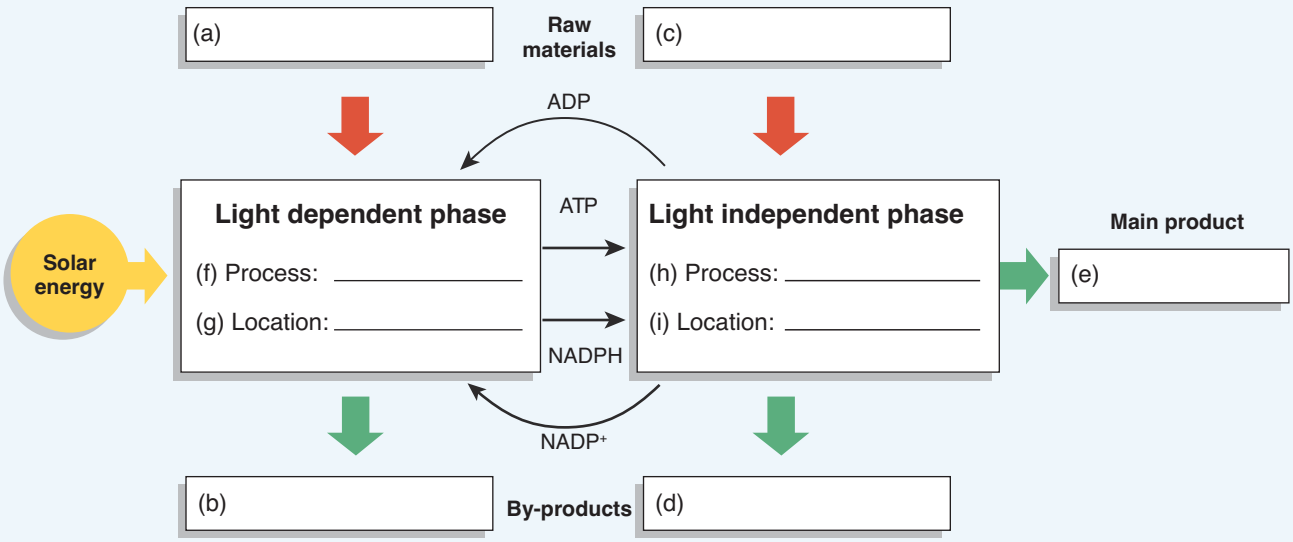
   (a) Carbon dioxide: _____

   (b) Oxygen: _____

   (c) Hydrogen: _____

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4. Use the information on the previous page to fill in the diagram below, including the raw material (inputs), products (outputs), and processes.



5. In two experiments, radioactively-labelled oxygen (shown in red) was used to follow oxygen through the photosynthetic process. The results of the experiment are shown below:

   Experiment A: $6CO_2 + 12H_2\mathbf{O} + \text{sunlight energy} \rightarrow C_6H_{12}O_6 + 6\mathbf{O}_2 + 6H_2O$

   Experiment B: $6C\mathbf{O}_2 + 12H_2O + \text{sunlight energy} \rightarrow C_6H_{12}\mathbf{O}_6 + 6O_2 + 6H_2O$

   From these results, what would you conclude about the source of the oxygen in:

   (a) The carbohydrate produced? _____

   (b) The oxygen released? _____

6. Name the products that triose phosphate is converted into: _____

   _____

7. Describe what happens during:

   (a) The light dependent phase of photosynthesis: _____

   _____

   _____

   (b) The light independent phase of photosynthesis: _____

   _____

   _____

8. What is the function of each of the following in photosynthesis:

   (a) ATP: _____

   (b) NADPH: _____

   (c) Light: _____

   (d) Chlorophyll: _____

   (e) Water: _____

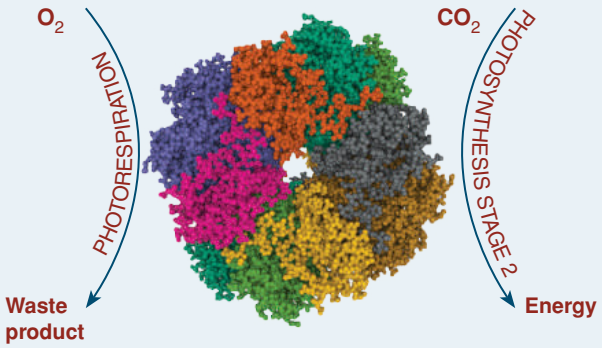
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46 Adaptations for Maximising Photosynthesis

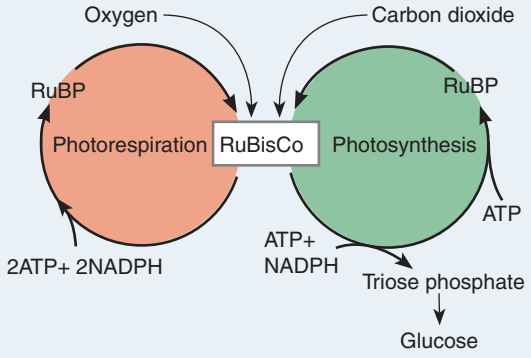
Key Idea: RuBisCo is an inefficient enzyme and some plant groups have evolved ways to reduce the mistakes it makes and so increase the efficiency of photosynthesis.

RuBisCo is an enzyme involved in the fixation of carbon in photosynthesis. It is very inefficient at this process. Not only does it carry out a very low number of reactions per second, but 20% of the those reactions involve oxygen instead of carbon dioxide (a process called photorespiration). This produces molecules the plant can't use and so costs the plant energy. Some groups of plants have evolved processes that separate oxygen from the air from carbon dioxide and so improve the efficiency of RuBisCo.

RuBisCo is not specific



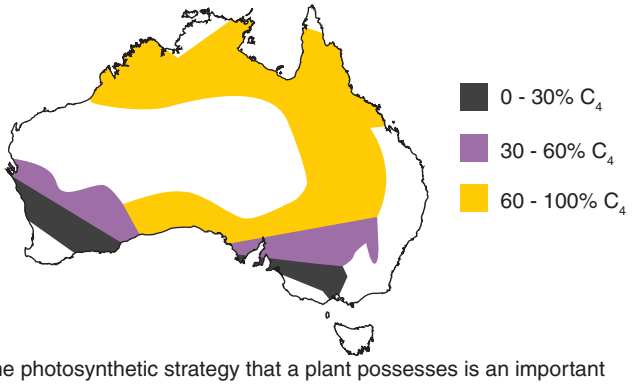
RuBisCo catalyses the carboxylation (adding of carbon) of the molecule ribulose-1,5-bisphosphate (RuBP) in the first step of the light independent reactions in photosynthesis. Unfortunately it does not always discriminate between carbon dioxide and oxygen and can add oxygen to RuBP instead. The plant then wastes energy in breaking down the resulting molecule in a process called photorespiration.



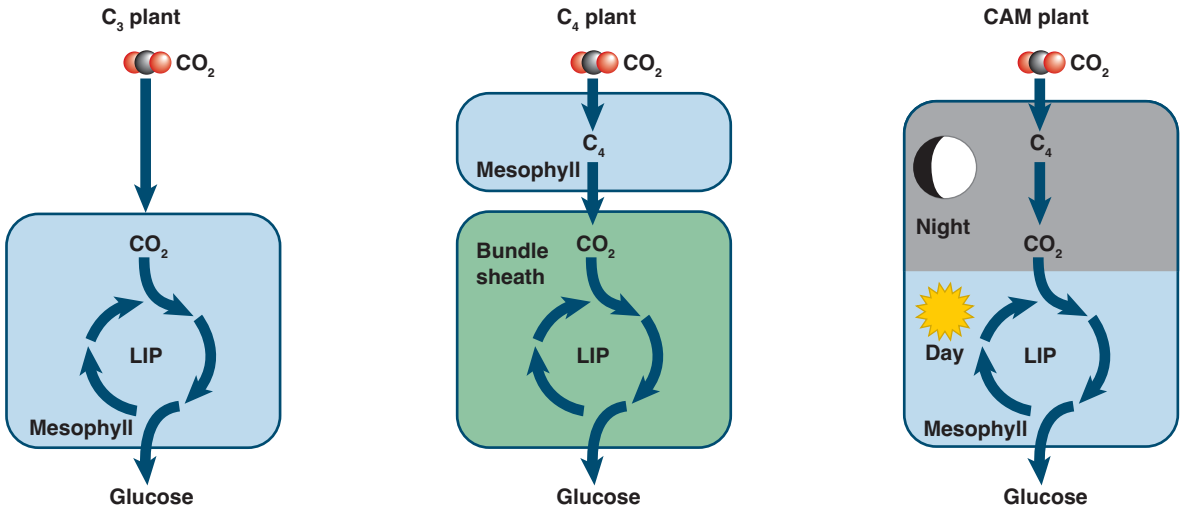
Photosynthetic strategies

- In about 85% of plants on Earth, the first detectable compound made in photosynthesis is a 3-carbon compound called glycerate 3-phosphate (GP). These plants are called C_3 plants.
- However, in some plants a 4-carbon molecule is made first and a unique leaf anatomy allows CO_2 to concentrate around RuBisCo, reducing photorepsiration and increasing photosynthetic efficiency. These plants, which include cereals and tropical grasses, are called C_4 plants. They have high rates of photosynthesis, thriving in environments with high light levels and warm temperatures. The high productivity of C_3 plants at high temperatures gives them a competitive advantage in tropical climates. CAM plants also produce oxaloacetate as their first photosynthetic compound but produce it at night, storing carbon dioxide until light is available.

% C_4 plants across Australia



The photosynthetic strategy that a plant possesses is an important factor in determining its distribution. Many of the enzymes of C_4 plants have optimum temperatures well above 25°C, so they thrive in hot tropical and sub-tropical climates. Under these conditions, they out-compete most C_3 plants because they achieve higher photosynthetic rates. The proportion of C_4 plants in Australia is greatest near the tropics and arid interior.



In C_3 plants carbon dioxide is fixed directly from the air. Stomata must be open (which lets air in, but also water out). The light independent phase (LIP) occurs in the leaf mesophyll (where light is also captured). This exposes RuBisCo to oxygen, reducing photosynthetic efficiency.

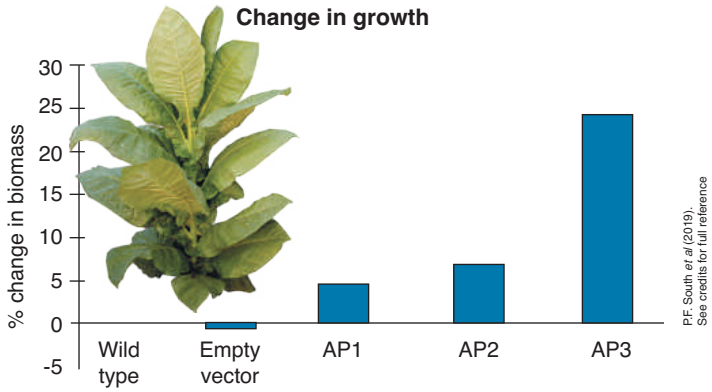
In C_4 plants, carbon dioxide is fixed in a two step process. Carbon dioxide is used to produce oxaloacetate in the leaf mesophyll. This diffuses deeper into the leaf. CO_2 is released to RuBisCo deeper in the leaf away from oxygen, increasing efficiency.

In CAM plants stomata are opened at night to take in CO_2 (stored as oxaloacetate). This reduces water loss on hot days. During the day stomata are closed, reducing RuBisCo exposure to oxygen and again increasing photosynthetic efficiency.

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Increasing photosynthesis efficiency

- About 20% of the time RuBisCo uses oxygen instead of CO_2, producing a plant toxic molecule. The plant wastes energy passing the molecule along a biochemical pathway in order to break it down and recover the original useful molecule. As the temperature increases this problem becomes worse.
- Recently scientists genetically engineered cells of a tobacco plant (a C_3 plant) to shortcut the photorespiration pathway. They used *Agrobacterium tumefaciens* to insert genes from various organisms, including *E. coli*, *Chlamydomonas reinhardtii,* and *Arabidopsis thaliana*, into tobacco plant cells. Three strains of engineered tobacco plant were produced, each with an alternative photorespiration pathway.



- By short-cutting the photorespiration pathway, photosynthetic efficiency would increase and the pant could divert more energy to growth. This could lead to larger, more efficiently grown crops, producing more food for a growing world population.
- The plants, AP1, AP2, and AP3 were grown and tested. The strain AP3 produced the best result. First generation plants had a biomass increase of 20%. Second generation AP3 plants had a biomass average increase of about 24% and up to 37%.

1. Explain why C_4 plants have a competitive advantage over C_3 plants in the tropics: _____

2. Explain why CAM plants have a competitive advantage over C_4 and C_3 plants in arid situations: _____

3. Explain what C_4 plants achieve by carrying out the light independent reactions inside the bundle sheath:

4. Study the map of Australia on the previous page showing the distribution of C_4 plants. Explain the distribution pattern in terms of their competitive advantage and the environmental conditions required for this advantage:

5. Suggest why increasing the ambient CO_2 concentration increases the rate of photosynthesis in C_3 plants:

6. Why would genetically engineering a crop plant to short circuit the photorespiration pathway potentially increase crop yields?

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47 Investigating Photosynthetic Rate

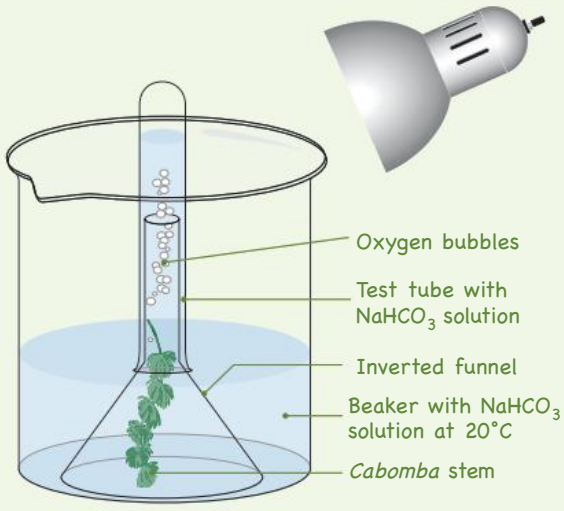
Key Idea: Measuring the production of oxygen provides a simple way to measure the rate of photosynthesis.

The rate of photosynthesis can be investigated by tracking the substances involved in photosynthesis. These include measuring the uptake of carbon dioxide, the production of oxygen, or the change in biomass over time. Measuring the rate of oxygen production provides a good approximation of the photosynthetic rate and is relatively easy to carry out.

Investigation 4.1 Investigating photosynthetic rate

See appendix for equipment list.

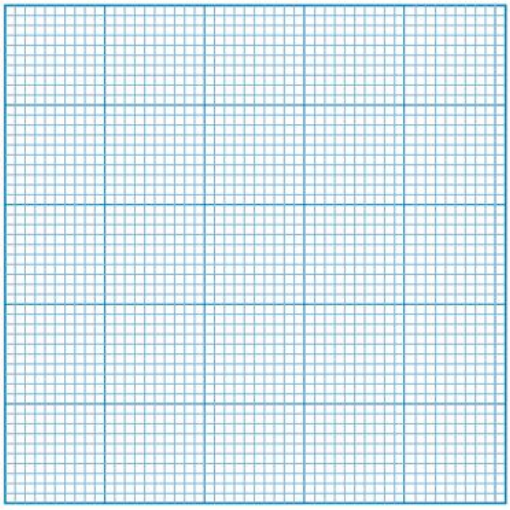
1. Weigh 0.8–1.0 grams of *Cabomba aquatica* stem on a balance. Cut the stem underwater and invert to ensure a free flow of oxygen bubbles.
2. Place into a beaker filled (at approximately 20°C) with a solution containing 0.2 mol/L sodium hydrogen carbonate (to supply carbon dioxide).
3. Invert a funnel over the *Cabomba* and then invert a test tube filled with the sodium hydrogen carbonate solution on top to collect any gas produced.
4. Place the beaker at distances 20, 25, 30, 35, 40, 45, and 50 cm from a 60W light source. Measure the light intensity with a lux meter at each interval.
5. Leave the *Cabomba* stem to acclimatise to the new light level for 5 minutes before recording data. Count the bubbles for a period of three minutes at each distance and record.



6. Alternatively the volume of gas captured in the test tube can be measured and recorded.

Distance (cm)	Light intensity (lx)	Bubbles counted in three minutes	Bubbles per minute	Volume (mL)
50 cm				
45 cm				
40 cm				
35 cm				
30 cm				
25 cm				
20 cm				

1. Use the data to draw a graph of the bubble produced per minute vs light intensity:



2. Why is measuring light intensity directly in lux better than inferring light intensity from the measured distance?

_____

_____

_____

3. The sample of gas collected during the experiment was tested with a glowing splint. The splint reignited when placed in the gas. What does this confirm about the gas produced?

_____

4. Why is measuring gas collected rather than bubbles produced a more accurate why of recording data?

_____

_____

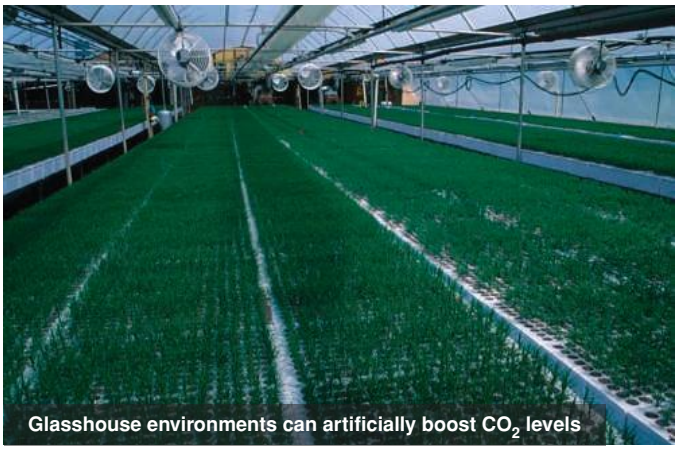
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48 Factors Affecting Photosynthesis

Key Idea: Environmental factors, such as light availability and carbon dioxide level, affect photosynthetic rate.

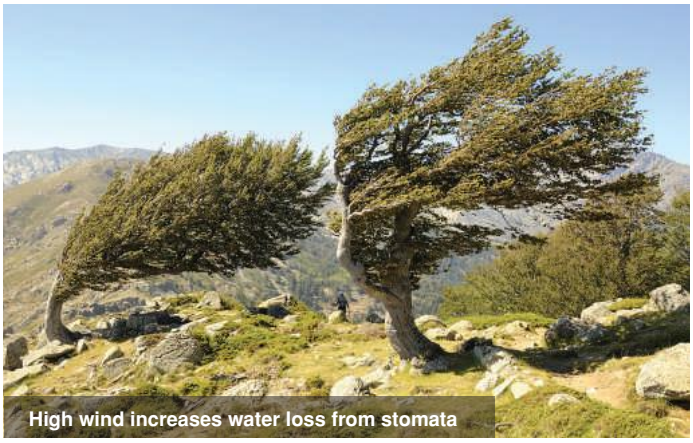
The rate at which plants can photosynthesise is dependent on environmental factors, particularly light and carbon dioxide (CO_2), but also temperature. In the plant's natural environment, fluctuations in these factors (and others) influence photosynthetic rate, so that the rate varies daily and seasonally. The effect of each factor can be tested experimentally by altering one while holding the others constant. Usually, either light or CO_2 level is limiting. Humans can overcome the limitations of low light or CO_2 by growing plants in a controlled environment.

- The rate of photosynthesis is affected by abiotic (non-living) factors in the environment, particularly light intensity, temperature, and CO_2 level. These factors are termed **limiting factors**, because they influence achievable photosynthetic rate.
- The two graphs (right) illustrate the effect of these variables on the rate of photosynthesis in cucumber plants. Figure A shows the effect of increasing light intensity at constant temperature and CO_2 level. Figure B illustrates how this response is influenced by CO_2 concentration and temperature. 30°C is at the upper range of tolerance for many plants.

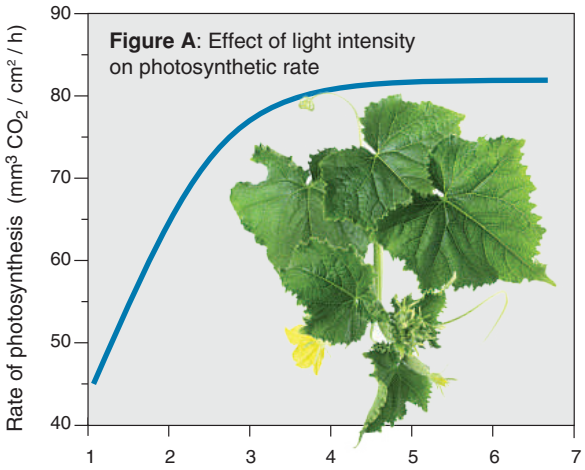


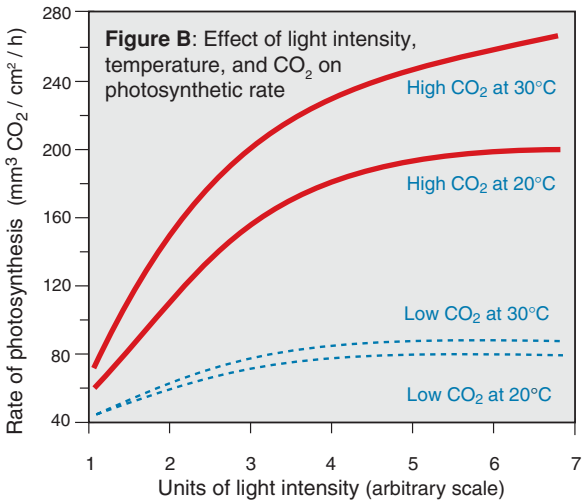
Glasshouse environments can artificially boost CO_2 levels

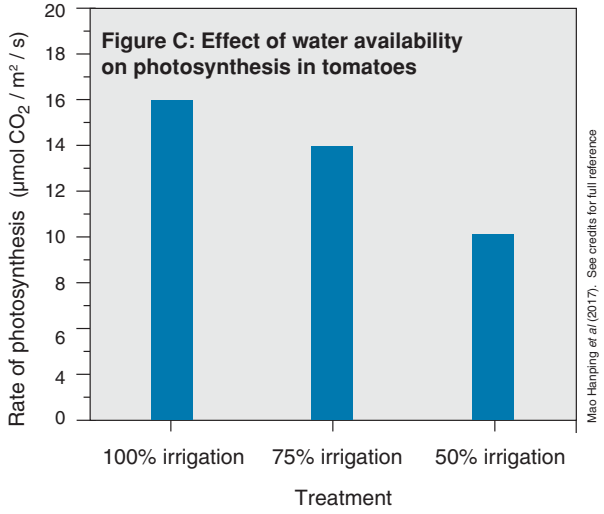
- Water is one of the two reactants in the general equation for photosynthesis. It follows that water availability should affect the rate of the photosynthesis. However, because water makes up so much of the plant, the effect of reduced water does not directly affect the rate of photosynthesis (water is always available).
- Conditions that cause water loss from the plant (e.g. increased wind speed or reduced humidity) also cause the plant to close its stomata. Plants acquire CO_2 from the atmosphere through their stomata. This means that the conditions that cause water stress also reduce CO_2 uptake and lower photosynthetic rate. Figure C shows the effect of water availability on the rate of photosynthesis in tomatoes.



High wind increases water loss from stomata







Mao Hanping *et al* (2017). See credits for full reference

1. Why does photosynthetic rate decline when the CO_2 level is reduced? _____

_____

_____

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2. Based on the figures opposite, summarise and explain the effect of each of the following factors on photosynthetic rate:

   (a) CO_2 concentration: _____

   _____

   (b) Light intensity: _____

   _____

   (c) Temperature: _____

   _____

3. Why might hot, windy conditions reduce photosynthetic rates? _____

_____

_____

_____

4. Design an investigation to determine the effect of temperature on photosynthetic rate. Attach a diagram if you wish:

_____

_____

_____

_____

_____

_____

_____

_____

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_____

_____

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_____

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_____

_____

_____

5. Describe how greenhouses can be used to increase the growth of crops: _____

_____

_____

_____

_____

_____

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49 Overcoming Limiting Factors in Photosynthesis

Key Idea: Growing plants in controlled environments allows abiotic factors to be manipulated in order to maximise yield.

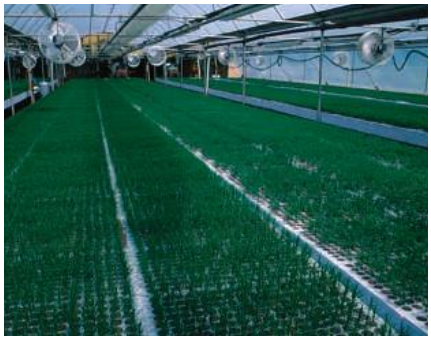
Manipulating abiotic factors can maximise crop yields by maximising photosynthetic rate and reducing losses to pests, disease, and competition. For example, covering the soil with black plastic reduces weed growth, increases soil temperature, and boosts production. Greater control of the abiotic conditions is achieved by growing crops in a controlled-environment system such as a greenhouse. Temperature, carbon dioxide (CO_2) concentration, and light intensity are optimised to maximise the rate of photosynthesis and therefore production. Greenhouses also allow specific abiotic factors to be manipulated to trigger certain life cycle events such as flowering. CO_2 enrichment dramatically increases the growth of greenhouse crops providing that other important abiotic factors (such as nutrients) are not limiting.



The growing environment can be controlled or modified to varying degrees. Black plastic sheeting can be laid over the soil to control weeds and absorb extra solar heat. Tunnel enclosures (above) may be used to reduce light intensity and airflow, prevent frost damage, and reduce damage by pests.



Large, commercial greenhouses have elaborate computer-controlled watering systems linked to sensors that measure soil moisture, air temperature, and humidity. Coupled with a timer, they deliver optimal water conditions for plant growth by automatically operating the irrigation system.

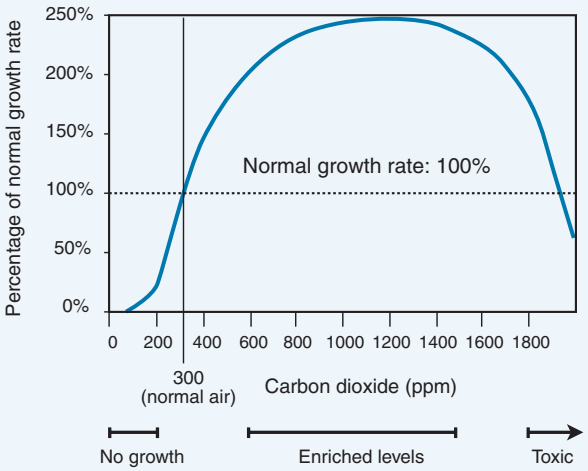


Air flow through a greenhouse is essential to providing a homogeneous air temperature. Air flow also ensures an even distribution of CO_2 throughout the enclosure. Airflow from one end of the enclosure to the other is maintained by fans all blowing in the same direction.

Carbon dioxide enrichment

- Carbon dioxide (CO_2) is a raw material used in photosynthesis. If the supply of CO_2 is cut off or reduced, plant growth and development are curtailed. The amount of CO_2 in air is normally 0.03% (250-330 ppm). Most plants will stop growing when the CO_2 level falls below 150 ppm. Even at 220 ppm, a slowing of plant growth is noticeable (see graph, right).
- Controlled CO_2 atmospheres, which boost the CO_2 concentration to more than 1000 ppm, significantly increase the rate of formation of dry plant matter and total yield (e.g. of flowers or fruit).
- Extra carbon dioxide can be generated (at a cost) by burning hydrocarbon fuels, using compressed, bottled CO_2 or dry ice, or by fermentation or decomposition of organic matter.

The effect of CO_2 concentration on plant growth



1. Explain why CO_2 enrichment has the capacity to radically increase crop production: _____

_____

_____

2. State the two primary considerations influencing the economic viability of controlled environments: _____

_____

_____

3. List the abiotic factors that are controlled in a greenhouse environment: _____

_____

_____

_____

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50 Chapter Review: Did You Get It?

1. (a) Write the process of photosynthesis as:

   A word equation: _____

   A chemical equation: _____

   (b) Where does photosynthesis occur? _____

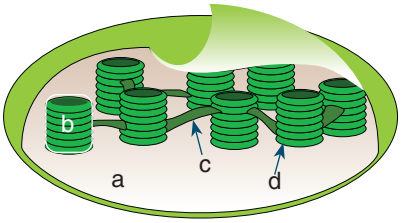
2. Using the word list, identify the features (a)-(d) of a chloroplast shown on the diagram below right.
   Word list: granum, stroma, thylakoid disc, stroma lamellae

   (a) _____

   (b) _____

   (c) _____

   (d) _____



   (e) In which region(s) do the light dependent reactions take place? _____

   (f) In which region(s) do the light independent reactions take place? _____

3. Students investigated the effect of different light wavelengths (colour) on the rate of photosynthesis. They used a leaf disc assay in which the rate of photosynthesis is measured indirectly by the time it takes for prepared leaf discs (right) to float to the surface when placed in an illuminated beaker of sodium hydrogen carbonate. The results are tabulated below, right:

   (a) Why do you think photosynthesising leaf discs would float?

   _____

   _____

   _____

Light colour	Time taken for 10 discs to float (s)
Blue	162
Red	558
Green	998
White	694



Preparing the leaf discs by applying pressure to a sodium hydrogen carbonate solution.

   (b) Generate a working hypothesis for this experiment:

   _____

   _____

   _____

   (c) Which light colour(s) was/were the most effective at driving photosynthesis? Explain: _____

   _____

   _____

   (d) Which light colour was the least effective at driving photosynthesis? _____

   (e) Did the results of this experiment support your hypothesis? Explain: _____

   _____

5. Describe the difference in photosynthesis between C_3, C_4, and CAM plants: _____

   _____

   _____

   _____

   _____

   _____

   _____

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CHAPTER 5

Cellular Respiration

Key terms

acetyl coA
alcoholic fermentation
anaerobic metabolism
ATP
ATP synthase
cellular respiration
cristae
electron transport chain
ethanol
FAD/$FADH_2$
fermentation
glucose
glycolysis
Krebs cycle
lactic acid fermentation
link reaction
matrix
mitochondrion
NAD/NADH
oxidative phosphorylation
pyruvate
substrate level phosphorylation

The role of cellular respiration

Key skills and knowledge

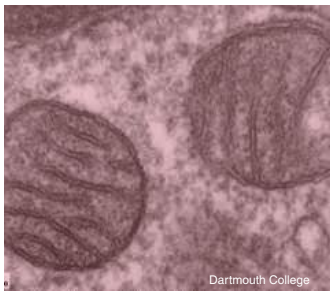
Activity number

- ☐ 1 Recall the role of ATP in the energy transformations of living systems. Describe the purpose of cellular respiration in living organisms. **51**
- ☐ 2 Use a schematic diagram to explain how ATP supplies energy for biochemical reactions. How is ATP regenerated after it has been involved in reactions? **52**
- ☐ 3 Write the overall equation for cellular respiration in words and chemical notation. **53**

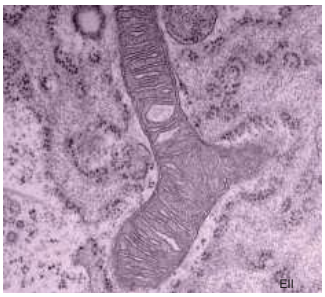
The steps in cellular respiration

Key skills and knowledge

- ☐ 4 Describe the structure and role of mitochondria, including the cristae (folded inner membranes) and matrix. **53**
- ☐ 5 Identify the main steps in cellular respiration to include glycolysis, Krebs cycle, and electron transport chain (ETC). Understand that glycolysis is an almost universal pathway in cells and can yield ATP in anaerobic conditions. **53**
- ☐ 6 Describe the main inputs, outputs, and location of glycolysis, the Krebs cycle, and the electron transport chain, including ATP yield. **53**
- ☐ 7 PRAC Use a simple respirometer to investigate respiration rate, e.g. in germinating seeds, under different conditions. **54**







Anaerobic pathways

Key skills and knowledge

- ☐ 8 Describe the location, inputs, and outputs of anaerobic pathways for ATP generation in eukaryotes to include alcoholic fermentation in yeast and lactic acid fermentation in mammalian muscle. Contrast the electron acceptor in each case. **55**
- ☐ 9 Compare and explain ATP yield from fermentation and aerobic respiration. **55**
- ☐ 10 PRAC Investigate the effect of substrate type on rate of fermentation in yeast. **56**

Factors affecting cellular respiration

Key skills and knowledge

- ☐ 11 Describe factors affecting the rate of cellular respiration, including temperature, glucose availability, and oxygen concentration. **57**
- ☐ 12 PRAC Investigate the effect of temperature on aerobic respiration in yeast. Yeast respire aerobically when sugars are limited. Can you explain why? **57**

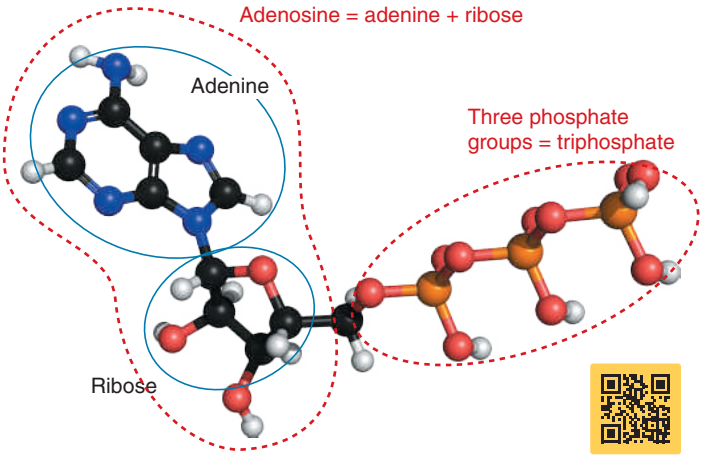
51 The Role of ATP in Cells

Key Idea: ATP transports chemical energy within the cell for use in metabolic processes.

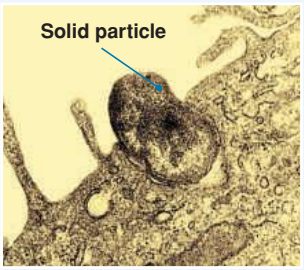
All organisms require energy to be able to perform the metabolic processes required for them to function and reproduce. This energy is obtained by cellular respiration, a set of metabolic reactions which ultimately convert biochemical energy from 'food' into the nucleotide **adenosine triphosphate** (ATP). ATP is considered to be a universal energy carrier, transferring chemical energy within the cell for use in metabolic processes such as biosynthesis, cell division, cell signalling, thermoregulation, cell mobility, and active transport of substances across membranes.

Adenosine triphosphate (ATP)

- The ATP molecule consists of three components; a purine base (**adenine**), a pentose sugar (**ribose**), and **three phosphate groups** which attach to the 5' carbon of the pentose sugar. Adenine + ribose form adenosine (the "A" in ATP). The structure of ATP is shown right.
- The bonds between the phosphate groups contain electrons in a high energy state which store a large amount of energy. The energy is released during ATP hydrolysis. Typically, hydrolysis is coupled to another cellular reaction to which the energy is transferred. The end products of the reaction are adenosine diphosphate (ADP) and an inorganic phosphate (Pi).
- Note that energy is released during the formation of bonds during the hydrolysis reaction, not the breaking of bonds between the phosphates (which requires energy input).



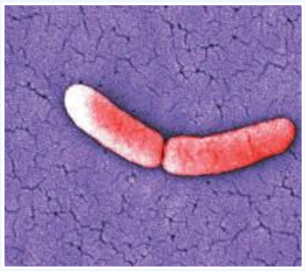
ATP powers metabolism



The energy released from the removal of a phosphate group of ATP is used for active transport of molecules and substances across the plasma membrane e.g. **phagocytosis** (above) and other active transport processes.



Mitosis, as seen in this stained onion cell, requires ATP to proceed. Formation of the mitotic spindle and chromosome separation both require the energy provided by ATP hydrolysis to occur.



ATP is required when bacteria divide by binary fission (above). For example, ATP is required in DNA replication and to synthesise components of the peptidoglycan cell wall.



Not all of the energy released in the oxidation of glucose is captured in ATP. The rest is lost as heat. This heat energy can be used to maintain body temperature. Thermoregulatory mechanisms such as shivering and sweating also use ATP.

1. What process produces ATP in a cell? _____

2. Identify the three distinct elements of the space filing model of ATP, labelled (a)-(c) below right:

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

3. Which two of the elements you labelled in question 2 make up adenosine? _____

4. Explain why thermoregulation requires the expenditure of energy:

   _____

   _____

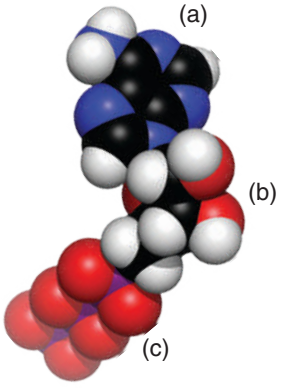
   _____

   _____

5. Describe one other process in a cell that requires ATP: _____

   _____

   _____



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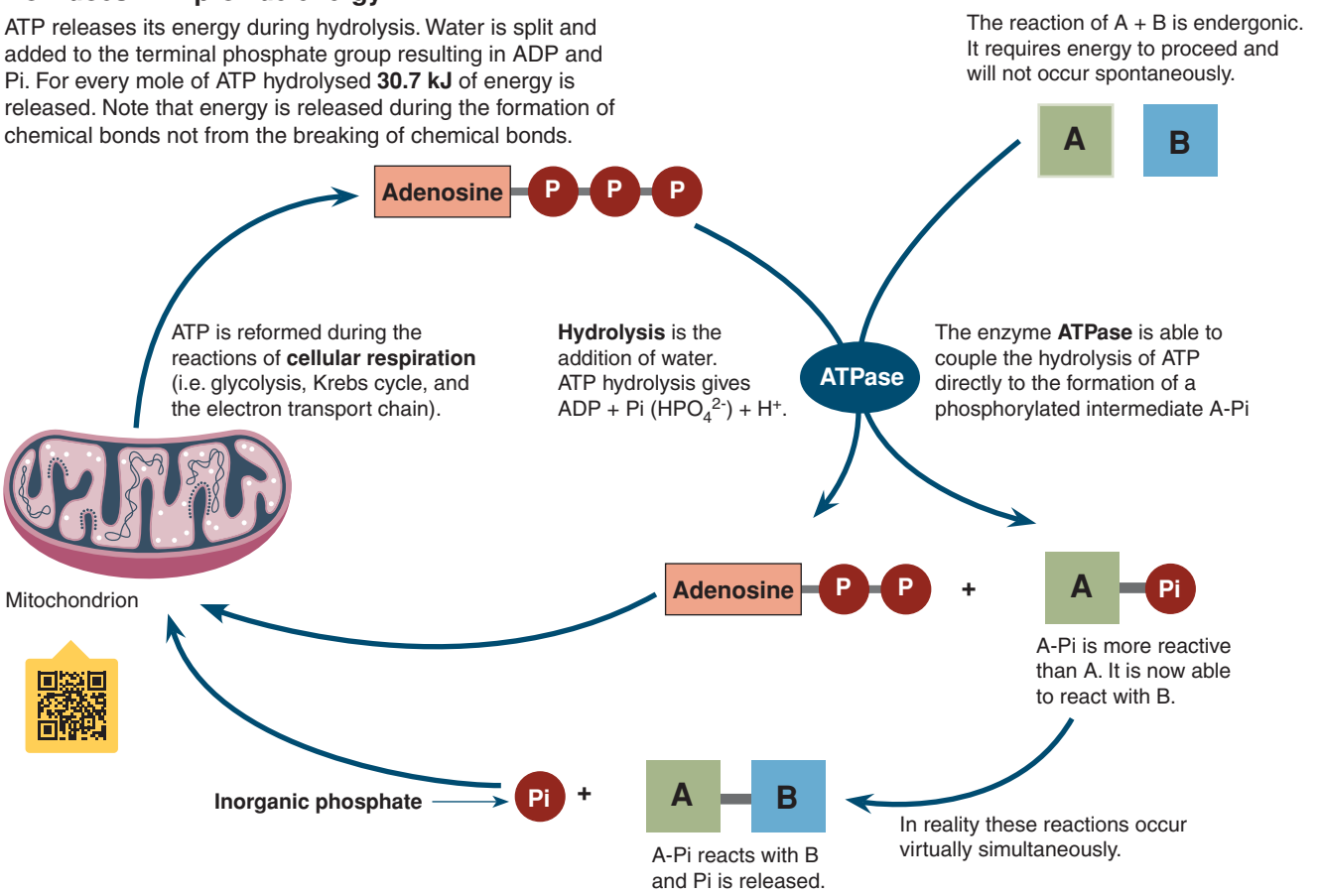
52 ATP and Energy

Key Idea: ATP is the universal energy carrier in cells. Energy is stored in the covalent bonds between phosphate groups.

The molecule ATP (adenosine triphosphate) is the universal energy carrier for the cell. ATP can release its energy quickly by hydrolysis of the terminal phosphate. This reaction is catalysed by the enzyme ATPase. Once ATP has released its energy, it becomes ADP (adenosine diphosphate), a low energy molecule that can be recharged by adding a phosphate. The energy to do this is supplied by the controlled breakdown of glucose in cellular respiration.

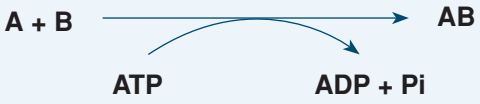
How does ATP provide energy?

ATP releases its energy during hydrolysis. Water is split and added to the terminal phosphate group resulting in ADP and Pi. For every mole of ATP hydrolysed **30.7 kJ** of energy is released. Note that energy is released during the formation of chemical bonds not from the breaking of chemical bonds.



Note! The phosphate bonds in ATP are often referred to as high energy bonds. This can be misleading. The bonds contain *electrons in a high energy state* (making the bonds themselves relatively weak). A small amount of energy is required to break the bonds, but when the intermediates recombine and form new chemical bonds a large amount of energy is released. The final product is less reactive than the original reactants.

In many textbooks the reaction series above is simplified and the intermediates are left out:



1. (a) How does ATP supply energy to power metabolism? _____

   _____

   _____

   (b) In what way is the ADP/ATP system like a rechargeable battery? _____

   _____

   _____

2. What respiratory substrate provides the energy for reforming ATP? _____

3. During the many metabolic reactions occurring in the body, most of the energy in the initial respiratory substrate is lost as heat. What is the purpose of this heat?

   _____

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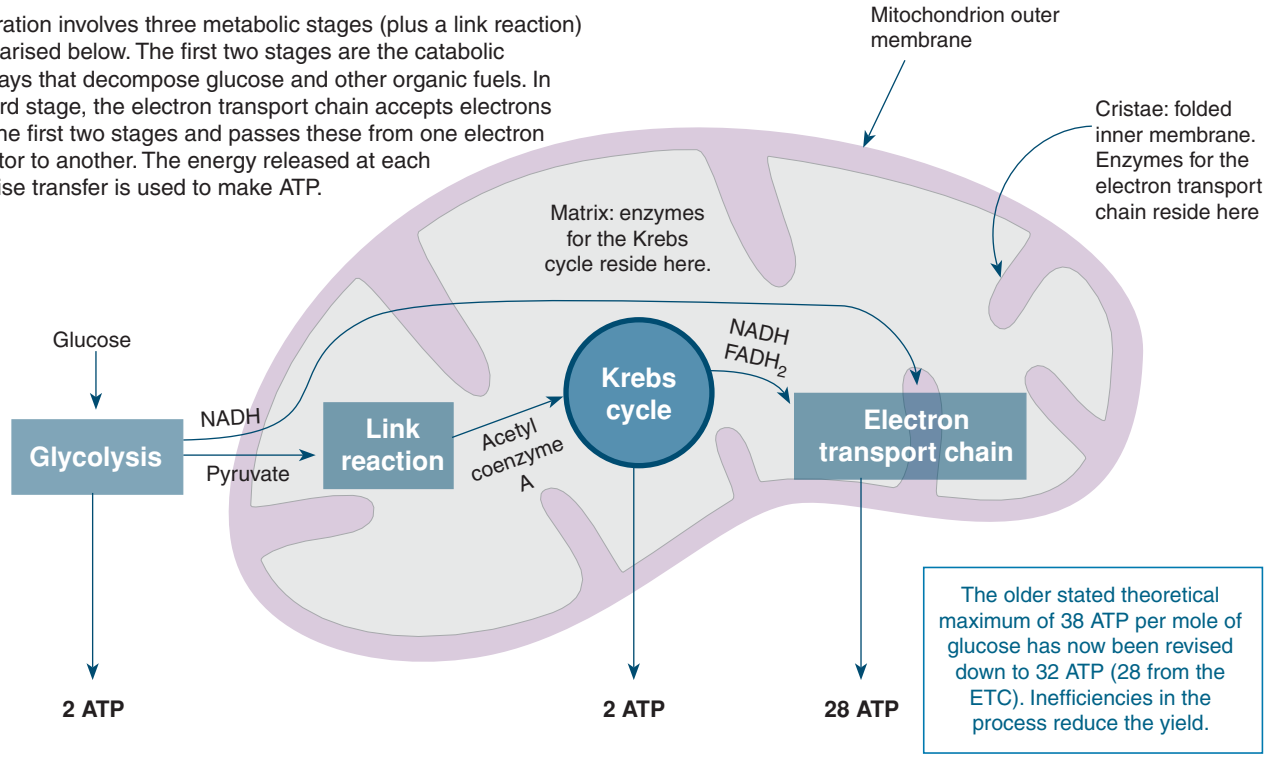
53 Cellular Respiration: Inputs and Outputs

Key Idea: During cellular respiration, the energy in glucose is transferred to ATP in a series of enzyme controlled steps.

The oxidation of glucose is a catabolic, energy yielding pathway. The breakdown of glucose and other organic fuels to simpler molecules is coupled to ATP synthesis. Glycolysis and the Krebs cycle supply electrons to the electron transport chain (ETC), which drives **oxidative phosphorylation**. The conversion of pyruvate (the end product of glycolysis) to **acetyl CoA** links glycolysis to the Krebs cycle. Most of the ATP generated in cellular respiration is produced by oxidative phosphorylation when NADH + H^+ and $FADH_2$ donate electrons to the electron carriers in the ETC. At the end of the chain, electrons are passed to molecular oxygen, reducing it to water. Electron transport is coupled to ATP synthesis.

Overview of cellular respiration

Respiration involves three metabolic stages (plus a link reaction) summarised below. The first two stages are the catabolic pathways that decompose glucose and other organic fuels. In the third stage, the electron transport chain accepts electrons from the first two stages and passes these from one electron acceptor to another. The energy released at each stepwise transfer is used to make ATP.



The older stated theoretical maximum of 38 ATP per mole of glucose has now been revised down to 32 ATP (28 from the ETC). Inefficiencies in the process reduce the yield.

The general equation for cellular respiration

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{energy}$$

1. Describe precisely in which part of the cell the following take place:

   (a) Glycolysis: _____

   (b) The link reaction: _____

   (c) Krebs cycle reactions: _____

   (d) Electron transport chain: _____

2. Write a word equation for the general equation for cellular respiration: _____

   _____

3. What is the total number of ATP produced from one glucose molecule? _____

4. Describe three functions of glycolysis in the process of cellular respiration: _____

   _____

   _____

5. Describe two functions of the Krebs cycle in the process of cellular respiration: _____

   _____

   _____

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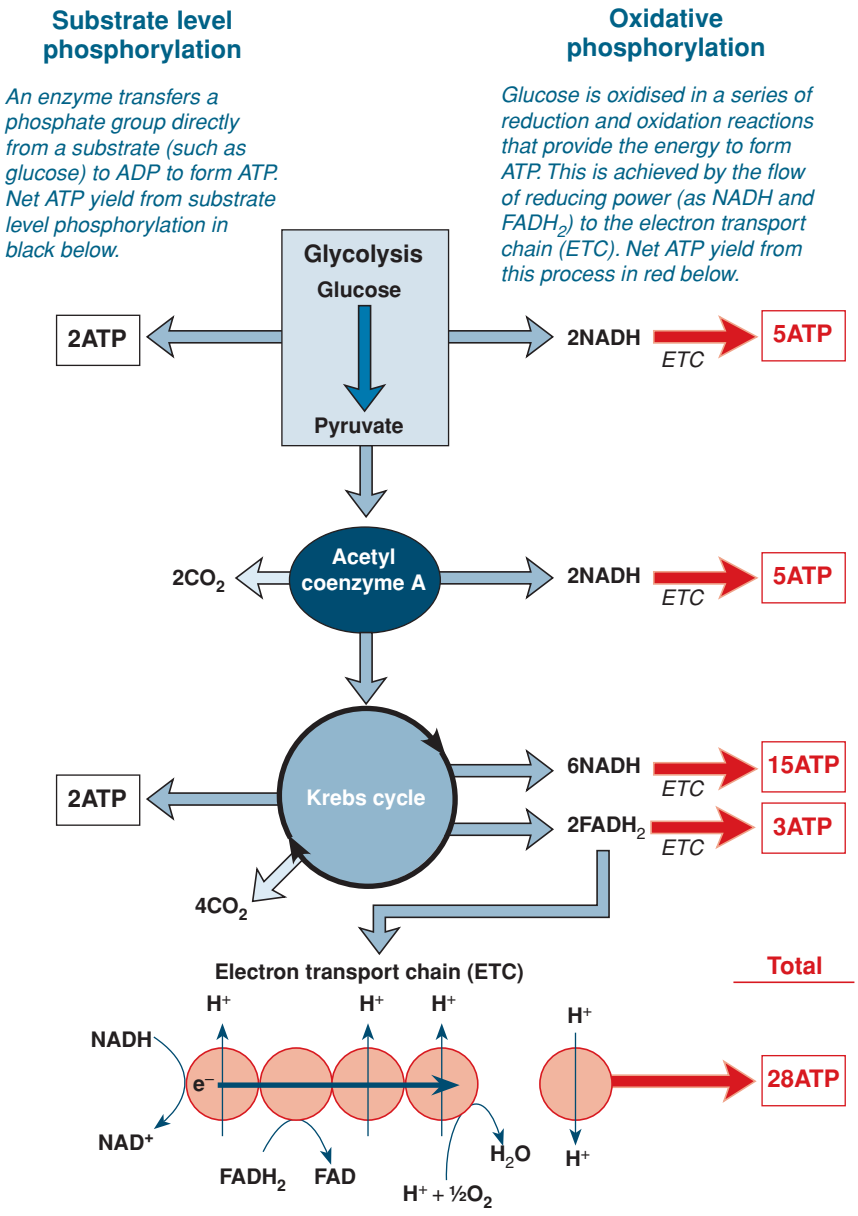
Steps in cellular respiration

Glycolysis
Glycolysis is the beginning of cellular respiration. It takes glucose and produces two pyruvate molecules, each of which can then enter the Krebs cycle. Glycolysis initially uses two ATP but produces four ATP. NADH is produced for use in the electron transport chain. **The numbers shown are for one glucose molecule.**

Link reaction
The link reaction removes CO_2 from pyruvate and adds coenzyme A, producing the 2C molecule acetyl coenzyme A, which enters the Krebs cycle. NADH is also produced and flows to the electron transport chain.

Krebs cycle
In the Krebs cycle, acetyl coenzyme A is attached to the 4C molecule oxaloacetate and coenzyme A is released. Oxaloacetate is eventually remade in a cyclic series of reactions that produce more NADH and $FADH_2$ for the electron transport chain. Two ATP are also made by substrate level phosphorylation.

Electron transport chain
Electrons carried by NADH and $FADH_2$ are passed to a series of electron carrier enzymes embedded in the inner membrane of the mitochondria. The energy from the electrons is used to pump H^+ ions across the inner membrane from the matrix into the intermembrane space. These are allowed to flow back to the matrix via the enzyme ATP synthase which uses their energy to produce ATP. The electrons are coupled to H^+ and oxygen at the end of the electron transport chain to form water.



6. (a) What is substrate level phosphorylation? _____

_____

(b) How many ATP are produced this way during cellular respiration (per molecule of glucose)? _____

7. (a) What is oxidative phosphorylation? _____

_____

(b) How many ATP are produced this way during cellular respiration (per molecule of glucose)? _____

8. Which parts of cellular respiration produce CO_2? _____

_____

9. Describe how ATP is produced in the electron transport chain: _____

_____

_____

_____

10. What is the purpose of NADH and $FADH_2$ in cellular respiration? _____

_____

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54 Measuring Respiration

Key Idea: Respiration is the process by which cells convert energy in glucose to usable energy, which is stored in the molecule ATP. The process uses oxygen, which can be quantified using a simple respirometer.

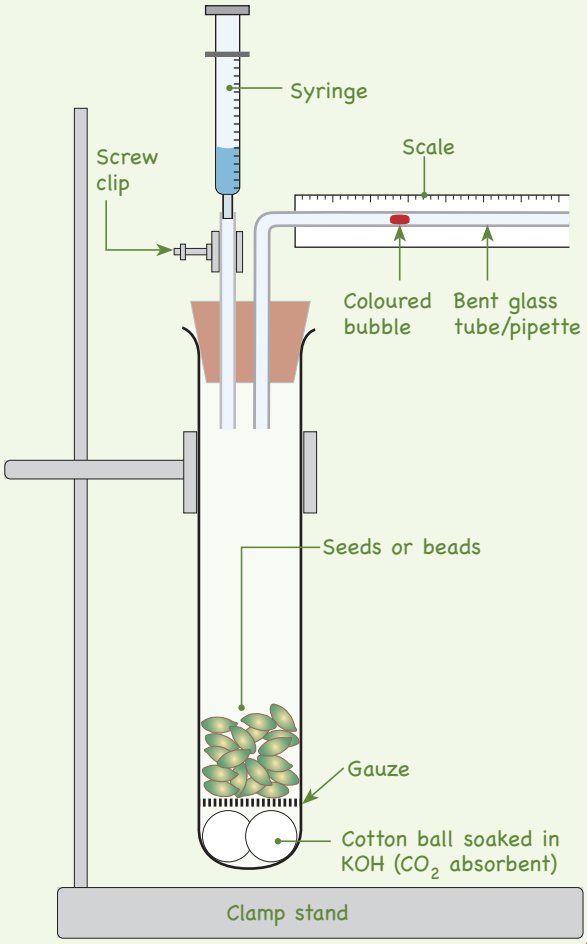
A respirometer can be used to measure the amount of oxygen consumed by an organism during cellular respiration and so can be used to measure respiration rate. A simple respirometer is shown in the diagram below. The carbon dioxide produced during respiration is absorbed by the potassium hydroxide. As the oxygen is used up, the coloured bubble in the glass tube moves. Measuring the movement of the bubble (e.g. with a ruler or taped graph paper) allows an estimation of the change in volume of gas and therefore the rate of cellular respiration.

Investigation 5.1 Measuring respiration in germinating seeds

See appendix for equipment list.

Caution is required when handling potassium hydroxide as it is caustic and can cause chemical burns. You should wear protective eyewear and gloves.

1. Work in groups of four to set up three respirometers using the set up shown right as a guide.
2. Collect three boiling tubes and place two cotton balls in the bottom of each. Label the tubes A, B, and C.
3. Use a dropper to add 15% potassium hydroxide (KOH) solution on the cotton balls until they are saturated (there should be no liquid in the boiling tube). Add the same amount of KOH to the cotton balls in each boiling tube.
4. Place gauze on top of the cotton balls in each tube. This prevents the KOH coming into contact with the seeds and killing them.
5. Quarter fill tube A with germinated bean seeds. These seeds will be damp because they have been germinated under damp paper towels for four days.
6. Quarter fill tube B with ungerminated (dry) seeds.
7. Quarter fill tube C with glass beads.
8. Place a two-hole stopper firmly in each boiling tube. In one hole insert a bent glass tube or bent pipette. In the second hole insert a tube that can be clamped shut using a screw clip.
9. Use a dropper or fine pipette to place a drop of coloured liquid into the bent tube/pipette of each set up. Attach a syringe to the clamped tube. Open the screw clip and use the syringe to draw the coloured bubble into the middle of the bent tube/pipette.
10. Place all three tubes in a water bath at 25°C. Secure them with a clamp stand or in racks.
11. Leave the apparatus to equilibrate for 10 minutes.
12. At the end of the equilibration period, close the screw clip on the boiling tubes. Mark the position of the bubble with a marker pen. This is your time zero position.
13. Start the timer.
14. Use a ruler or the pipette's scale (if there is one) to measure the distance the colored bubble moved at 5, 10, 15, 20, and 25 minutes.
15. Record your results on the table at the top of the next page.



CARE!

Respirometers of this sort are very sensitive to poor procedure because the volumes involved are so small.

Be very careful with your set-up and when taking readings. Have one person responsible taking the measurements of the bubble movement.

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Time (minutes)	Distance bubble moved (mm)		
	Germinated seeds (A)	Ungerminated seeds (B)	Glass beads (C)
0			
5			
10			
15			
20			
25			

1. What is the purpose of the test tube with the beads? _____

_____

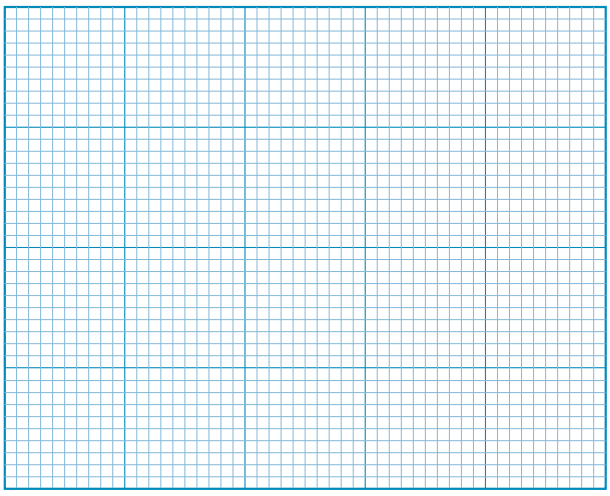
_____

2. (a) Calculate the corrected distance the bubble moved in tubes A and B by subtracting the distance moved in tube C from each value. Record these values in the table below.

   (b) Use the corrected distance the bubble moved to calculate the rate of respiration. Record this in the table below:

Time (minutes)	Corrected distance bubble moved (mm)		Rate (mm/min)	
	Germinated seeds (A)	Ungerminated seeds (B)	Germinated seeds (A)	Ungerminated seeds (B)
0				
5				
10				
15				
20				
25				

(c) Plot the rate of respiration on the grid (right). Include appropriate titles and axis labels:



(d) What does your plot show? _____

_____

_____

_____

_____

_____

_____

_____

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3. Why does the bubble in the capillary tube move? _____

4. What conclusion can you make about cellular respiration in germinated and ungerminated seeds?





5. How would you have to modify the experiment if you were measuring respiration in a plant instead of seeds?

6. Explain the purpose of the following in the experiment:

   (a) KOH: _____

   (b) Equilibration period: _____

   (c) The ungerminated seeds: _____

7. A student decided to repeat the respirometer experiment but used maggots instead of seeds. Their results are shown on the table (right).

   (a) Calculate the rates and record them in the table:

   (b) Graph the rates:

   (c) Describe the results: _____

Time (minutes)	Distance bubble moved (mm)	Rate (mm/min)
0	0	
5	25	
10	65	
15	95	
20	130	
25	160	

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55 Anaerobic Pathways

Key Idea: Glucose can be metabolised aerobically and anaerobically to produce ATP. The ATP yield from aerobic processes is higher than from anaerobic processes.

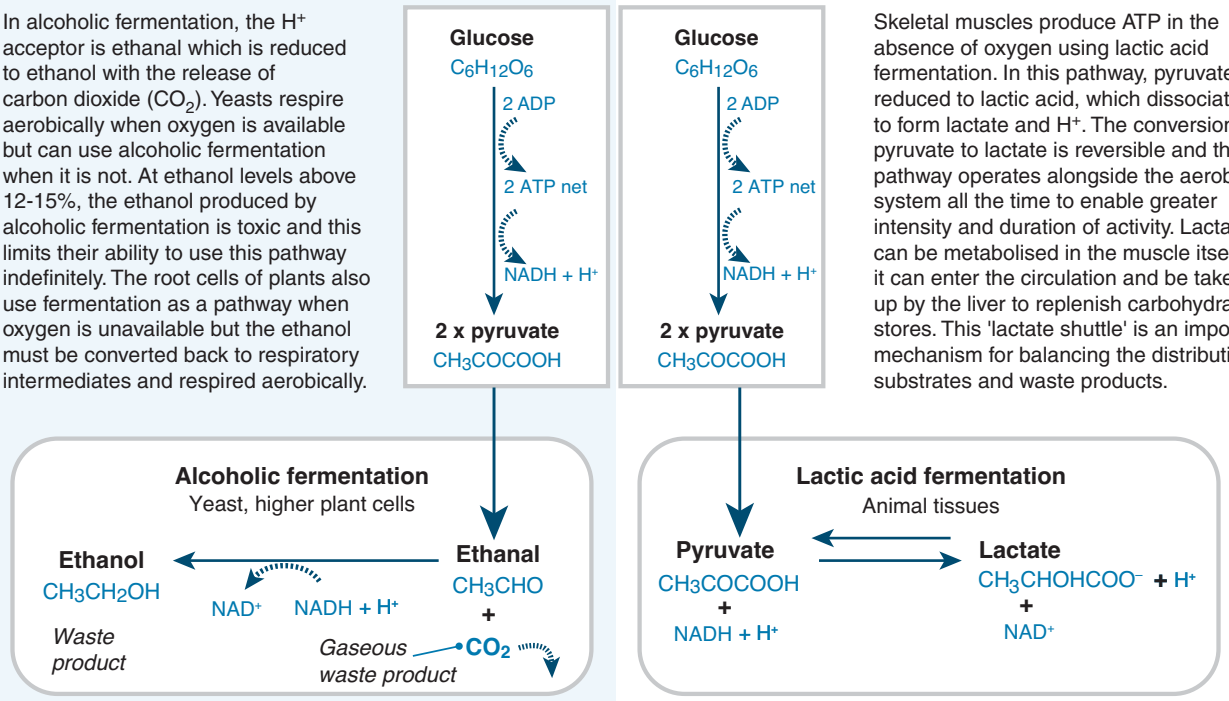
Aerobic respiration occurs in the presence of oxygen. Organisms can also generate ATP when oxygen is absent by using a molecule other than oxygen as the terminal electron acceptor for the pathway. In alcoholic fermentation in yeasts, the electron acceptor is ethanal. In lactic acid fermentation, which occurs in mammalian muscle even when oxygen is present, the electron acceptor is pyruvate itself.

Alcoholic fermentation

In alcoholic fermentation, the H^+ acceptor is ethanal which is reduced to ethanol with the release of carbon dioxide (CO_2). Yeasts respire aerobically when oxygen is available but can use alcoholic fermentation when it is not. At ethanol levels above 12-15%, the ethanol produced by alcoholic fermentation is toxic and this limits their ability to use this pathway indefinitely. The root cells of plants also use fermentation as a pathway when oxygen is unavailable but the ethanol must be converted back to respiratory intermediates and respired aerobically.

Lactic acid fermentation

Skeletal muscles produce ATP in the absence of oxygen using lactic acid fermentation. In this pathway, pyruvate is reduced to lactic acid, which dissociates to form lactate and H^+. The conversion of pyruvate to lactate is reversible and this pathway operates alongside the aerobic system all the time to enable greater intensity and duration of activity. Lactate can be metabolised in the muscle itself or it can enter the circulation and be taken up by the liver to replenish carbohydrate stores. This 'lactate shuttle' is an important mechanism for balancing the distribution of substrates and waste products.





The alcohol and CO_2 produced from alcoholic fermentation form the basis of the brewing and baking industries. In baking, the dough is left to ferment and the yeast metabolises sugars to produce ethanol and CO_2. The CO_2 causes the dough to rise.



Yeasts are used to produce almost all alcoholic beverages (e.g. wine and beers). The yeast used in the process breaks down the sugars into ethanol (alcohol) and CO_2. The alcohol produced is a metabolic by-product of fermentation by the yeast.



Andrea Braakhuis, Wintec

The lactate shuttle in vertebrate skeletal muscle works alongside the aerobic system to enable maximal muscle activity. Lactate moves from its site of production to regions within and outside the muscle (e.g. liver) where it can be respired aerobically.

1. Describe the key difference between aerobic respiration and fermentation: _____

_____

_____

2. (a) Refer to pages 91-92 and determine the efficiency of fermentation compared to aerobic respiration: _____%

(b) Why is the efficiency of these anaerobic pathways so low? _____

_____

3. Why can't alcoholic fermentation go on indefinitely? _____

_____

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56 Investigating Yeast Fermentation

Key Idea: Brewer's yeast preferentially uses alcoholic fermentation when there is excess sugar. The CO_2 released can be collected as a measure of fermentation rate.

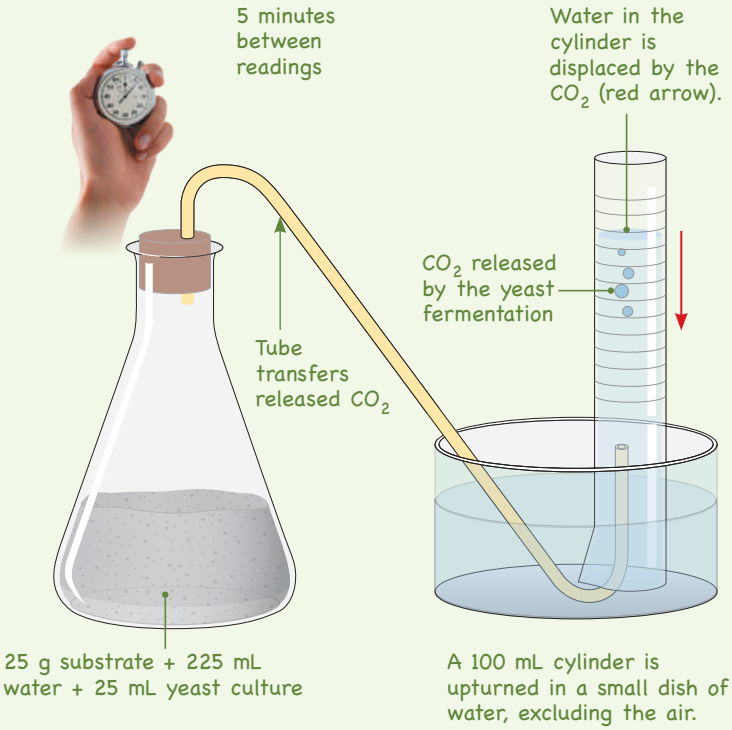
Brewer's yeast is a facultative anaerobe (meaning it can respire aerobically or use fermentation). One would expect glucose to be the preferred substrate, as it is the starting molecule in cellular respiration, but brewer's yeast can use a variety of sugars, including disaccharides (two unit sugars), which can be broken down into single units. The rate at which yeast (*Saccharomyces cerevisiae*) metabolises carbohydrate substrates is influenced by temperature, solution pH, and type of carbohydrate available. High levels of sugars suppress aerobic respiration in yeast, so yeast will preferentially use fermentation in the presence of excess substrate.

Investigation 5.2 Investigating fermentation in yeast

See appendix for equipment list.

Work in pairs for this activity. Your teacher will assign you a substrate to investigate.

1. Make a yeast culture by dissolving 10 g of active yeast into 50 mL of water at 24°C.
2. In a conical flask boil 225 mL of tap water then cool to room temperature (24°C). This removes any dissolved oxygen from the water.
3. Add 25 g of substrate (glucose, maltose, sucrose, lactose, or none). Stir carefully to dissolve (stirring too vigorously will cause oxygen to dissolve back into the water).
4. Then add 25 mL of the source yeast culture to the conical flask solution.
5. Add a thin layer of paraffin oil over the solution in the conical flask to create a anaerobic environment.
6. Stopper the conical flask and set up a measuring cylinder to capture any gas as in the diagram right.
7. Start timing and record the change in gas volume every five minutes for 1 hour. Record the results for your substrate in the table. Pool data as a class and use it to complete the table below.



Substrate / Time (min)	Cumulative volume of carbon dioxide collected (mL)				
	None	**Glucose**	**Maltose**	**Sucrose**	**Lactose**
0					
5					
10					
15					
20					
25					
30					
35					
40					
45					
50					
55					
60					

1. Write the equation for the fermentation of glucose by yeast:

_____

_____

2. Using the final values (60 minutes) collected from the class, calculate the rate of CO_2 production per minute for each substrate:

(a) None: _____

(b) Glucose: _____

(c) Maltose: _____

(d) Sucrose: _____

(e) Lactose: _____

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3. Use the tabulated data to plot an appropriate graph of the results on the grid provided:



4. Identify the independent variable: _____

5. (a) Summarise the results of the fermentation experiment: _____

_____

_____

_____

(b) Which substrate produced the most CO_2, and explain why: _____

_____

_____

_____

(c) Were fermentation rates lower on maltose and sucrose than on glucose? Was this what you expected? Suggest an explanation (you may have to do some research on these molecules to find out the answer):

_____

_____

_____

(d) Did any substrate produce no CO_2? Can you suggest why? _____

_____

6. Predict what would happen to CO_2 production rates if the yeast cells were respiring aerobically: _____

_____

_____

_____

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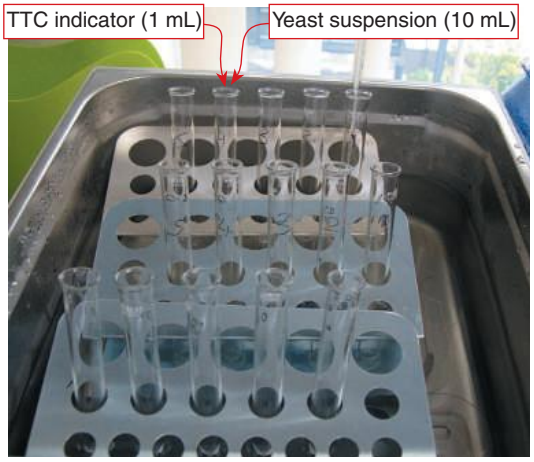
57 Factors Affecting Cellular Respiration

Key Idea: Glucose availability, oxygen concentration, and temperature are all factors affecting the rate of respiration.

Aerobic cellular respiration is a series of chemical reactions catalysed by enzymes. The overall equation for all these reactions is $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$. It then follows that like any chemical reaction the rate of the reaction can be affected by the concentration of the reactants (glucose and oxygen) and environmental factors such as temperature.

The effect of temperature on dehydrogenase activity

- During cellular respiration, dehydrogenase enzymes remove hydrogens from glucose and pass them to hydrogen acceptors. The indicator triphenyl tetrazolium chloride (TTC) is a colourless hydrogen acceptor and intercepts the H^+ produced during respiration.
- TTC turns red when reduced (accepts H^+) so the rate of colour change of TTC can be used to indicate the rate of respiration of cells in a test tube. To quantify the colour change, samples are placed in a colorimeter and the light absorbance measured.
- The effect of temperature on the respiration rate of yeast cells was investigated using the TTC indicator method. Three test tubes of a yeast cell suspension (10 g yeast in 1.5 g/L glucose) containing TTC were placed in water baths at each of 25°C, 40°C, and 55°C. At this concentration of glucose and in the presence of oxygen, yeast will respire aerobically. Absorbances at 1.5, 3.0, and 4.5 hours are tabulated below. A yeast-glucose control at 25°C included no TTC. This is a positive control because it is used to validate the procedure (yeast will grow).



The Science Floor (via Flickr), Tre Briercliffe with permission

	Tube number (absorbance)									
	25°C			40°C			55°C			Control
Time (hr)	1	2	3	4	5	6	7	8	9	10
1.5	1.13	1.02	1.20	2.34	2.33	2.29	4.11	4.05	4.17	0.40
3.0	1.96	1.88	2.04	5.85	5.89	5.80	8.86	8.90	8.82	0.51
4.5	2.76	2.69	2.81	7.84	7.88	7.80	9.77	9. 87	9.74	0.62

1. Calculate the mean absorbance for each of the times and temperatures and enter the values in the table below:

	Mean absorbance		
Time (hr)	25°C	40°C	55°C
1.5			
3.0			
4.5			

2. Use the table in (1) to plot a graph of absorbance over time for the three temperatures measured:

3. (a) Why did the absorbance of the control tube change?

_____

_____

(b) How could the control have been improved?

_____

_____

4. How does temperature affect the respiration rate of yeast? _____

_____

5. What does you think would happen if the experiment was done at 70°C? _____

_____

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The effect of glucose and oxygen concentrations on cellular respiration

- Glucose and oxygen are the two key reactants in respiration: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$

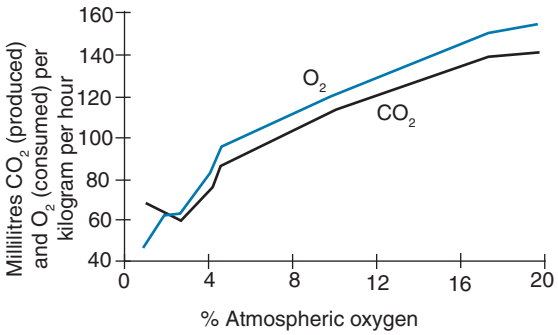
6. Given no-limiting oxygen, what would be the effect of increasing the concentration of glucose on the rate of respiration?

7. Given non-limiting glucose, what would be the effect of increasing the concentration of oxygen on the rate of respiration?

8. These effects tend to flatten out or plateau at higher concentrations of glucose and oxygen. Why might this be?

9. If the concentration of oxygen became very low, how would you expect the cell to deal with this?

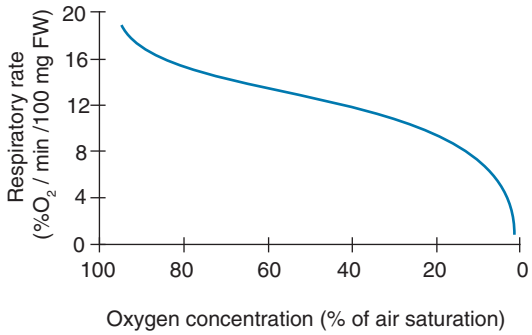
10. Study the graph below:



(a) As the percentage of atmospheric oxygen increases, what happens to the production of carbon dioxide?

(b) As the percentage of atmospheric oxygen increases, what happens to the consumption of oxygen?

11. A study of respiration in pea roots sealed the roots within an electrode with atmospheric oxygen to study the effect of reducing oxygen availability on respiration rate.



(a) What is the relationship between atmospheric oxygen and respiration rate?

(b) Why was percentage of oxygen reducing over the course of the experiment?

(c) Would would giving the plant more glucose have had an effect on the outcome of the experiment? Why?

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58 Chapter Review: Did You Get It?

1. Match each term to its definition, as identified by its preceding letter code.

alcoholic fermentation .....

electron transport chain .....

glycolysis .....

Krebs cycle .....

lactic acid fermentation .....

link reaction .....

mitochondria .....

A A series of reactions that converts glucose into pyruvate. The energy released is used to produce ATP.

B Also known as the citric acid cycle. Part of a metabolic pathway involved in the chemical conversion of carbohydrates, fats and proteins to CO_2 and water to generate a form of usable energy (ATP).

C Organelles responsible for producing the cell's ATP. They appear oval in shape with an outer double membrane and a convoluted interior membrane.

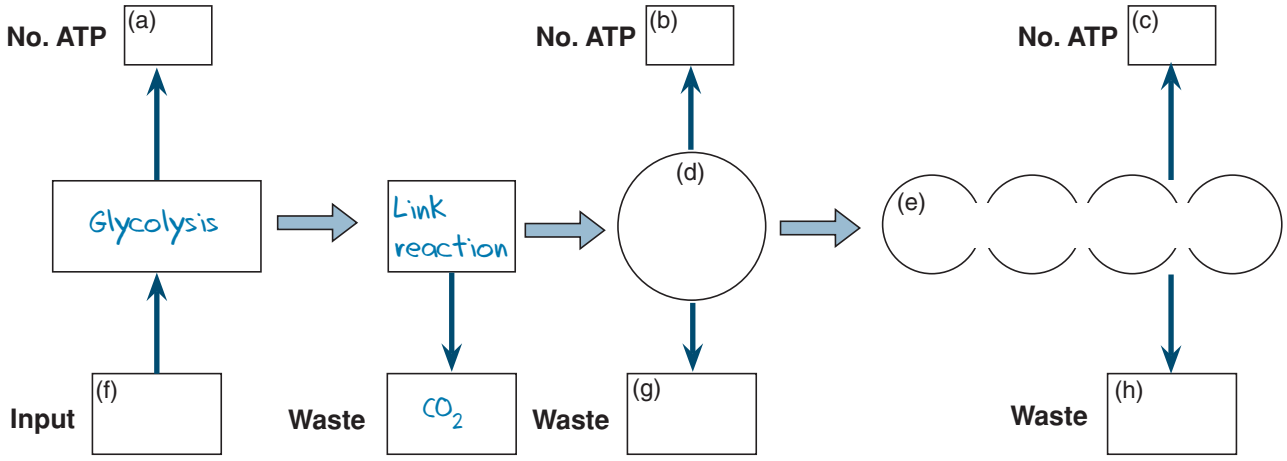
D The stage in cellular respiration where pyruvate enters the mitochondrion and carbon dioxide is removed.

E An anaerobic pathway where ethanal acts as the electron acceptor and the end product is ethanol.

F Chain of enzyme-based redox reactions, which passes electrons from high to low redox potentials. The energy released is used to pump protons across a membrane and produce ATP.

G An anaerobic pathway occurring in the skeletal muscle of mammals. Pyruvate is reduced to lactic acid.

2. Complete the diagram of cellular respiration below by filling in the boxes below:

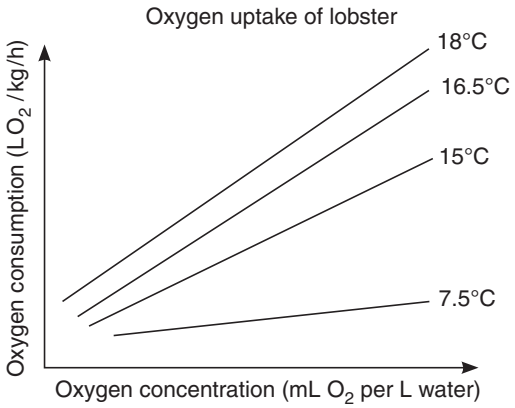


3. (a) Where does glycolysis occur in the cell? _____

(b) Where does the Krebs cycle occur in the cell? _____

(c) Where is the electron transport chain located in a cell? _____

4. Study the graph of oxygen consumption of lobsters, right.



(a) What happens to the rate of oxygen consumption as oxygen concentration increases?

_____

_____

(b) What happens to the rate of oxygen consumption as temperature increases?

_____

(c) Give a reason for the relationship between the rate of oxygen consumption and water temperature in lobsters.

_____

_____

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CHAPTER 6

Applications of Biochemical Pathways

Uses and applications of CRISPR-Cas9 technology

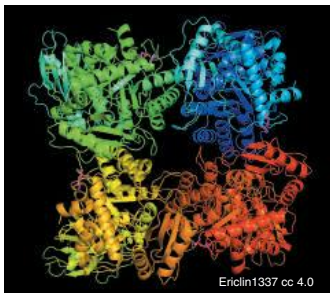
Key skills and knowledge

Activity number

Key terms

anaerobic fermentation
CRISPR-Cas9
biofuel
biogas
in-vitro
in-vivo

- ☐ 1 Recall how the CRISPR-Cas9 complex is used to make specific targeted changes to genomes. Recall its potential applications, including in genetic engineering. **24**
- ☐ 2 Describe how CRISPR-cas9 technologies can be used to modify plant genomes. What types of genes are targeted and why? Distinguish between *in-vivo* and *in-vitro* formation of a CRISPR-Cas9 complex to modify plant genomes. **59**
- ☐ 3 Describe the potential uses and applications of CRISPR-Cas9 technologies in improving photosynthetic efficiencies and crop yields. Which crop species are likely candidates for CRISPR-Cas9 modification and why? How feasible are these genetic modifications in terms of their widespread application? **59**







Applications of anaerobic fermentation

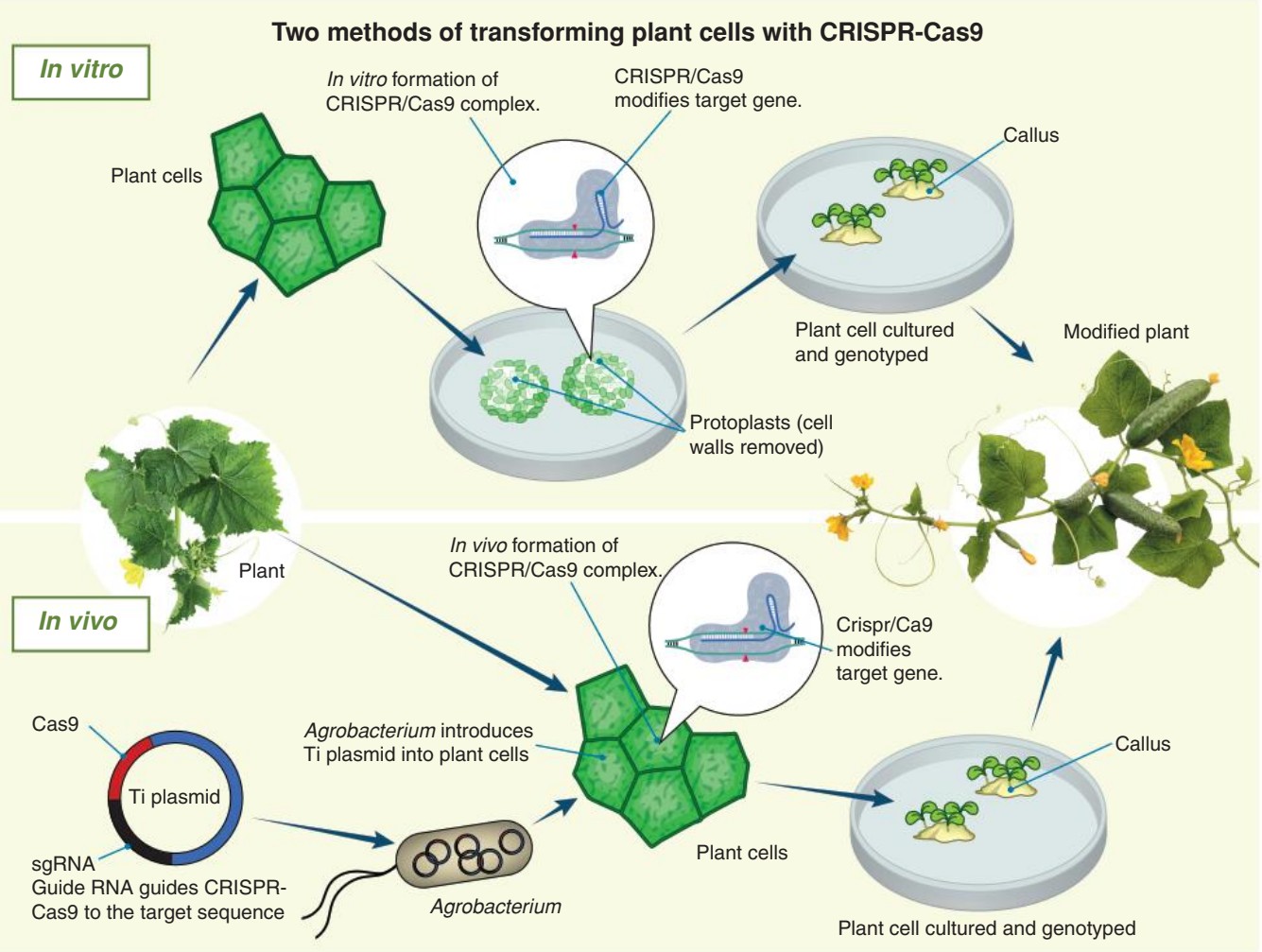
Key skills and knowledge

- ☐ 4 Recall how microorganisms such as yeasts utilise carbohydrate sources using an anaerobic pathway called fermentation. What the final electron acceptor in the absence of oxygen and what is the product of this fermentation? **60**
- ☐ 5 Describe the uses and applications of anaerobic fermentation of biomass for biofuel production. Do you think biofuel is carbon neutral? Why or why not? Suggest why the uptake of biofuels in Australia is low and variable across the country. **60**
- ☐ 6 What other fuel is produced using anaerobic fermentation (anaerobic digestion) and what are its uses? **60**
- ☐ 7 **TEST** Design a methodology to use genetic engineering to increase food security. Analyse and evaluate GE as a solution to global food security issues. **61**

59 Improving Productivity Using Technology

Key Idea: Increasing productivity by improving photosynthetic efficiency could help to increase food and fuel production.

The CRISPR-Cas9 tool is finding increasing use in crop science. Plant genes can be strategically edited with specific goals relating to crop production. These efforts have focussed on different aspects of plant physiology and the approach depends on the crop type and the goal. Universal goals include improving disease resistance or increasing the rate of photosynthesis. Others are targeted to specific plants, such as faster ripening in fruit, removing caffeine from coffee beans, or decreasing the trans-fat content of soy. Some gene edits are relatively simple, involving a single gene, but others are more difficult as they require insertion of multiple genes to modify or build new metabolic pathways.



- There are two common ways to edit a plant genome with CRISPR/Cas 9: *in vitro* (CRISPR-Cas9 complex formed outside the cell) and *in vivo* (CRISPR-Cas9 complex formed inside the cell). The *in vitro* method (upper schematic) inserts the CRISPR-Cas9 complex directly into plant protoplasts (cells without cell walls). The cell DNA is then edited directly by the inserted complex.
- The *in vivo* method (lower schematic) uses *Agrobacterium* to deliver a modified Ti plasmid into the plant cells. The Ti plasmid inserts the genes required to introduce CRISPR-Cas9 into the plant DNA. The cell's machinery then produces the CRISPR-Cas9 protein and RNA, which then edits the cell's DNA.

Genes and traits targeted by CRISPR-Cas9

- Crop science has targeted many different plant traits. Each is targeted to enhance some biological aspect that will improve the crop, save energy in growing it, or have it last longer once harvested.

Crop species	Target gene	Target trait
Rice	Gn1a, GS3, DEP1	Enhanced grain number, larger grain size, dense erect panicles (grain flower clusters)
Rice	OsSWEET13	Bacterial blight resistance
Wheat	GW2	Increased grain weight and protein content
Maize, potato	Wx1	High amylopectin content
Cucumber	eIF4E	Virus resistance
Mushroom	PPO	Anti-browning phenotype
Tomato	SP5G	Earlier harvest time
Soybean	ALS	Herbicide resistance



Rice is a commonly engineered crop

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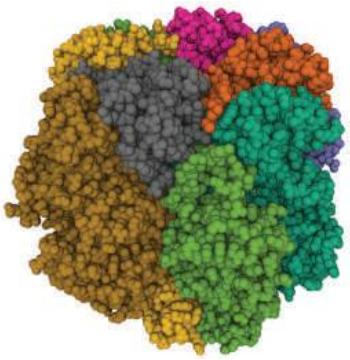
Improving photosynthesis

- A major focus in gene editing and genetic modification is improving the efficiency of photosynthesis. This may involve targeting the photosynthetic pathway or often on RuBisCo. RuBisCo catalyses carbon fixation in the first step of the Calvin cycle, but is very inefficient.
- RuBisCo consists of two types of protein subunit, called the large chain and the small chain. The functional enzyme is made of eight large subunits (L) and eight small subunits (S). The genes encoding these subunits are the target for modification by CRISPR-Cas9 complexes.
- Proof of concept was shown when CRISPR-Cas9 was used to knock out the genes encoding the RuBisCo small subunit in model tobacco plants. The plants showed a 93% decrease in RuBisCo content and accumulated only 10% of the biomass of untransformed plants.
- This showed that the CRISPR-Cas9 complex was able to target the correct genes and could later possibly be used to insert genes that may improve RuBisCo's efficiency.

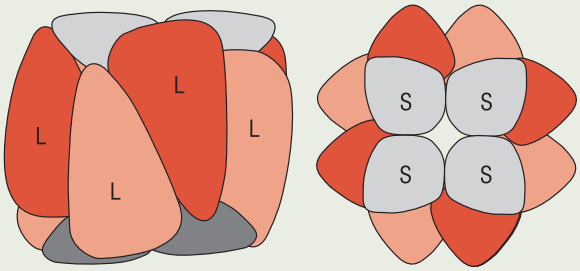
Further prospects for improving photosynthesis and productivity

- Improving the catalytic activity of RuBisCo.
- Increasing the amount of RuBisCo in the leaf.
- Enhancing the CO_2 concentration around Rubisco by introducing the C_4 pathway into C_3 plants.
- Improving RuBisCo's affinity for CO_2.
- Enhancing chloroplast electron transport rate.
- Increasing the thermostability of RuBisCo activase, an enzyme involved in activating RuBisCo.
- Insertion of genes from more efficient plants.

1RXO, Activated Spinach Rubisco, Taylor T.C, Mol*



RuBisCo space filling model. Large subunits in ochre, greens, and purple. Small subunits in grey orange, pink, and gold.



Schematic structure of RuBisCo showing the 8 large (L) and 8 small (S) subunits. The large sub-units carry the catalytic sites. The small sub-units, are necessary for its function and have a regulatory role.

1. Suggest why it has become very important for researchers to look at ways of boosting crop productivity? _____

2. Describe the two common ways of using CRISPR for gene editing in plants:

   (a) _____

   (b) _____

3. White button mushrooms have had the PPO gene edited to reduce the amount of polyphenol oxidase, which turns food brown when exposed to air (it is also present in apples). How might editing this gene reduce food wastage?

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60 Applications of Anaerobic Fermentation

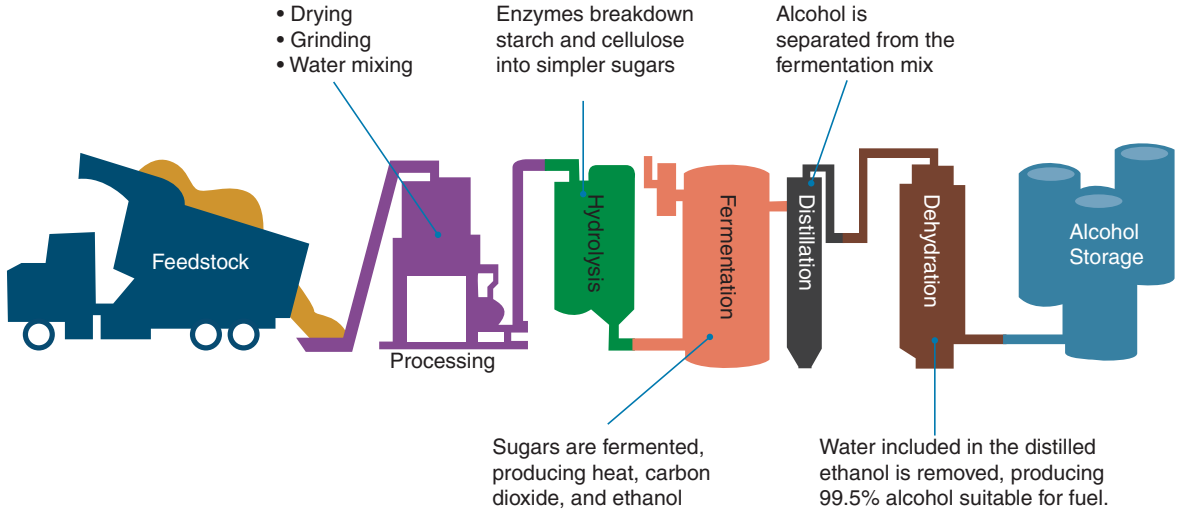
Key Idea: Anaerobic fermentation can be used to produce fuels that can be used as alternatives for fossil fuels.

Biofuels are any fuels derived from biological materials. They have been used since humans began burning wood. Modern biofuels are mostly based around microbial fermentation of carbohydrate sources (e.g. corn) to produce ethanol and methane. It has been hoped biofuels might be able to provide a carbon neutral fuel source, with any CO_2 produced by burning the fuel being removed by the production of plant biomass, which will then be used to produce more biofuel.

Alcoholic fermentation produces ethanol for biofuel

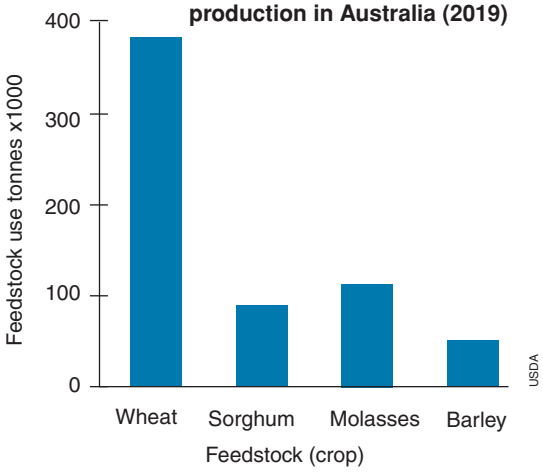
- Ethanol is an important industrial chemical. It has properties that make it useful in both food production and industry. Ethanol has been proposed as a replacement for fossil fuels such as gasoline because it burns well as a fuel, it can be stored easily, it is easy to produce in large quantities, and it can be produced from plant material.

Producing ethanol from feedstock



- In Australia, consumption of ethanol as biofuel is relatively low. Consumption in 2020 was around 220 million litres, down from around 239 million litres in 2019. Biofuel production capacity has not increased since 2010.
- All biofuels (including bioethanol, and biodiesel) contributed to only 0.5% of transport fuel energy in 2017. Blended gasoline (E10 gasoline containing 10% ethanol) use has declined over recent years to 1.4% of gasoline use in 2019.
- New South Wales and Queensland are the only two states in Australia that mandate ethanol blended fuel. Not surprisingly, they have the highest consumption of ethanol blended fuel. However, their use of ethanol blended fuel is well below the mandated target.
- Victoria is the only other state in Australia that sells ethanol blended fuel. Its consumption is just 0.7 percent of gasoline sales.
- Ethanol is often promoted as a carbon neutral fuel. However it requires large amounts of fuel to grow, harvest, transport, and distill the crop. Ethanol also contains only two thirds the energy of the equivalent volume of gasoline.

Feedstock use for ethanol production in Australia (2019)



Sales of gasoline and ethanol fuel for motor vehicle use in Australia, 2019

Fuel use (millions of litres)						
Gasoline type	**Premium (96-97)**	**Premium (98+)**	**Regular (<95)**	**Ethanol blended fuel (10% ETOH)**	**Total**	**Ethanol content (of fuel total)**
New South Wales	1045.9	1350.8	1773.5	1400.6	5570.8	2.51%
Queensland	361.0	579.3	1972.2	649.3	3561.8	1.82%
Victoria	400.6	738.3	3248.6	344.1	4731.6	0.73%
South Australia	87.9	140.5	941.1	0.0	1169.5	0.0%
Western Australia	215.4	241.1	1359.2	0.0	1815.7	0.0%
Tasmania	46.7	3.1	324.3	0.0	374.1	0.0%
Northern Territory	21.6	0.6	102.3	0.0	124.5	0.0%
Totals	2178.9	3054.3	9721.6	2393.5	17,348.3	1.38%

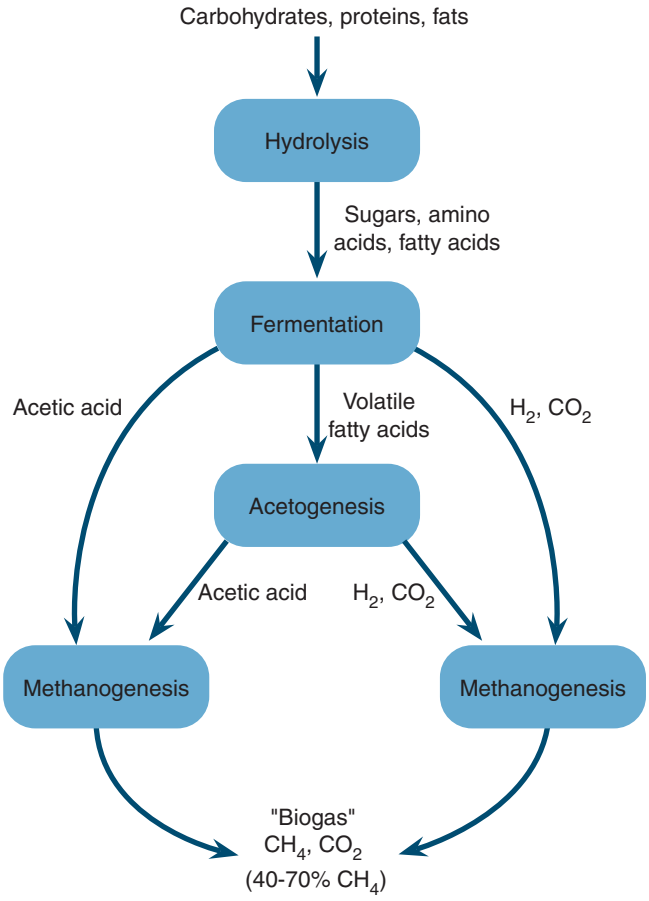
USDA

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Producing methane

- Ethanol is not the only fuel that can be produced by anaerobic fermentation. Hydrogen gas is produced by a different anaerobic pathway. Methane producing bacteria combine hydrogen and carbon dioxide to produce methane (a process called methanogenesis). The methane can then be used as a high energy fuel and as a starting product for the synthesis of various chemicals.
- The process of producing methane from biological material is often called anaerobic digestion.
- Biogas can be produced from the same material as ethanol, but it can also be produced from material that is not suitable for ethanol production, such as sewage and manure.





1. Why is ethanol a useful industrial chemical? _____

2. Which is the most used crop for ethanol production in Australia? _____

3. Which Australian state uses the most blended ethanol fuel? _____

4. What is the total percentage of ethanol in Australian gasoline fuel use? _____

5. Ethanol is often promoted as a carbon neutral fuel. Why and in what way might this not be entirely true?

6. Why might biogas production be useful in the disposal of sewage at a local level? _____

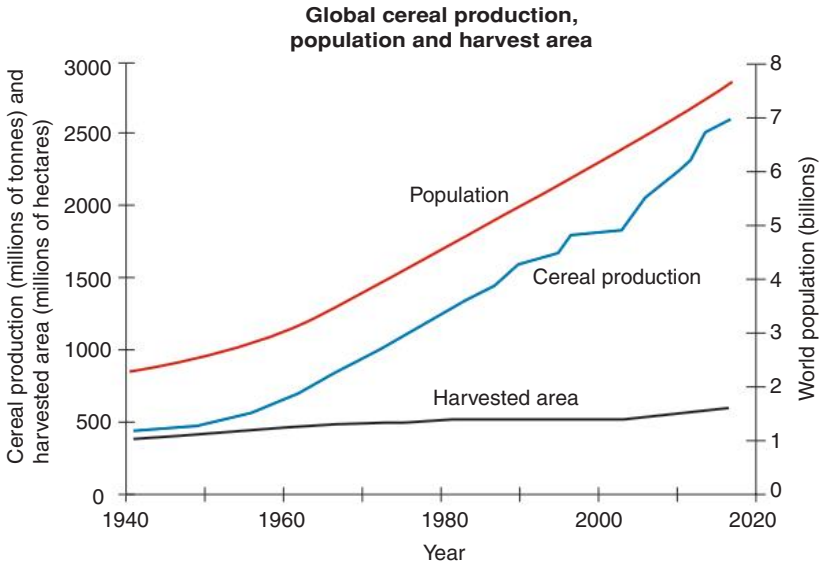
7. The energy density of gasoline is 34.4 megajoules per litre (MJ/L). The energy density of E10 (ethanol blended gasoline) is 33.2 MJ/L. How might this affect E10's use as a fuel in a car compared to regular gasoline?

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61 Food Security

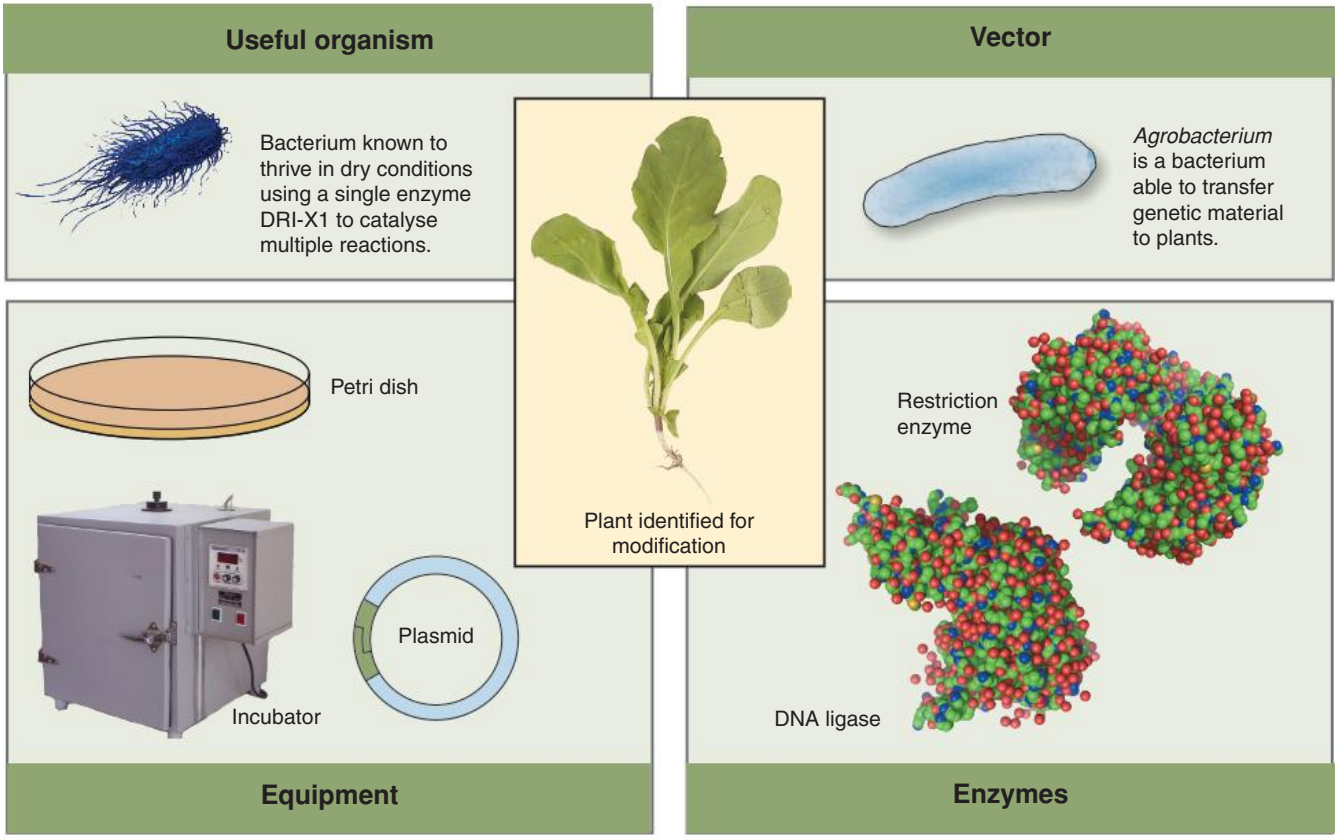
Assessment Task, Outcome 2: *Analysis and evaluation of a contemporary bioethical issue*

- By 2050 the global population will reach over 9 billion people. Food supplies will need to increase by at least 50% to support this population growth. The solution to the problem of food production is complicated. Most of the Earth's arable land has already been developed and currently uses 37% of the Earth's land area, leaving little room to grow more crops or farm more animals.
- Development of new fast growing and high yield crops appears to be part of the solution, but many crops can only be grown under a narrow range of conditions or they are susceptible to disease. Moreover, the farming and irrigation of some areas is difficult, costly, and can be environmentally damaging. Genetic modification of plants may help to solve some of these looming problems by producing plants that will require less intensive cultivation or that will grow in areas previously considered not arable.
- In the 1950s, the world population was about 2.5 billion. During the 1950s and 60s a series of improvements in plant breeding and crop development (known as the Green Revolution) greatly increased the food produced without increasing the land needed.
- However the world's population has continued to increase to the point where per capita food production is beginning to fall, despite increased harvests. Any increase in food security produced by the Green Revolution has effectively gone. A new "Gene Revolution" is needed to reestablish food security.
- Without a new increase in crop yield a greater harvest area will be needed. This can only come from the removal of wild lands such as forests.



Engineering a solution

- A solution to the possible future food crisis is to genetically engineer food crops to maximise growth under adverse conditions. Standard selective breeding techniques could be used to do this, but in some plants this may not be possible or feasible and it may require more time than is available. A selection of genetic tools and organisms with useful characteristics are described.
- Your task is to use the items shown to devise a technique to successfully create a plant that could be successfully farmed in semi-desert environments such as sub-Saharan Africa or parts of the Australian Outback. Answer the questions on the following page first and use them to help you to produce a complete outline of your technique on a separate sheet. Attach it to this page.



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1. Describe a process to isolate the required gene, insert it into the vector, and then finally into the target plant:

2. How would the modified plant strains be grown up and identified?

3. Many people are against GM crops. How might you convince these people that your new GM crop will benefit us all?

Increasing rice production



- Rice is currently a staple food for about 50% of the world's population. Increasing its supply will be a major step forward in improving global food security as the global population continues to grow.
- Rice is a C_3 plant. As such, its photosynthetic pathway is not especially efficient. C_4 plants make up just a few percent of plant species but they account for nearly 25% of global plant productivity. This is due to their more efficient photosynthetic pathway and under tropical conditions they out compete C_3 plants. Corn is an example of a C_4 plant.
- For several years, an international group of scientists have been researching how to genetically engineer rice to make it into a plant with C_4 photosynthetic pathways. This meant inserting the relevant genes from maize into rice. In 2020, they succeeded in creating a gene construct for five enzymes and having it expressed in transgenic rice plants.
- There are still many years to go before the project will be completed but the hope is that the final C_4 rice will have a 50% greater yield than rice available today, using the same area of land and less water than current varieties.
- However, already there are concerns being raised about C_4 rice. Most genetically engineered plant crops to date have lower fitness than wild or unmodified crops (they only survive with the help of human input). If they escape into the wild or cross pollinate with other plants the offspring are likely to be out-competed by other plants. However, in the case of rice, the C_4 pathways could give genetically engineered rice a marked advantage over its unmodified C_3 ancestor. Were it to escape into the wild or cross pollinate there is a possibility it could out-compete other plants in some situations and change the environment.

4. How might C_4 rice aid food security in the near to mid future?

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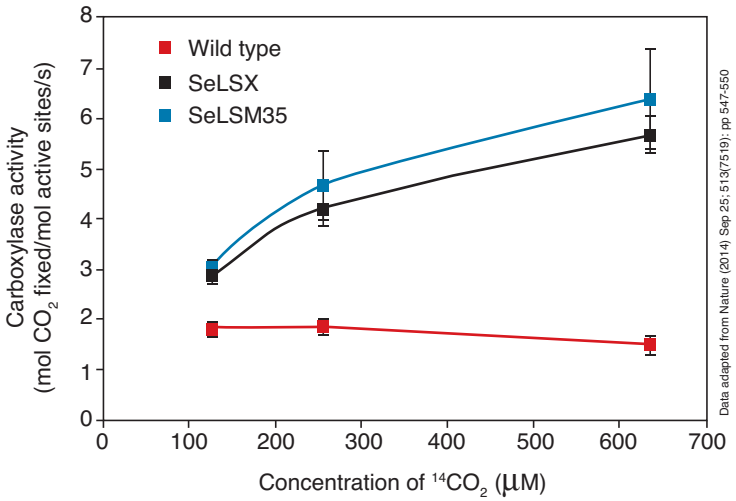
62 Chapter Review: Did You Get It?

1. Researchers have investigated a number of ways to improve productivity, including the genetic modification of Rubisco. Cyanobacteria have a CO_2 concentrating mechanism (CCM), which allows them to produce a CO_2 rich environment around Rubisco. This improves photosynthetic productivity because there is less oxygen reacting with Rubisco.

   Two genes (SeLSX and SeLSM35) from the cyanobacterium *Synechococcus elongatus* PCC7942 were transplanted into the chloroplast DNA of the tobacco plant.

   The graph on the right shows the carbon fixation results of the two modified tobacco plants compared against the wild type.

Mean carboxylase activity (± standard deviation) as a measure of carbon dioxide fixation in tobacco plants.



1. Why has it become important for researchers to look at ways of boosting photosynthesis productivity? _____

2. (a) How do cyanobacteria improve their photosynthetic activity? _____

   (b) Describe the effect of the transplanted cyanobacteria genes on Rubisco activity in tobacco plant: _____

3. Increasing plant productivity is not just about increasing the rate or amount of photosynthesis. Identify two traits that could be modified to improve plant productivity and explain why these would improve plant productivity:

4. Explain how biofuels can help in reducing wastes and dependence on fossil fuels: _____

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63 Synoptic Questions: Unit 3, Area of Study 2

1. Aerobic cellular respiration is essential for providing usable energy for cells. Outline this process in eukaryotes. Your discussion should include reference to the purpose of aerobic cellular respiration, the location of the main stages, and the raw material(s) and final product(s):

2. Photosynthesis is a process by which autotrophs such as green plants use light to produce carbohydrate molecules. Outline the process of photosynthesis in plants and explain how its rate is affected by specific environmental factors (you may use extra paper if required). Your discussion should include reference to the location of photosynthesis in plant cells, the raw material(s) and final product(s), and factors that affect photosynthetic rate.

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3. Enzymes are essential for catalysing biological reactions.

   (a) Describe the induced fit model of enzyme action: _____

   (b) Draw a diagram in the space below to show how a metabolic pathway can be regulated by feedback inhibition:

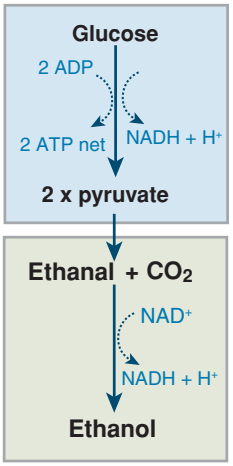
4. What is the role of RuBisCo in photosynthesis? Describe the adaptations of plants to enhance its role in photosynthesis:

5. Describe some of the factors that affect photosynthesis and explain how greenhouses help to overcome these to improve crop production:

6. (a) Identify the overall process pictured right: _____

   (b) What process is represented in the blue box? _____

   (c) Explain how the process you identified in (a) can be used to produce useful products:

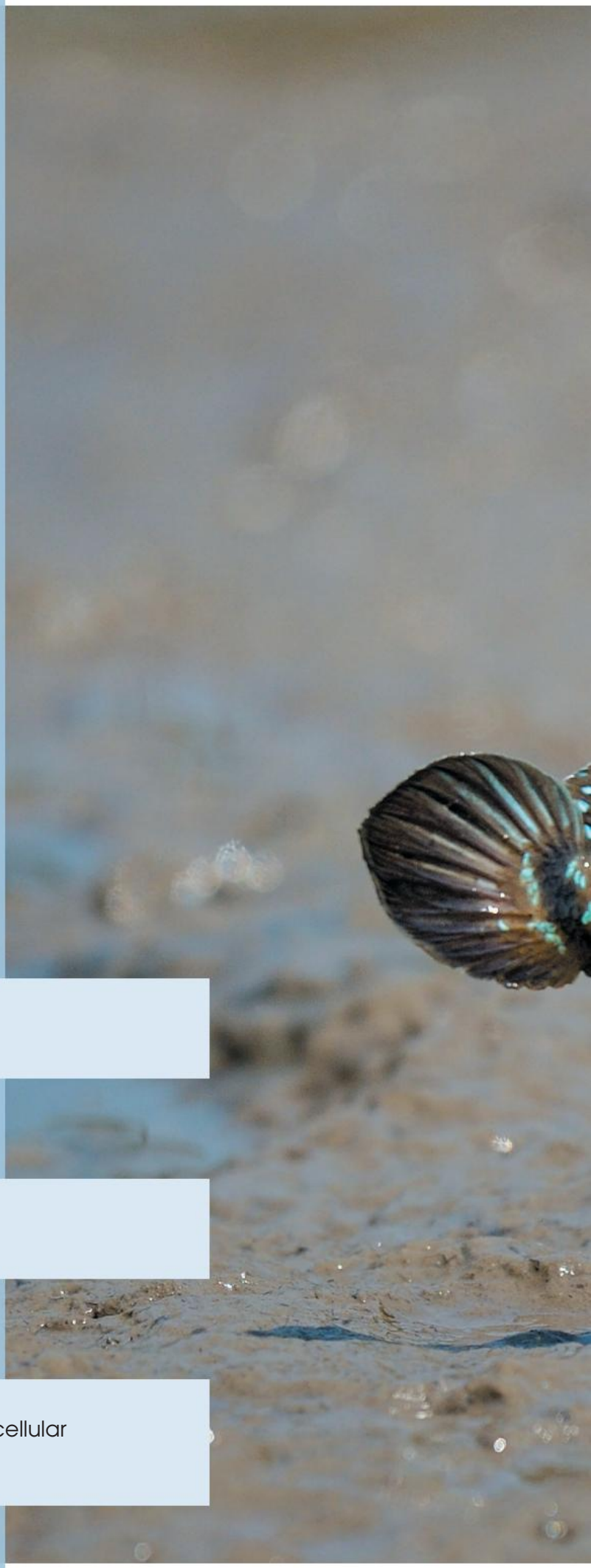


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UNIT

04

How does life change and respond to challenges?



Area of Study 1

How do organisms respond to pathogens?

Area of Study 2

How are species related over time?

Area of Study 3

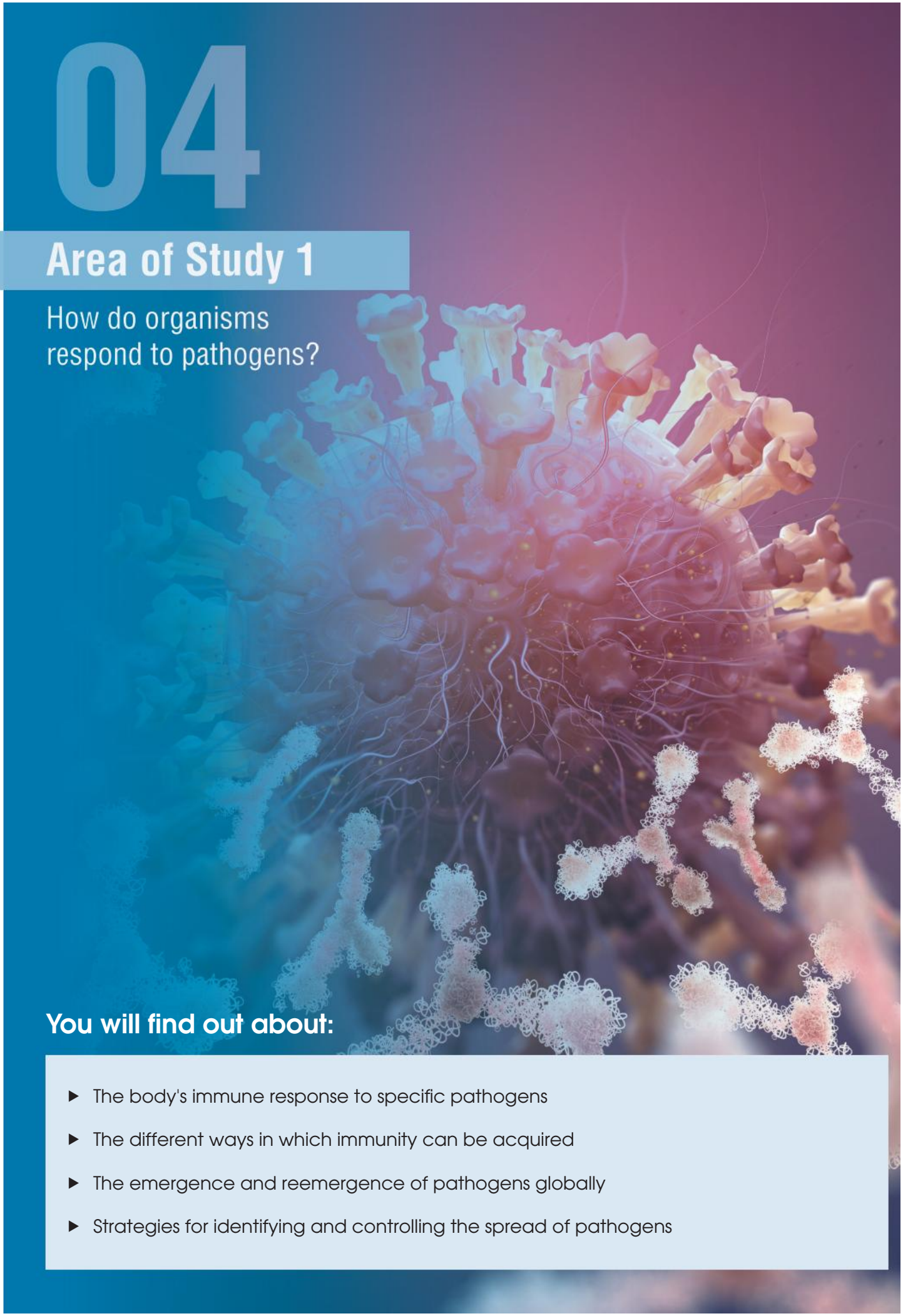
How is scientific inquiry used to investigate cellular processes and/or biological change?



04

Area of Study 1

How do organisms respond to pathogens?



You will find out about:

- The body's immune response to specific pathogens
- The different ways in which immunity can be acquired
- The emergence and reemergence of pathogens globally
- Strategies for identifying and controlling the spread of pathogens

CHAPTER 7

Responding to Antigens

Key terms

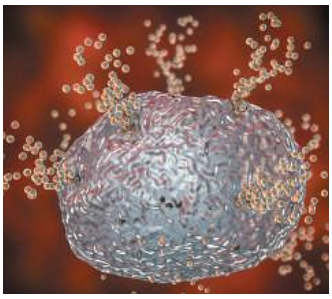
allergen
antigen
cellular pathogen
complement proteins
dendritic cell
eosinophil
histamine
infection
inflammation
innate (=non-specific) immune response
interferon
leucocyte
macrophage
mast cell
MHC
microbiome
neutrophil
non-cellular pathogen
phagocyte
self antigen
non-self antigen

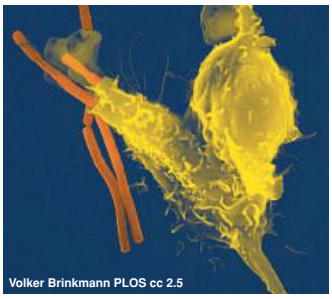
Barriers against infection

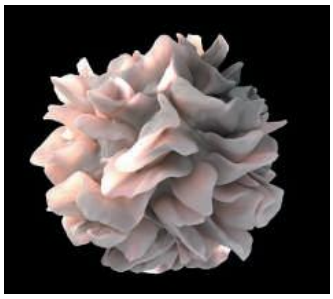
Key knowledge

Activity number

- ☐ 1 Recognise pathogens as disease-causing agents with antigenic properties. Distinguish between cellular and non-cellular pathogens. Describe the role of pathogens as a source of non-self antigens. — 64
- ☐ 2 Describe the range of physical defences against pathogen invasion in plants and animals. Include reference to physical barriers, mucous membranes and secretions, and mechanical defences, such as the mucociliary escalator. — 65
- ☐ 3 Describe the range of chemical defences against pathogen invasion in plants and animals. Include reference to antimicrobial substances and release of chemicals in the inflammatory response. — 66
- ☐ 4 Describe the role of the normal microflora (the microbiome) in preventing pathogenic infection in plants and animals. — 67







The innate immune response

Key skills and knowledge

- ☐ 5 Describe the features and roles of the components of the innate (or non-specific) immune response to antigens, including the steps in inflammatory response. Include reference to the role of: — 68 69
  (a) Complement proteins in the plasma in stimulating phagocyte activity.
  (b) Interferons and their role in activating natural killer T cells and macrophages.
  (c) Mast cells and the release of histamine.
  (d) Macrophages and neutrophils in phagocytosis.
  (e) Eosinophils and their role in the inflammatory response
  (f) Dendritic cells in processing and presenting antigenic material.

Antigens and the immune response

Key skills and knowledge

- ☐ 6 Distinguish between non-self antigens, self-antigens, and allergens and explain the importance of the distinction. — 71
- ☐ 7 Explain how the body distinguishes self from non-self. Include reference to the role of the major histocompatibility complex (MHC) and its role in self-recognition. Explain why the microbiome of the body is tolerated, whereas other microorganisms are attacked. Identify when the self-recognition system is suppressed (naturally or artificially) and explain why. — 64 72
- ☐ 8 Recall the difference between cellular and non-cellular pathogens. Describe the role of antigen-presenting cells, such as dendritic cells and macrophages, in initiating an immune response by processing and presenting antigens to lymphocytes — 72

64 Pathogens and Antigens

Key Idea: Antigens are substances capable of producing an immune response. Some antigens are pathogens, infectious agents that cause disease.

An **antigen** is any substance that causes an immune response in an organism. Most antigens are foreign material and originate from outside the organism (e.g. a microbe), but sometimes an antigen can originate from inside your own body. The presence of an antigen usually stimulates an immune response, this causes the antigen to be destroyed. **Pathogens** are disease-causing agents and they are an example of a foreign antigen. They, or their toxins, cause their host to have an immune response. Pathogens can be **cellular pathogens** (living organisms) or **non-cellular pathogens** (non-living organisms).

Cellular pathogens

Cellular pathogens are living organisms. They have all the cellular machinery to carry out their life process and reproduce. Bacteria and eukaryotic pathogens (fungi, protists, and parasitic worms) are cellular pathogens. Cellular pathogens cause a wide range of diseases in plants and animals. The severity of the diseases they cause varies greatly, some are a mild inconvenience (e.g. athlete's foot) while some can be fatal (e.g. TB).

Non-cellular pathogens

Non-cellular pathogens (viruses and prions) are non-living entities that lack the cellular machinery needed to carry out metabolism on their own.



CDC: Janice Haney Carr

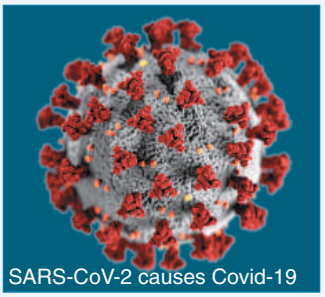
Mycobacterium tuberculosis



"Ringworm" caused by *Tinea*



Malaria parasite (intracellular)



SARS-CoV-2 causes Covid-19

CDC

Bacterial pathogens

Pathogenic bacteria can be transmitted through food, water, air, or by direct contact. Bacteria have historically caused widespread, devastating diseases, but the discovery and use of antibiotics and aseptic techniques has reduced these.

Fungal pathogens

Pathogenic fungi are more common in plants than in animals. They spread by spores and the infections they cause are generally chronic (long-lasting, low grade) infections because fungi grow relatively slowly. Some, however, can be fatal.

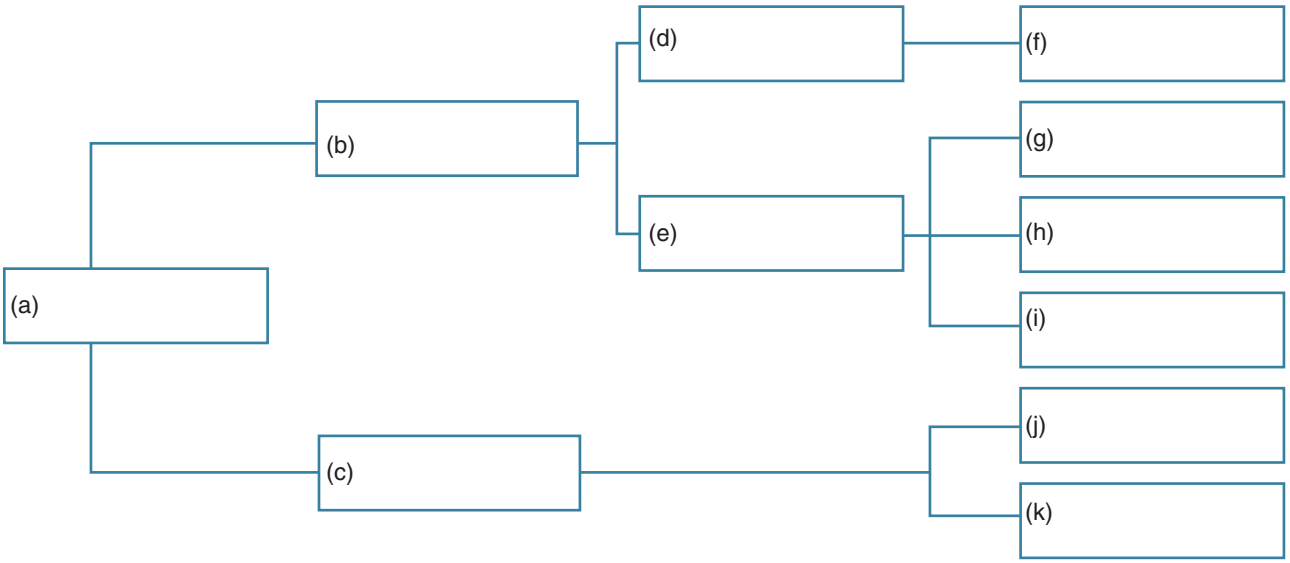
Protistan pathogens

Protistans are a large and diverse group of eukaryotes. A number of species are significant pathogens of animals or plants. Pathogenic protists have very complex life cycles, often involving a number of different hosts and several different life stages.

Viruses and prions

Viruses and prions (misfolded infectious proteins) rely on the host's metabolism to reproduce. Viruses cause a wide variety of diseases, including Covid-19. Prions cause fatal nervous diseases in mammals including BSE in cattle and kuru in humans.

1. Use the template below to categorise pathogens. Use the following word list to help you: *Cellular pathogen, virus, prokaryote, fungi, non-cellular pathogen, bacteria, pathogen, protist, parasitic worm, prion, eukaryote.*



2. Explain the difference between cellular pathogens and non-cellular pathogens: _____

_____

_____

_____

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65 Physical Defences in Plants and Animals

Key Idea: Physical defences against pathogens include physical barriers, mucous membranes and secretions, and mechanical defences, such as ciliary activity in animals.

Most organisms have a tiered system of defences to provide resistance against disease. Physical defences are the first line of defence against pathogens. Physical defences are a non-specific form of defence, they are always present and are designed to stop as many pathogens as possible from entering the body. In animals, physical defences fall into three categories: physical barriers, mucous membranes and secretions, and mechanical defences. Plants also have a range of physical barriers to protect them from pathogens.

Physical defences in animals



Jpbarrass public domain

Physical barriers: Undamaged skin provides a waterproof, physical barrier to stop pathogens entering the body. Your skin is covered in microbes, but unless the skin is cut or grazed, the microbes cannot cross the skin and enter your body. Even if your skin is damaged, it quickly begins to form a scab as it heals (above). This reduces pathogen entry into the body.



Mucous membranes and secretions: The eyes, mouth, nose, and urinary tract are entry points for pathogens. However, secretions (tears, saliva, and mucus) help keep pathogens out by washing them away. Some entry points are lined with mucous membranes. The cells of the mucous membrane secrete sticky mucus to trap pathogens and prevent them causing harm.



Mechanical defences: These physically remove microbes from areas where they might cause infection. Tiny hair-like cilia move in a wave-like motion to carry mucus-trapped pathogens out of the body. This "mucociliary escalator" moves trapped pathogens in the airways (above) away from the lungs, where the material can be expelled or swallowed and destroyed in the stomach.

Physical defences in plants

- Plants have physical defences that protect them from infection by pathogens.
- Many trees and shrubs are covered in bark. This tough external covering forms a physical barrier, which pathogens find difficult to cross.
- The addition of pectin (a type of carbohydrate) and a layer of cellulose (the cell wall) adds strength and has the added benefit of providing extra layers to protect against infection.
- Many plants have leaves covered in a waxy cuticle. The cuticle is another physical barrier, reducing pathogen entry into the plant tissue via the leaf.



lookscloser CC 2.0

Woody stems and bark
Trees and woody shrubs, like the juniper wattle, have thick coverings with waxy suberin, which limit pathogen entry to the inner tissues. The spiny, reduced leaves protect it from being eaten, but also limit the pathways for pathogen entry.



Impervious waxy cuticle
All plants have a waxy cuticle, even if it is thin. The cuticle is made up of fatty acids so all leaves are negatively charged and hydrophobic. These properties repel many spores and microbes and make the leaf environment less suitable for fungal invasion.

1. What features of physical barriers make them an important first line of defence against pathogens?

2. What are the similarities between the way human skin and plant bark reduce pathogen entry?

3. Some plants leaves secrete wax in response to the presence of pathogens. How is this different from the other physical barriers in plants?

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66 Chemical Defences In Plants and Animals

Key Idea: All plants and animals have chemical defences to defend against pathogens. Some mechanisms are always present while others are stimulated by an attack.

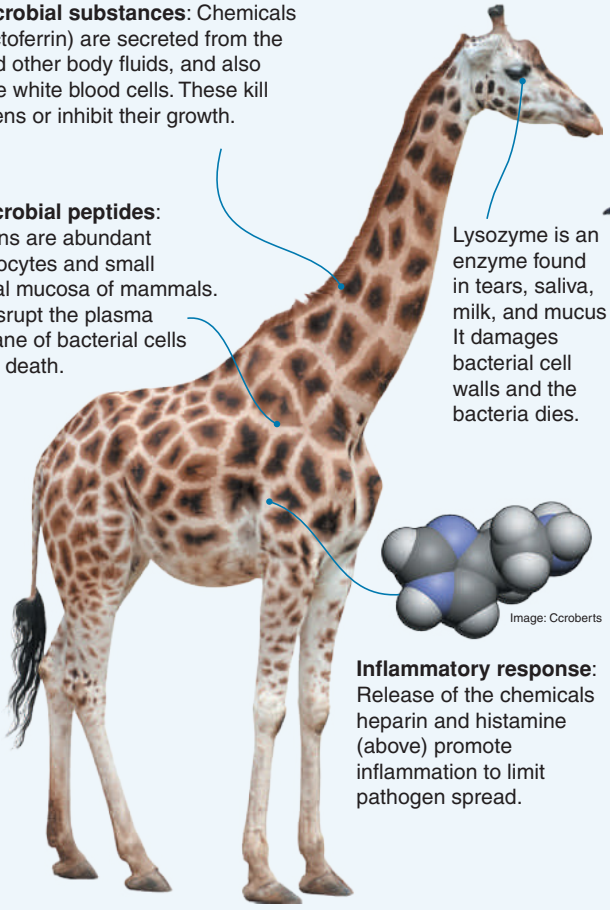
Living organisms are under constant attack from pathogens. As a result plants and animals have evolved a wide range of chemical defences to protect themselves from pathogens and limit the damage they can do. All plants and animals have innate (non-specific) defences, whereas vertebrates also have specific immune responses, directed against particular pathogens and invoked by pathogen contact. The chemical defences of plants not only protect them from pathogens, but may also inhibit browsing or plant-plant competition.

Chemical defences in animals

Vertebrate innate defences

Antimicrobial substances: Chemicals (e.g. lactoferrin) are secreted from the skin and other body fluids, and also by some white blood cells. These kill pathogens or inhibit their growth.

Antimicrobial peptides: Defensins are abundant in phagocytes and small intestinal mucosa of mammals. They disrupt the plasma membrane of bacterial cells causing death.



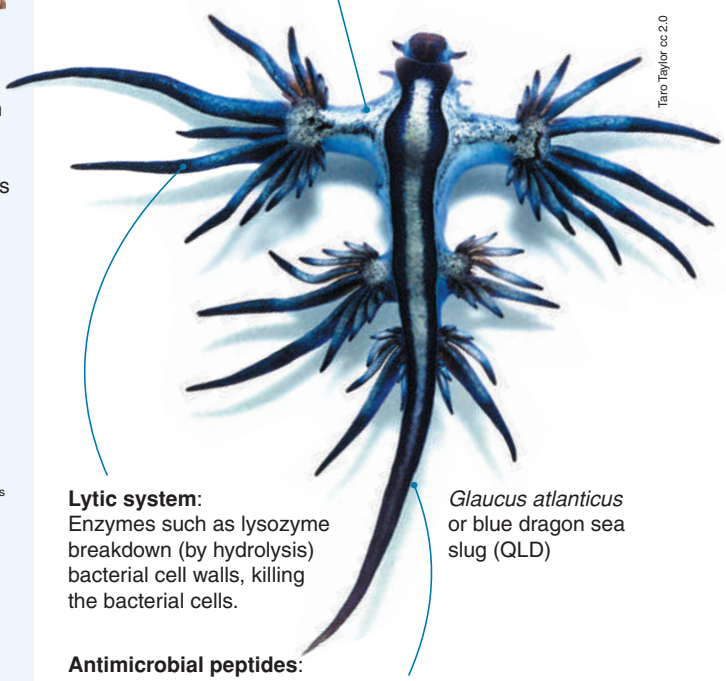
Lysozyme is an enzyme found in tears, saliva, milk, and mucus It damages bacterial cell walls and the bacteria dies.

Image: Ccroberts

Inflammatory response: Release of the chemicals heparin and histamine (above) promote inflammation to limit pathogen spread.

Invertebrate innate defences

ProPO system: Microbial compounds induce the prophenoloxidase (proPO) defence system. proPO produces a cascade, and the final product, melanin, encases and kills the pathogen. The system is present in most invertebrates.



Taro Taylor cc 2.0

Lytic system: Enzymes such as lysozyme breakdown (by hydrolysis) bacterial cell walls, killing the bacterial cells.

Glaucus atlanticus or blue dragon sea slug (QLD)

Antimicrobial peptides: The antimicrobial peptide **defensin** binds to the cell membrane of pathogens, and causes damage by puncturing the membrane. The pathogen becomes "leaky" and dies.

1. (a) Describe the advantage of having multiple (non-specific) defence responses: _____

_____

_____

_____

(b) Describe a disadvantage of having only general (non-specific) defence responses: _____

_____

_____

2. Compare and contrast the non-specific defences of vertebrate and invertebrate animals: _____

_____

_____

_____

_____

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Chemical defences in plants

Passive defences

Passive defences are always present and are not the result of contact with a pathogen or grazer. Plants have both physical and chemical defences to deter pathogens. However, if the physical defence is breached, the chemical defences protect the plant against further damage.



The powdery mildew infecting this plant is a fungus

Many plants produce a range of antimicrobial and antifungal chemicals and enzymes to kill or inhibit the growth of pathogens. Some of these compounds cover the surface of the plant, killing pathogens before they enter the plant. Other compounds act internally.

Many native Australian plants have antimicrobial properties, which are exploited by humans for medicinal purposes. The antimicrobial properties of the emu bush have long been used in traditional Australian Aboriginal medicine to treat infections of the skin, eyes, and throat. Four antimicrobial compounds have been isolated from emu bush.

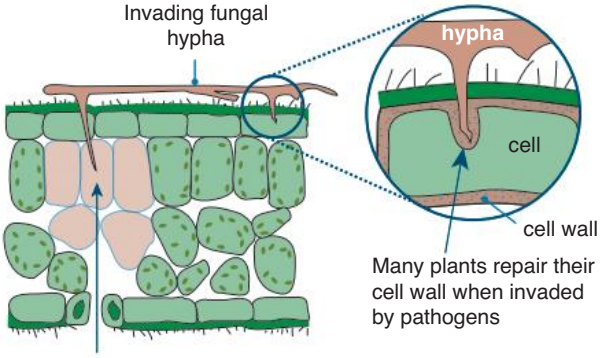


Emu bush (*Eremophila alternifolia*)

Geoff Derrin CC 4.0

Active defences

Once infected, a plant responds actively to prevent any further damage. **Active defences** are invoked only after a pathogen has been recognised, or after wounding or attack by a herbivore. This makes biological sense because active defences are costly to produce and maintain. Active defences work through a variety of mechanisms including slowing pathogen growth, puncturing the cell wall, disrupting metabolism, or killing cells by release of reactive oxygen species such as hydrogen peroxide (H_2O_2).



Many plants produce an enzyme-activated **hypersensitive response** when invaded by pathogens. This leads to the production of reactive nitric oxide and cell death. Cell death in the infected region limits the spread of the pathogen.



Sealing off infected areas gives rise to abnormal swellings called **galls** (oak gall, left and bulls-eye galls on a maple leaf, right). These galls limit the spread of the parasite or the infection in the plant.

Ell

3. (a) Distinguish between passive and active defence mechanisms in plants: _____

_____

_____

_____

(b) Why are most plant defensive chemicals produced only after a pathogen is detected? _____

_____

4. How are galls effective in reducing the spread of infection in some plants? _____

_____

_____

5. What similarities are there between the active defence mechanisms of plants and the immune responses of animals?

_____

_____

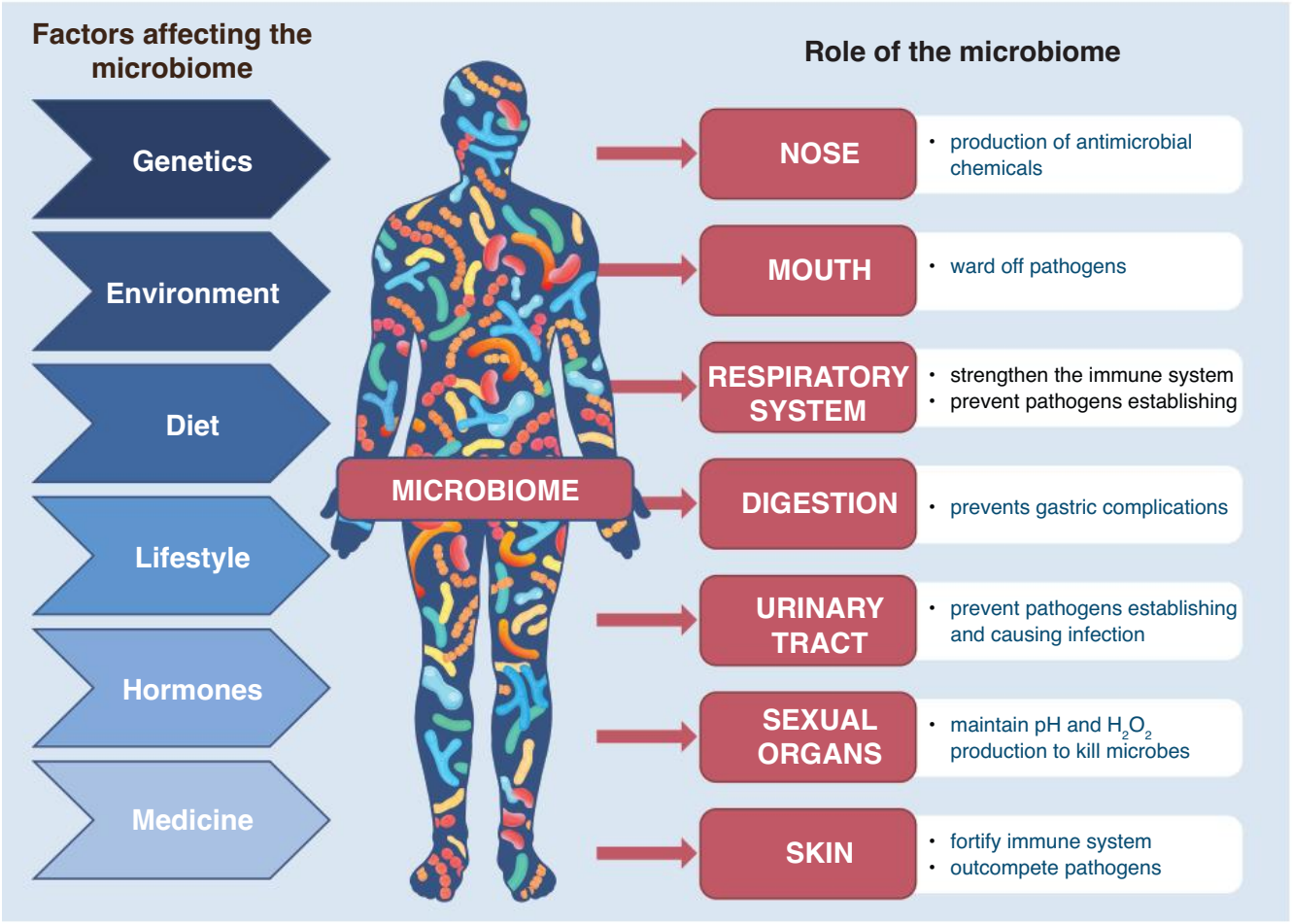
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67 The Protective Microbiome

Key Idea: The presence of beneficial microbes can prevent pathogenic microbes establishing and causing harm.

In humans, microbial populations begin to establish on your skin and in your gut and other areas of the body soon after birth. Usually these microbial populations are beneficial. They assist in overall good heath and prevent pathogenic microbes becoming established and causing disease. The "good" microbes do this by out-competing pathogens for space or nutrients, and therefore limit pathogen numbers. Many factors influence the normal microbiome (below, left) and disruptions to it can allow pathogens to become established and cause disease. For example, if antibiotics kill off the beneficial gut microbes, diarrhoea-causing pathogens can become established and cause a person to become very sick.



Microbial protection in plants

- Pathogenic microbes are responsible for plant disease, including crop diseases and diseases spread by insect vectors. The presence of beneficial bacteria and fungi can help prevent pathogenic microbes establishing and causing disease. A beneficial root microbiome can also promote good plant health (e.g. through improved nitrogen uptake).
- Scientists grew thale cress in the presence of individual beneficial microbes. They then introduced pathogens to the root system to study how plant health was affected. The study showed that the presence of beneficial microbial root associations (especially bacteria) restricted pathogen growth in the roots of thale cress (keeping the plant healthier).



1. Identify the two main ways the beneficial microbiome prevents pathogens establishing:

_____

_____

_____

2. What evidence is there that the plant root microbiome is important for reducing plant disease? _____

_____

_____

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68 The Body's Defences: An Overview

Key Idea: The human body has a tiered system of defences that together provide resistance against disease.

The human body has a range of physical, chemical, and biological defences that provide **resistance** against pathogens. The first line of defence consists of external barriers to prevent pathogens entering the body. If this fails, a second line of defence targets any foreign bodies that enter. Lastly, the specific immune response provides a targeted third line of defence against the pathogen. The defence responses fall into two broad categories, the innate and the adaptive immune responses. The **innate** (or non-specific) response make up the first and second lines of defence. It protects against a broad range of non-specific pathogens. This response is present in all animals. It involves blood proteins (e.g. complement), inflammation, and phagocytic white blood cells. The **adaptive** (or specific) immune response is the third line of defence. It is specific to identified pathogens and is present only in vertebrates. It involves defence by specific T cells (cellular immunity) as well as antibodies, which neutralise foreign antigens (humoral immunity).

1st line of defence

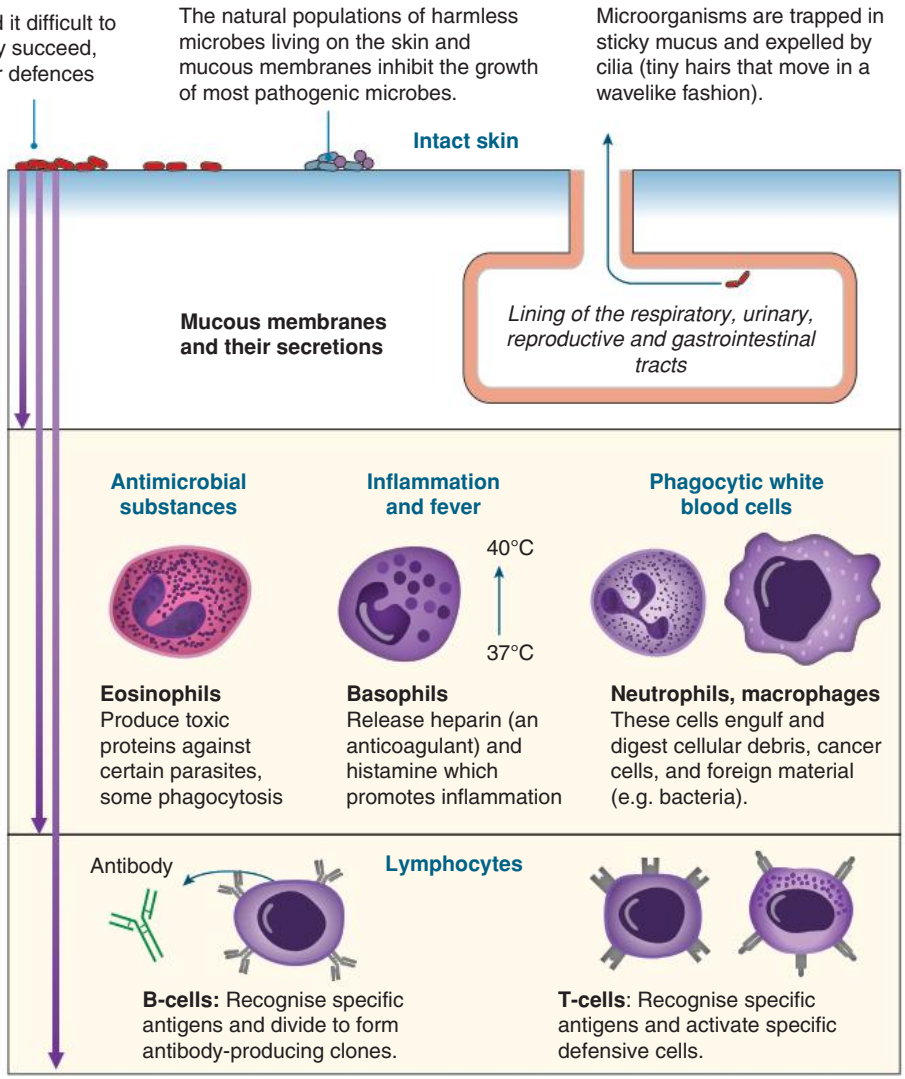
The skin provides a physical barrier to the entry of pathogens. Healthy skin is rarely penetrated by microorganisms. Its low pH is unfavourable to the growth of many bacteria and its chemical secretions (e.g. sebum, antimicrobial peptides) inhibit growth of bacteria and fungi. Tears, mucus, and saliva also help to wash bacteria away.

2nd line of defence

A range of defence mechanisms operate inside the body to inhibit or destroy pathogens. These responses react to the presence of any pathogen, regardless of which species it is. White blood cells are involved in most of these responses. It includes the complement system whereby blood plasma proteins work together to bind pathogens and induce inflammation to help fight infection.

3rd line of defence

Once the pathogen has been identified by the immune system, **lymphocytes** (specialised white blood cells) launch a range of specific responses to the pathogen, including the production of defensive proteins called **antibodies**. Each type of antibody is produced by a B-cell clone and is specific against a particular antigen.



1. What are the differences between the innate and adaptive immune responses: _____

2. How does having a tiered defence help protect an organism from a pathogen? _____

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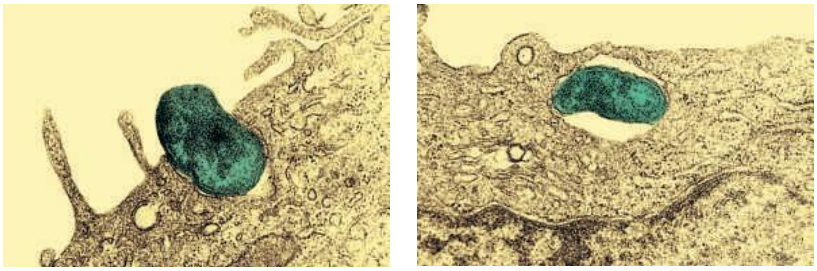
69 The Innate Immune Response

Key Idea: The innate immune response provides a rapid response to contain and destroy pathogens. Inflammation is an important part of this response.

The innate immune system provides protection against a pathogen, even if it has never encountered it before. The innate response is very fast and provides general protection (it is not antigen specific), but does not provide long lasting immunity. Many different cells and processes are involved. The primary outcome is to destroy and remove the cause of infection. This is achieved through containing the infection through inflammation and then recruiting immune cells to destroy the pathogen. During this process, a series of biochemical reactions (the complement system) is activated to destroy the pathogen and recruit immune cells to the site.

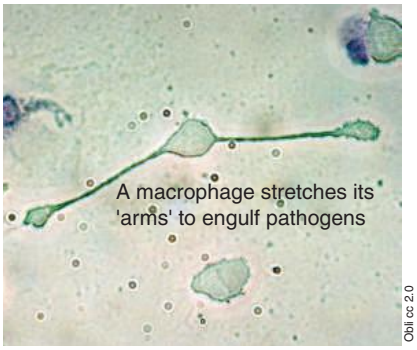
Phagocytic cells of the innate immune system

A phagocyte is any type of mobile white blood cell capable of phagocytosis. Phagocytes protect the body by engulfing and destroying antigenic material including harmful foreign particles, microbes, and dead or dying cells tagged for destruction Phagocytes move around the material to engulf it, then break it down into harmless fragments by enclosing it in a phagosome and digesting it. Macrophages, neutrophils, and dendritic cells are all phagocytes.



Photos: CDC

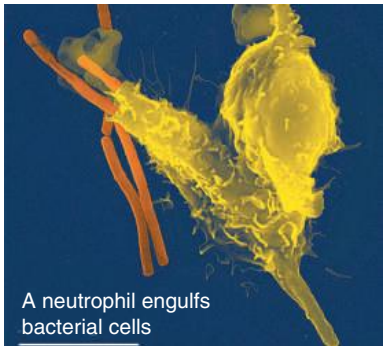
Photos above: A cell engulfs and ingests a bacterium by phagocytosis.



Obli cc 2.0

Macrophage

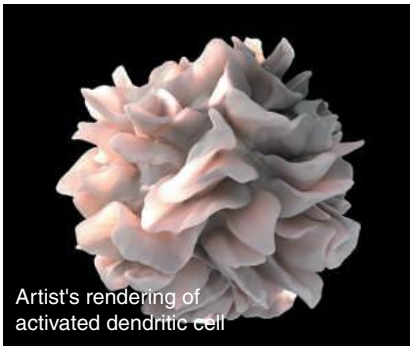
Macrophages are very large and are highly efficient phagocytes. They are found throughout the body and move using an amoeboid movement (above) to hunt down and destroy pathogens. Macrophages also have a role in recruiting other immune cells to an infection site and eliminating diseased and damaged cells.



Volker Brinkmann PLOS cc 2.5

Neutrophil

Neutrophils are the most abundant type of phagocyte and are usually the first cells to arrive at an infection site. They contain toxic substances that kill or inhibit the growth of extracellular pathogens such as bacteria and fungi. Neutrophils release cytokines, which amplify the immune response and recruit other cells to the infection site.



Dendritic cell

Dendritic cells are present in tissues that are in contact with the external environment (e.g. skin, and linings of the nose, lungs, and digestive tract). They act as messengers between the innate and adaptive immune systems by ingesting antigenic material and presenting them to the T cells of the immune system.

1. What feature do all phagocytic cells have in common? _____

2. Outline the role of the following phagocytes in the innate immune response:

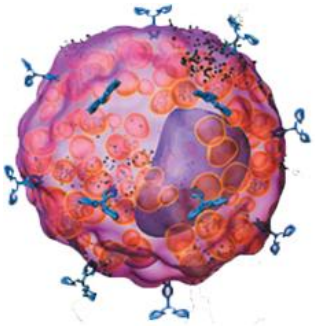
   (a) Macrophages: _____

   (b) Neutrophils: _____

   (c) Dendritic cells: _____

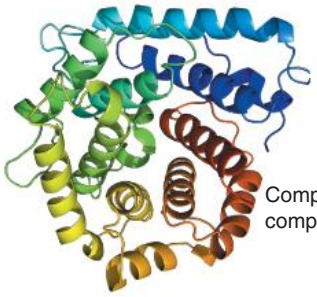
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Other cells and processes of the innate immune response



Mast cells

Mast cells contain a lot of histamine, a chemical involved in both inflammation and allergic responses. When a mast cell is activated, it releases histamine, which causes the surrounding blood vessels to dilate and become leaky. The increased permeability allows phagocytes to reach the site of infection easily.



Complement component 3 (C3)

Complement proteins

The complement system is made up of a number of different proteins. The proteins circulate as inactive precursors until they are activated. Complement proteins have three main roles: phagocytosis, attracting macrophages and neutrophils to the infection site, and rupturing the membranes of foreign cells.

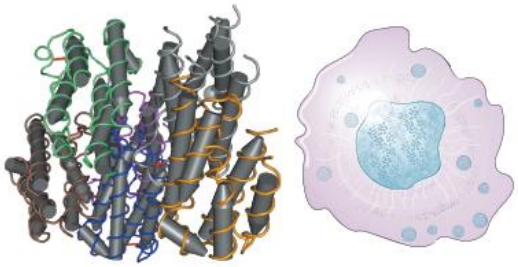


The process of inflammation

The inflammatory process is a protective response to pathogen invasion. It has several functions: (1) to destroy the cause of the infection and remove it and its products from the body; (2) if this fails, to limit the effects on the body by confining the infection to a small area; (3) replacing or repairing tissue damaged by the infection.

Interferons

Interferons are signalling proteins released by immune cells when viruses are detected. Interferons disrupt viral replication, reducing infection of the host's cells. Interferons also activate immune cells (e.g. natural killer T cells and macrophages) and cause them to increase in number. Interferons also increase the rate of antigen presentation by increasing the expression of major histocompatibility complex (MHC) antigens.



Interferons (model, above left) belong to a large class of proteins called cytokines. They activate natural killer T cells (illustration, above right).

Natural killer cells

Natural killer T cells (NK cells) are a white blood cell important in the destruction of cancer cells and cells infected with viruses. Cytokines (including interferon) activate NK cells. Once activated, the NK cells release a protein called perforin, which forms holes in the cell membrane of the target cell. A series of events causes the cell to die and any viral particles inside to be destroyed.

3. What are the three main roles the proteins of the complement system play in immunity?

   (a) _____

   _____

   (b) _____

   _____

   (c) _____

   _____

4. (a) Briefly explain what interferons are and what stimulates their production: _____

   _____

   _____

   (b) Explain the connection between interferons and natural killer cells: _____

   _____

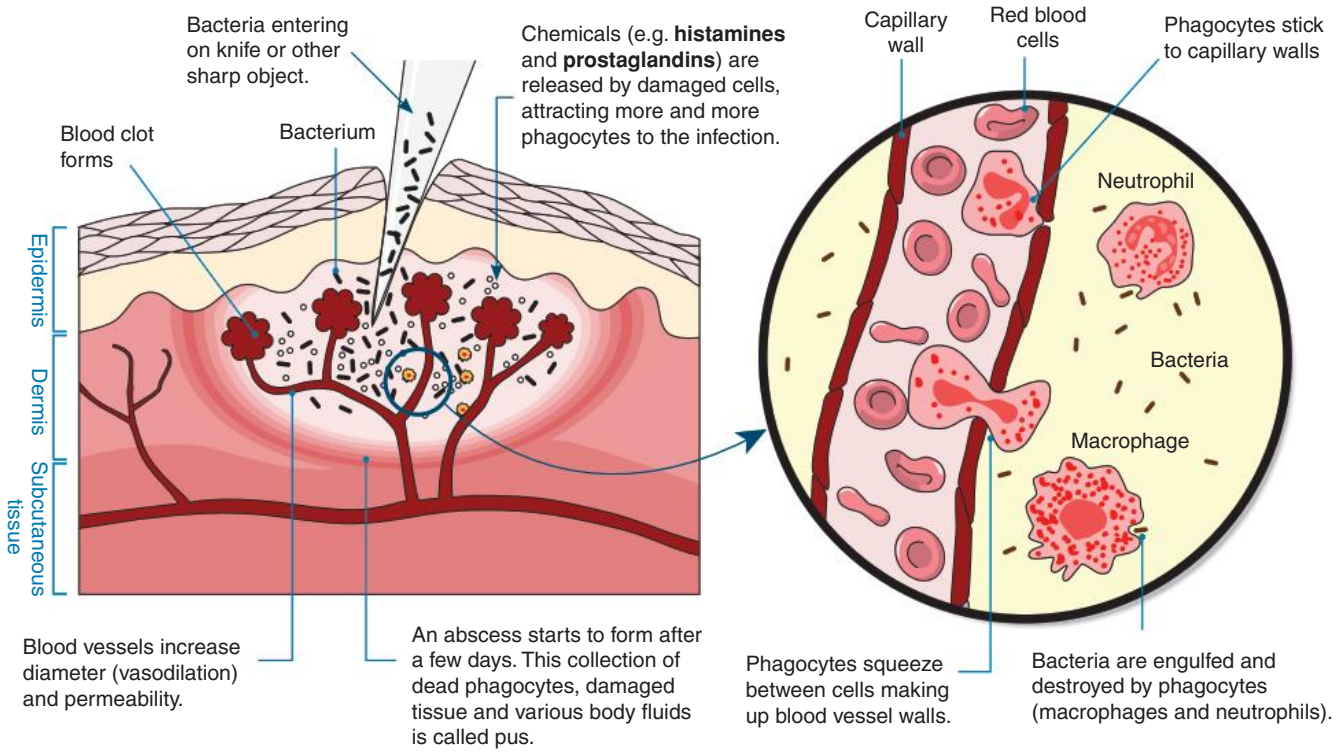
   _____

   _____

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The inflammatory response

The inflammatory response (inflammation) is triggered by the presence of a wide range of harmful factors including the presence of infectious agents (pathogens), damaged cells, toxic compounds, foreign objects, or physical damage (e.g. burns). It is therefore a very common response and an important component of innate immunity. The purpose of the inflammatory response is to remove the dangerous stimuli and to stimulate the beginning of the healing process. Body parts or tissue affected by inflammation are usually showing signs of heat, pain, redness, swelling, and loss of function.



Stages in the inflammatory response

Increased diameter and permeability of blood vessels

Blood vessels increase their diameter and permeability in the area of damage. This increases blood flow to the area and allows defensive substances to leak into tissue spaces.

Phagocyte migration and phagocytosis

Within one hour of injury, phagocytes appear on the scene. They squeeze between cells of blood vessel walls to reach the damaged area where they destroy invading microbes.

Tissue repair

Functioning cells or supporting connective cells create new tissue to replace dead or damaged cells. Some tissue regenerates easily (skin) while others do not at all (cardiac muscle).

5. Outline the three stages of inflammation and identify the beneficial role of each stage:

(a) _____

_____

(b) _____

_____

(c) _____

_____

6. What role do mast cells play in inflammation? _____

_____

_____

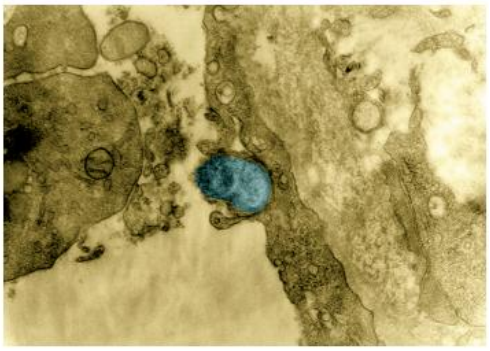
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70 Phagocytes and Phagocytosis

Key Idea: Phagocytes are mobile white blood cells that ingest and destroy extracellular foreign material and dead or dying cells.

Phagocytosis is the process by which a cell engulfs another cell or particle. Cells that do this are called phagocytes. All types of phagocytes (e.g. neutrophils, dendritic cells, and macrophages) are white blood cells. These specialised cells have receptors on their surfaces that can detect antigenic material, such as microbes. They then ingest the microbes and digest them, rendering them harmless. As well as destroying microbes, phagocytes also release substances called cytokines, which help to coordinate the overall response to an infection. Macrophages and dendritic cells also play an important role in processing and presenting antigens from ingested microbes to other cells of the immune system.



CDC

Stages in phagocytosis and destruction of a pathogen

1. **Detection and interaction**
Microbe coated in chemical markers is detected by the phagocyte, which attaches to it. Chemical markers coating the foreign material (e.g. a bacterial cell), mark it as a target for phagocytosis.

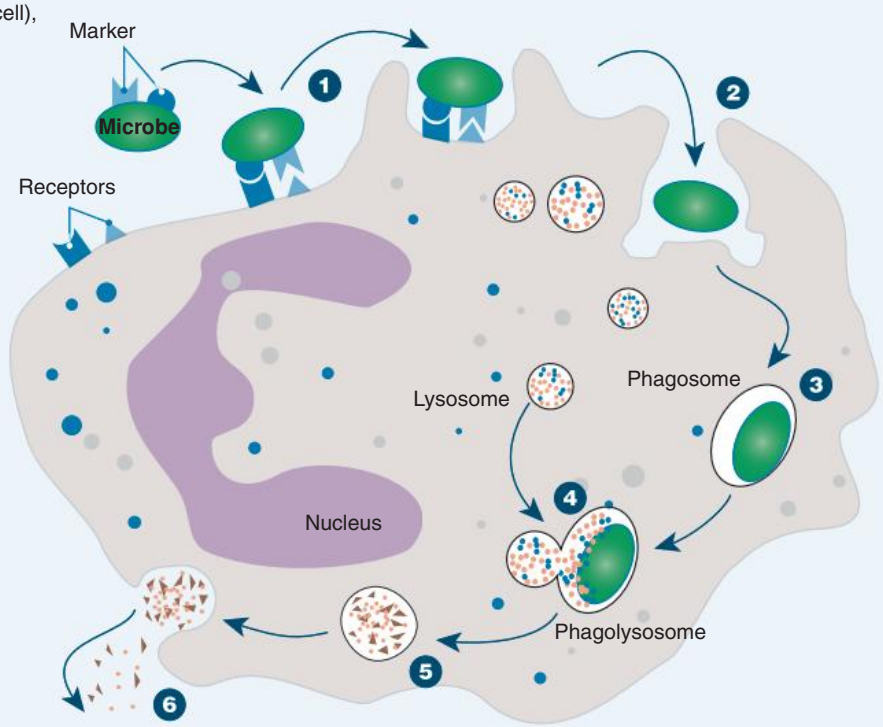
2. **Engulfment**
The markers trigger engulfment of the microbe by the phagocyte. The microbe is taken in by endocytosis.

3. **Phagosome forms**
A phagosome forms, enclosing the microbe in a membrane.

4. **Fusion with lysosome**
Phagosome fuses with a lysosome containing digestive enzymes. The fusion forms a phagolysosome.

5. **Digestion**
The microbe is broken down into its chemical constituents.

6. **Discharge**
Indigestible material is discharged from the phagocyte.



1. Explain the role of chemical markers and phagocyte receptors in enhancing phagocytosis: _____

_____

_____

2. What is the purpose of phagocytosis and how is involved in internal defence? _____

_____

_____

_____

_____

3. Why do think the foreign material has to be enclosed in a phagosome?_____

_____

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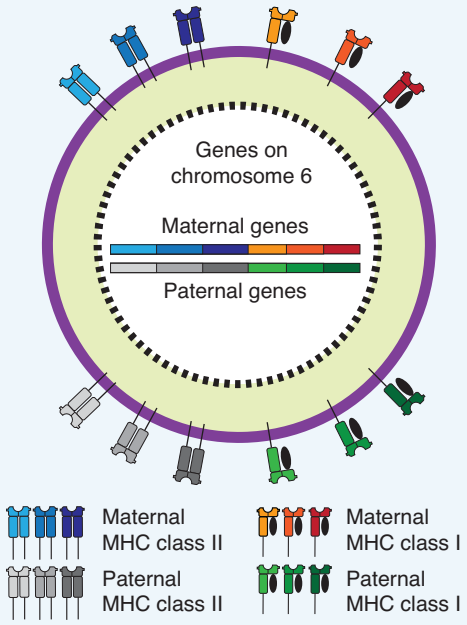
71 Recognising Antigens

Key Idea: It is important that the body can distinguish its own tissues from foreign material so that it does not attack itself.

Recall that an **antigen** is any substance that produces an immune response. Most antigens are **non-self antigens**. This means they are foreign and originate from outside the organism (e.g. bacteria or viruses). Sometimes an organism will react to its own cells and tissues. Antigens that originate from within the body are called **self-antigens**. Normally, because of the development of self-tolerance, the body recognises and does not attack its own tissues. However, in some instances, the immune system may mistakenly destroy its own tissues, causing an **autoimmune disease**. Allergens are specific antigens that cause a hypersensitivity reaction called an allergic response.

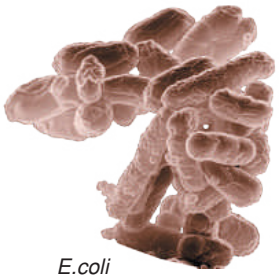
Distinguishing self from non-self

- Every type of cell has unique protein markers (antigens) on its surface. The type of antigen varies greatly between cells and between species. The immune system uses these markers to identify its own cells (self) from foreign cells (non-self). If the immune system recognises the antigen markers, it will not attack the cell. If the antigen markers are unknown, the cell is attacked and destroyed.
- The system responsible for this property is the major histocompatibility complex (**MHC**). The MHC is a cluster of tightly linked genes on chromosome 6. One set comes from the mother (maternal) and one set from the father (paternal). These genes code for MHC antigens that are attached to the surface of body cells. The main role of MHC antigens is to bind to antigenic fragments and display them on the cell surface so that they can be recognised by the cells of the immune system.
- Class I MHC antigens are found on the surfaces of almost all human cells. Class II MHC antigens occur only on macrophages and B-cells of the immune system, like the stylised neutrophil below.



Tolerance towards foreign bodies

- The human body has a very large population of resident microbes. Under normal conditions, *E.coli* in the gut form a protective layer preventing the colonisation of pathogenic bacteria. The microbial cells have foreign antigens but they are not attacked by the immune system because **tolerance** (the prevention of an immune response) has developed.



E.coli

- During pregnancy, specific features of the self recognition system are suppressed to allow the mother to tolerate a nine month relationship with a foreign body (the fetus).

Intolerance to tissue transplants

- The MHC is responsible for the rejection of tissue grafts and organ transplants. Foreign MHC molecules on the transplanted tissue are viewed as antigenic, causing the immune system to respond and the tissue to be rejected.
- To minimise rejection, attempts are made to match the MHC of the organ donor to that of the recipient as closely as possible. Immunosuppressant drugs are also used to minimise the immune response.



Kidney transplant

1. (a) Distinguish between non-self antigens and self antigens: _____

_____

_____

_____

(b) Why is it important that the body detects foreign antigens? _____

_____

_____

_____

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Types of antigens

Non-self antigens

Any foreign material provoking an immune response is termed a non-self antigen. Disease-causing organisms (pathogens) such as bacteria, viruses, and fungi are non-self antigens. The body recognises them as foreign and will attack and destroy them before they cause harm.



CDC

Influenzavirus

Pathogens have ways of avoiding detection. Mutations result in new surface antigens, delaying the immune response and allowing the pathogen to reproduce in its host undetected for a time (e.g. the flu virus, above). Some pathogens, e.g. the malaria-causing *Plasmodium*, switches off its surface antigens in order to enter cells undetected.

Self antigens

The body is usually tolerant of its own antigens. However, sometimes the self-tolerance system fails and the body attacks its own cells and tissues as though they were foreign. This can result in an autoimmune disorder in which tissue is destroyed, grows abnormally, or changes in function.

Autoimmune disorders, such as multiple sclerosis and rheumatoid arthritis, may be triggered by infection. The similarity of the pathogen and self antigens is thought to be behind this failure of self recognition.



Type 1 diabetes is the result of autoimmune destruction of the insulin-producing pancreatic cells. Patients must inject insulin to maintain normal blood glucose levels.

Allergens

Antigens that cause allergic reactions are called allergens. An allergic reaction is a very specific type of immune response in which the immune system overreacts to a normally harmless substance. An allergic response can produce minor symptoms (itching, sneezing, rashes, swelling) or life-threatening anaphylaxis (respiratory and cardiovascular distress).

Common allergens include dust, chemicals, mould, pet hair, food proteins, or pollen grains.



Kent Pryor

The swelling on the foot in the left of the photograph is a result of an allergic reaction to a bee sting.

2. How can pathogens avoid detection by the immune system? _____

3. (a) What is the nature and purpose of the major histocompatibility complex (MHC)? _____

   (b) Why is a self-recognition system important? _____

4. (a) What is immune tolerance? _____

   (b) When might tolerance to foreign antigens be beneficial or necessary? _____

5. Using examples, describe what happens when the body develops an inappropriate response to:

   (a) Self-antigens: _____

   (b) Normally non-antigenic substances: _____

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72 Recognising Cellular and Non-cellular Pathogens

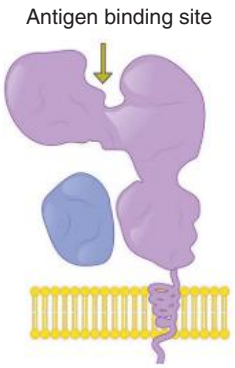
Key Idea: Antigen processing prepares and displays antigens for presentation to the T-cells of the immune system.

Antigen presenting cells (APCs) process and present antigens for recognition by T-cells. During antigen processing, the APC digests the foreign antigen into smaller peptide fragments. These fragments are then displayed on the surface of the APC by MHC receptors. The immune response evoked by the T-cells depends on which MHC receptor (MHCI or MHCII) is activated. Antigen presentation is necessary for T-cells to recognise infection or abnormal growth and activate other cells of the immune system. Dendritic cells, macrophages, and B-cells are APCs.

The role of MHC receptors

Recall there are two types of MHC receptors, class I and class II (right). Both have similar functions in that they display antigens on cell surfaces so that they can be recognised and processed by the T-cells of the immune system. T-cells can only recognise antigens if they are displayed by the MHC receptors. MHC receptors presenting no foreign antigens are ignored by T-cells, and are recognised as "self". Only MHC receptors with foreign antigens bound to them will attract T-cells and evoke an immune response.

The two classes of MHC receptors display different types of antigens. Class I MHC receptors display antigens from intracellular pathogens (e.g. viruses). Class II MHC receptors display antigens from pathogens that have been phagocytosed (e.g. bacteria).



Class I MHC
Presents antigens from within the cell (intracellular antigens) e.g. viral proteins

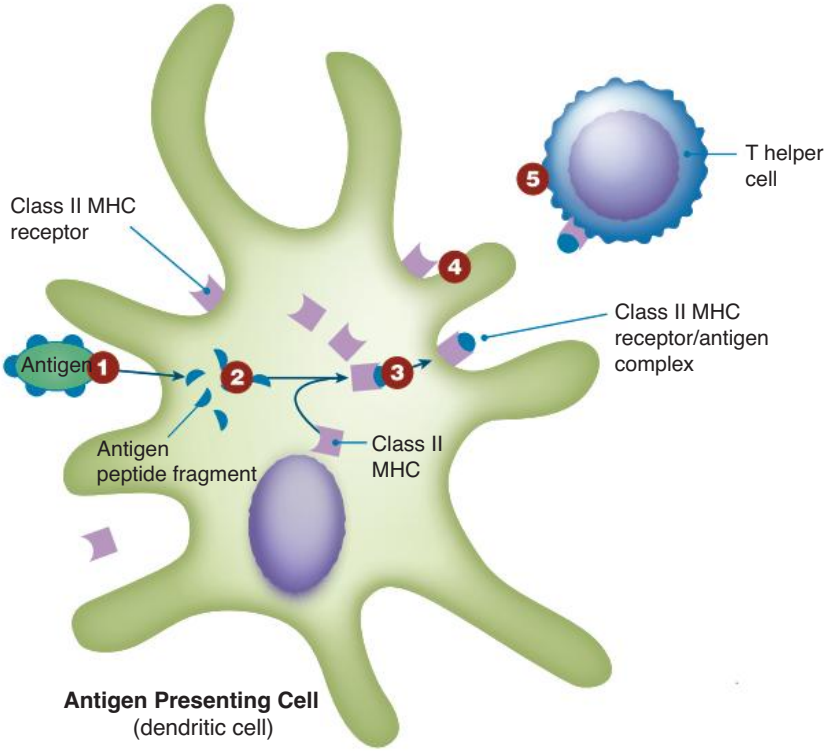


Class II MHC
Presents antigens from outside the cell (extracellular antigens) e.g. proteins from phagocytosed microbes.

An overview of antigen processing

The diagram on the right represents antigen processing of an extracellular peptide antigen via a class II MHC receptor.

1. An antigen presenting cell (APC) encounters an antigen.
2. The antigen is engulfed via phagocytosis and digested into short peptide fragments.
3. Class II MHC receptors bind the fragments and form a MHC-antigen complex.
4. The MHC-antigen complex is displayed on the surface of the APC.
5. A receptor on the T helper cell recognises the peptide as foreign. It binds and a series of events stimulate the adaptive immune response.



Antigen Presenting Cell
(dendritic cell)

1. What is the purpose of antigen processing? _____

_____

_____

2. Why do MHC receptors with no antigenic peptide bound not cause an immune response? _____

_____

_____

3. Describe the differences between class I and class II MHC receptors: _____

_____

_____

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73 Chapter Review: Did You Get It?

1. Contrast the innate and the adaptive immune responses with reference to the basic action and the cells involved:

2. The photograph on the right shows the effect of a pathogen infecting a human.



(a) Name the defensive response occurring: _____

(b) What is happening to the blood vessels at this location? _____

(c) Name the substance responsible for the change in the blood vessels:

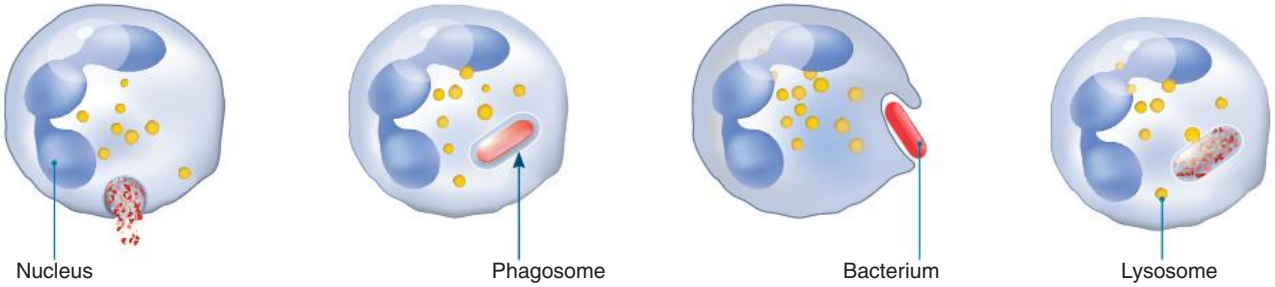
(d) What type of cell is the substance released from? _____

(e) During this response, the number of white blood cells increases/decreases (delete one).

(f) The process occurring here is an example of innate immunity / adaptive immunity (delete one).

3. Name the three types of phagocytes: _____

4. The images below show the stages of phagocytosis of a bacterium by a neutrophil. The stages are not in the correct order. Write numbers (1-4) underneath each stage to indicate the correct sequence of events (e.g. #4 would be the final stage). Briefly describe of what is occurring under each image.



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

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CHAPTER
8

Acquiring Immunity

Key terms

active immunity
adaptive (=specific) immune response
antibody (=immunoglobulin)
antigen
artificially acquired immunity
B cell (B lymphocyte)
cellular (cell mediated) immunity
clonal selection
dendritic cell
humoral immunity
immunised
immunity
immunological memory
infection
leucocyte
lymph
lymph node
lymphocyte
macrophage
MHC
naturally acquired immunity
passive immunity
phagocyte
primary response
secondary response
T cell (T lymphocyte)
T killer cell
T helper cell
thymus
vaccination

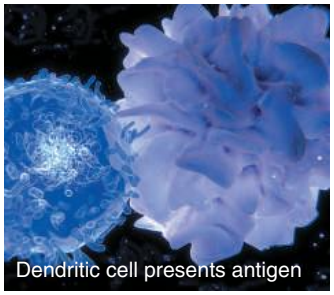
The role of the lymphatic system

Key skills and knowledge

Activity number

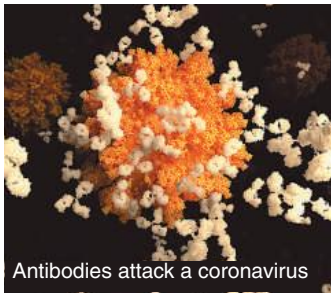
☐ 1 Outline the general structure of the lymphatic system, distinguishing between primary lymphoid organs and secondary lymphoid tissues. **74**

☐ 2 Explain the role of the lymphatic system in the immune response. Include reference to the role of lymph nodes as the site of antigen recognition by lymphocytes and the importance of the lymphatic system in transporting immune cells (e.g. dendritic cells) around the body. **74**


Dendritic cell presents antigen


Macrophage

Obli cc 2.0


Antibodies attack a coronavirus

The adaptive immune response

Key skills and knowledge

☐ 3 Describe the features and roles of the components of the adaptive immune response (cellular and humoral immunity), including the role of specificity and memory. Contrast the adaptive immune response against both extracellular and intracellular threats. **75**

☐ 4 Recognise clonal selection and the basis of immunological memory. Explain how the immune system is able to respond to the large range of potential antigens. **76**

☐ 5 Describe the actions of B-lymphocytes (B cells) and their antibodies in humoral immunity. Include reference to antibody structure and how this relates to function. Describe some of the ways in which antibodies can inhibit infection. **75 77**

☐ 6 Describe the actions of T-lymphocytes in cellular immunity to include T helper and T cytotoxic (killer) cells. **75**

Natural and artificial immunity

Key skills and knowledge

☐ 7 Distinguish between naturally acquired and artificially acquired immunity. **78**

☐ 8 Describe active and passive strategies for acquiring immunity. Using examples, explain how both active and passive immunity can be acquired naturally acquired or artificially. **78**

☐ 9 Describe primary and secondary responses to infection and explain how these responses form the basis of vaccination (immunisation) programmes. **78**

74 The Lymphatic System and Immunity

Key Idea: The lymphatic system transports lymph, a fluid rich in white blood cells, throughout the body to attack antigens.

The lymphatic system is a network of tissues and organs that collects the tissue fluid leaked from the blood vessels and transports it to the heart. The lymphatic system has an important role in immunity because the fluid (lymph) transported by the lymphatic system is rich in infection-fighting white blood cells called lymphocytes. The thymus and red bone marrow are the primary organs of the lymphatic system, but it has many secondary components too (below).

Components of the lymphatic system

Tonsils
A collection of secondary lymphoid tissues in the throat. They provide defence against ingested or inhaled pathogens and produce activated B and T cells.

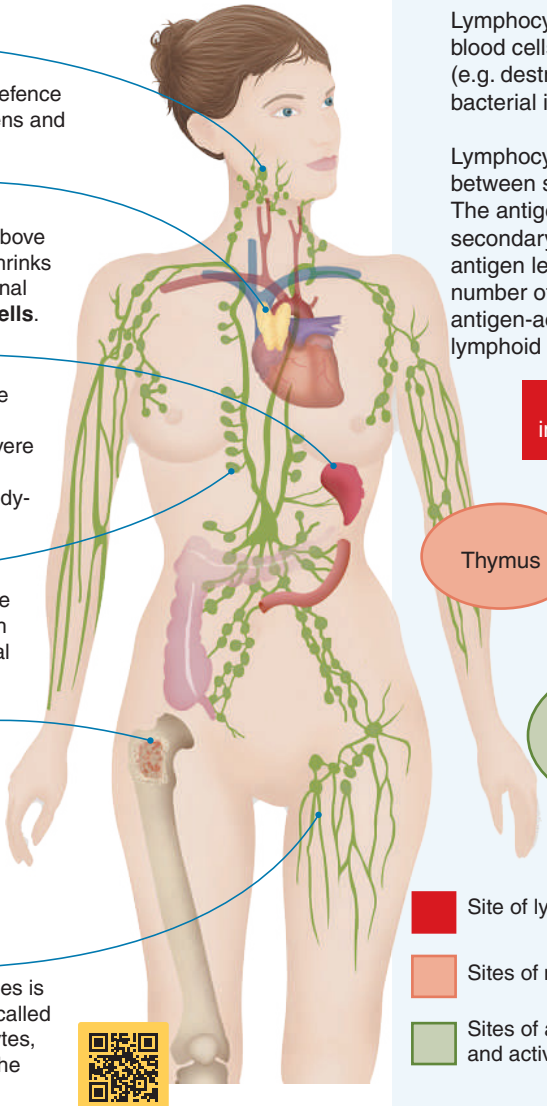
Thymus
A primary lymphoid organ located above the heart. It is large in infants and shrinks after puberty to a fraction of its original size. Important for maturation of **T-cells**.

Spleen
The largest mass of lymphatic tissue in the body. It stores and releases blood in case of demand (e.g. in severe bleeding), produces mature B-cells and antibodies, and removes antibody-coated antigenic material.

Lymph nodes
Ovoid masses of lymph tissue where lymphocytes are concentrated. Each node receives lymph through several incoming and outgoing vessels.

Red bone marrow
A primary lymphoid tissue where all the different kinds of blood cells (including white blood cells) are produced by cellular differentiation from stem cells. B cells also mature here.

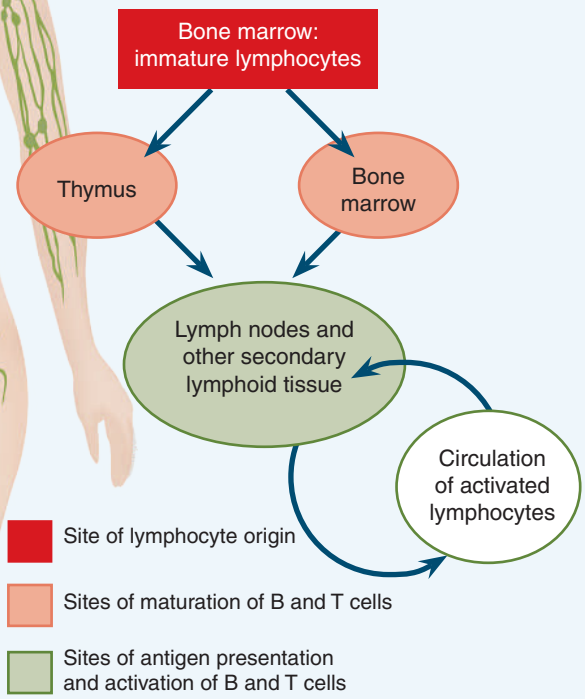
Lymphatic vessels
When the fluid leaking from capillaries is picked up by lymph capillaries, it is called **lymph**. The lymph, carrying leucocytes, flows in lymphatic vessels through the secondary lymphoid tissues.



The lymphatic system and immunity

Lymphocytes (T and B cells) are types of white blood cells,. They are important in fighting infection (e.g. destroying the microbes causing viral or bacterial infections).

Lymphocytes in circulation are constantly moving between sites where antigens may be encountered. The antigens are presented to T cells in the secondary lymphoid tissues. Recognition of the antigen leads to activation and rapid increase in number of both T and B cells. After several days, antigen-activated lymphocytes begin leaving the lymphoid tissue.



1. What is the general role of the lymphatic system in immunity? _____

_____

_____

_____

2. (a) What is the role of lymph nodes in the immune response? _____

_____

_____

_____

(b) Why do you think lymph nodes become swollen when someone has an infection? _____

_____

_____

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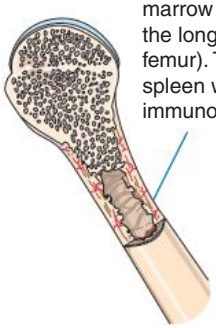
75 The Adaptive Immune Response

Key Idea: The adaptive immune response launches a specific immune response to antigens. It involves B cells and T cells.

Recall that the innate immune response responds the same way for every antigen it encounters. In contrast, the adaptive immune response reacts uniquely to each specific type of antigen it encounters. The adaptive immune response targets both extracellular antigens (e.g. bacteria) and intracellular antigens (e.g. viruses). There are two main components of the adaptive immune system: the humoral immune response and the cell mediated immune response. The **humoral immune response** is associated with the serum (the non-cellular part of the blood) and involves the action of antibodies secreted by **B cells** (B lymphocytes). The **cell-mediated immune response** is associated with the production of lymphocytes called **T cells** (T lymphocytes). Antigens are recognised by T cells only after antigen processing (opposite).

Lymphocyles and their functions

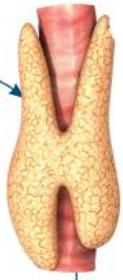
Bone marrow
B cells mature in the bone marrow in the shaft of the long bones (e.g. the femur). They migrate to the spleen where they become immunocompetent.



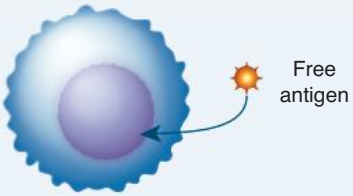
Stem cell
Stem cells in the bone marrow and fetal liver give rise to T cells and B cells.



Thymus gland
The thymus gland is located above the heart. It is large in infants but becomes smaller with age. Immature T cells move to the thymus to mature before migrating to other lymphatic organs.

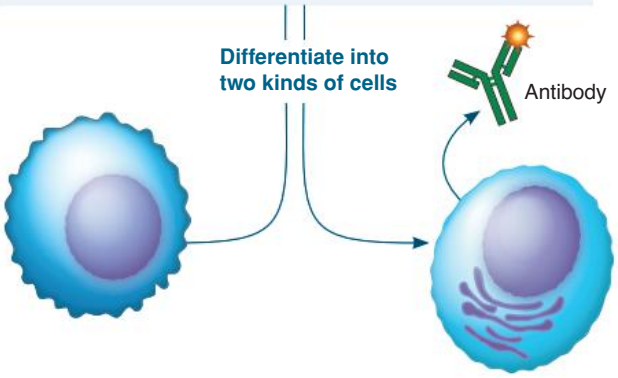


B CELLS



B cells recognise and bind antigens. Each B cell recognises one specific antigen. Helper T cells recognise specific antigens on B cell surfaces and induce their maturation and proliferation. A mature B cell may carry as many as 100,000 antigenic receptors embedded in its surface membrane. B cells defend against bacteria and viruses outside the cell and toxins produced by bacteria (free antigens).

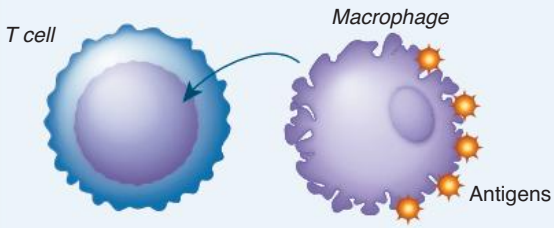
Differentiate into two kinds of cells



Memory cells
Some B cells differentiate into long-lived memory cells (see Clonal Selection). When these cells encounter the same antigen again (even years or decades later), they rapidly differentiate into antibody-producing plasma cells.

Plasma cells
When stimulated by an antigen (see Clonal Selection), some B cells differentiate into plasma cells, which secrete **antibodies** into the bloodstream. The antibodies then inactivate the circulating antigens.

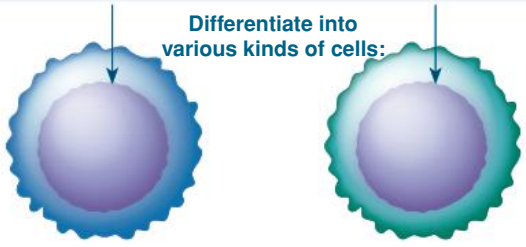
T CELLS



T cells respond only to antigen fragments that have been processed and presented by specialised antigen-presenting cells (dendritic cells and macrophages). They defend against:

- Intracellular bacteria and viruses
- Protozoa, fungi, flatworms, and roundworms
- Cancerous cells and transplanted foreign tissue

Differentiate into various kinds of cells:



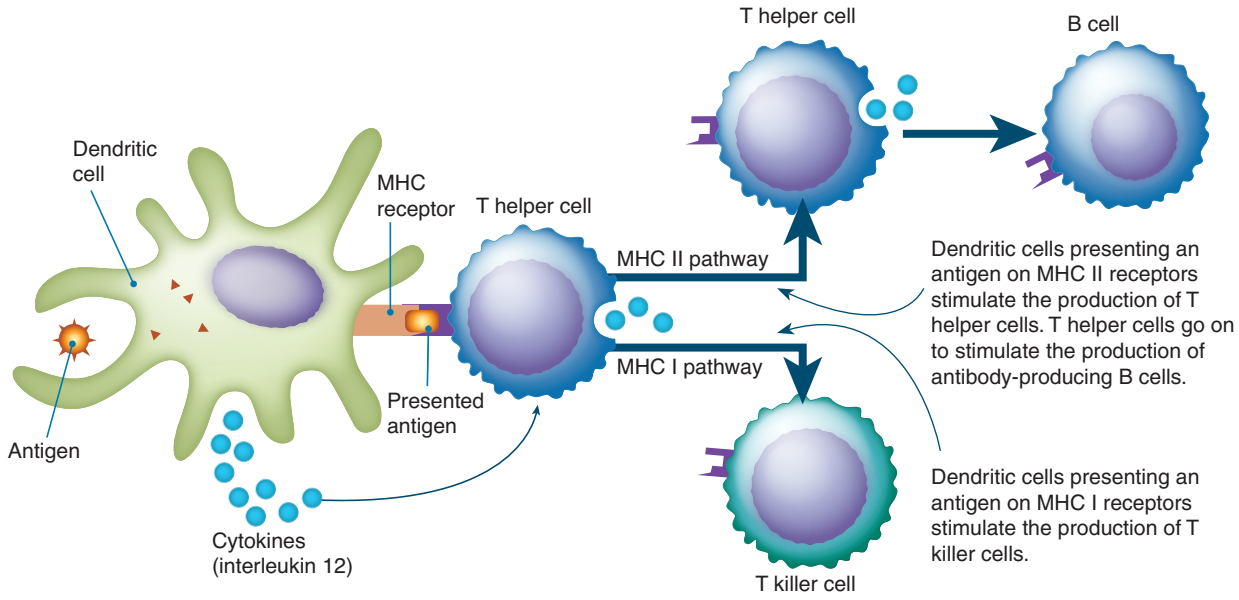
T helper cell activates T killer cells and other helper T cells. They are needed for B cell activation.

T killer cell destroys target cells on contact. Recognises tumour or virus-infected cells by their surface markers. They are also called T cytotoxic cells.

There are also other types of T cells:
T memory cells have encountered specific antigens before and can respond quickly and strongly when the same antigen is encountered again.
T regulator cells control the immune response by turning it off when no more antigen is present. They are important in the development of self tolerance.

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Dendritic cells stimulate the activation and proliferation of lymphocytes



- Dendritic cells (as well as macrophages and B cells) are specialised antigen-presenting cells (APC). Most nucleated cells have MHC I receptors but APC have both MHC I and MHC II receptors, making them so-called "professional" APC.
- Immature dendritic cells originate in the bone marrow and migrate to the lymph nodes. When the dendritic cell encounters an antigen, it presents it to a T helper cell (above). This stimulates the T helper cell to secrete chemicals called cytokines. Cytokines stimulate the activation and increase in number of T cells, activating the immune system against that specific antigen.

1. Describe the nature and general action of the two major divisions in the immune system:

   (a) Humoral immune system: _____

   _____

   _____

   (b) Cell-mediated immune system: _____

   _____

   _____

2. Explain how an antigen causes the activation and proliferation of T cells and B cells, including the role of dendritic cells:

   _____

   _____

   _____

   _____

3. In what way do dendritic cells act as messengers between the innate and the adaptive immune systems?

   _____

   _____

4. Describe the function of each of the following cells in the immune system response:

   (a) T helper cells: _____

   (b) T killer cells: _____

5. Suggest why the thymus gland is largest in infants: _____

   _____

   _____

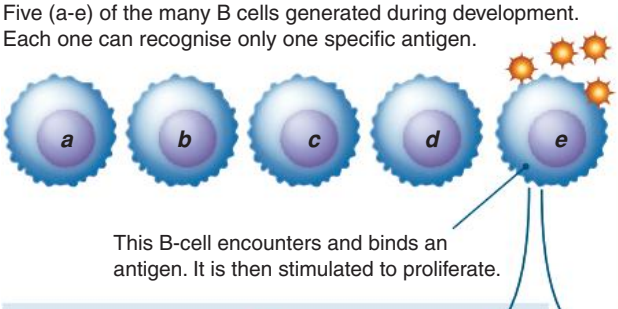
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76 Clonal Selection

Key Idea: Clonal selection theory explains how lymphocytes can respond to a large and unpredictable range of antigens.

The body has millions of B cells. Collectively they can respond to a large number of different antigens, but individual B cells recognise only one specific type of antigen. When an antigen is detected, the correct B cell is activated, multiples, and produces antibodies to destroy the antigen (below). This process is called the **clonal selection,** and it explains how the immune system can respond to a large range of potential antigens in the environment. In the same way, a T cell stimulated by a specific antigen will multiply and develop into different types of T cells.

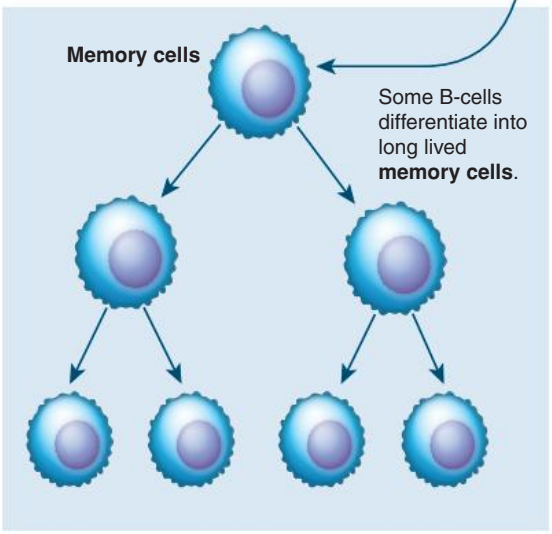
Five (a-e) of the many B cells generated during development. Each one can recognise only one specific antigen.



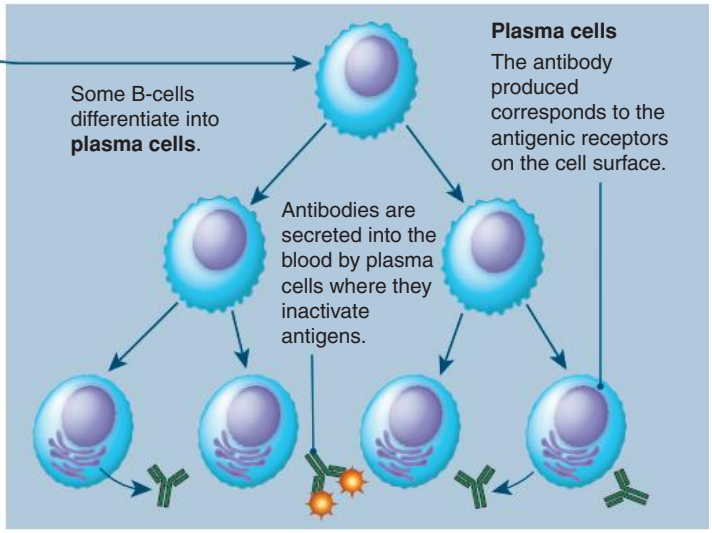
This B-cell encounters and binds an antigen. It is then stimulated to proliferate.

Clonal selection theory

Millions of B cells form during development. Antigen recognition is randomly generated, so collectively they can recognise many antigens, including those that have never been encountered. Each B cell has receptors on its surface for specific antigens and produces antibodies that correspond to these receptors. When a B cell encounters its antigen, it responds by multiplying and producing many clones that produce the same kind of antibody. This is called clonal selection because the antigen selects the B cells that will multiply.



Some B cells differentiate into long lived **memory cells**. These are retained in the lymph nodes to provide future immunity (**immunological memory**). If the antigen returns a second time, memory B cells react more quickly and vigorously than the first time the antigen appeared.



Plasma cells secrete antibodies specific to the antigen that stimulated their development. Each plasma cell lives for only a few days, but can produce about 2000 antibody molecules per second. During development, any B cells that react to the body's own antigens are destroyed in a process that leads to **self tolerance** (acceptance of the body's own tissues).

1. Describe how clonal selection results in the proliferation of one particular B cell clone: _____

2. (a) What is the function of the plasma cells in the immune system response? _____

   (b) Why do B cells only respond to a single type of antigen? _____

3. (a) Explain the basis of immunological memory: _____

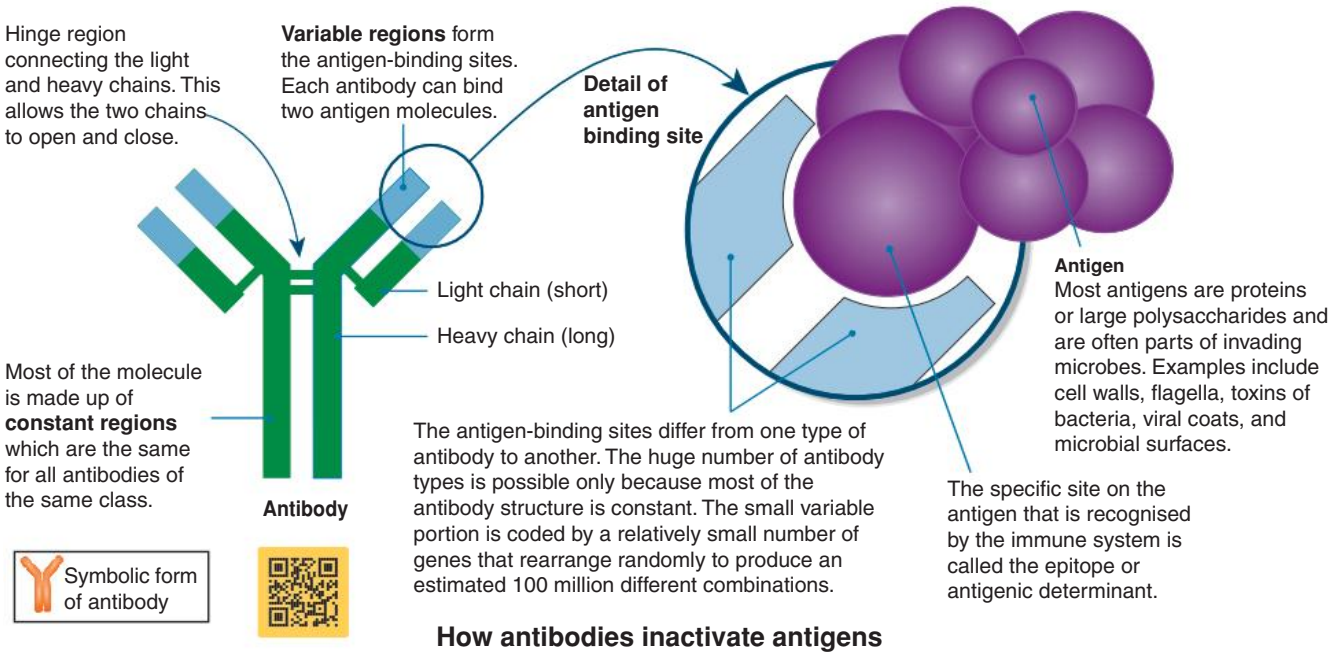
   (b) Why are B memory cells able to respond so rapidly to an encounter with an antigen long after an initial infection?

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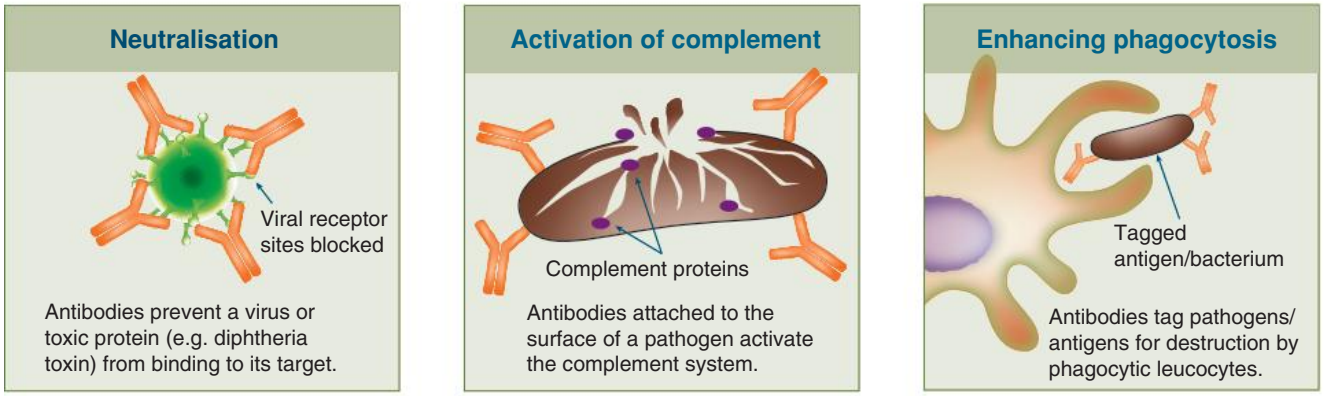
77 Antibodies

Key Idea: Antibodies are large, Y-shaped proteins, made by B cells, which destroy specific antigens.

Antibodies and antigens play key roles in the response of the immune system. Recall that antigens are foreign molecules which promote a specific immune response. Antigens include pathogenic microbes and their toxins, as well as substances such as pollen grains, blood cell surface molecules, and the surface proteins on transplanted tissues. **Antibodies** (also called immunoglobulins) are proteins made in response to antigens. They are secreted from plasma B cells into the plasma where they can recognise, bind to, and help destroy antigens. There are five classes of antibodies, each plays a different role in the immune response. Each type of antibody is specific to only one particular antigen.



How antibodies inactivate antigens



1. Describe the structure of an antibody, identifying the specific features of its structure that contribute to its function:

2. Use the diagram above to describe three ways in which antibodies help the immune system' to stop infection:

   (a)

   (b)

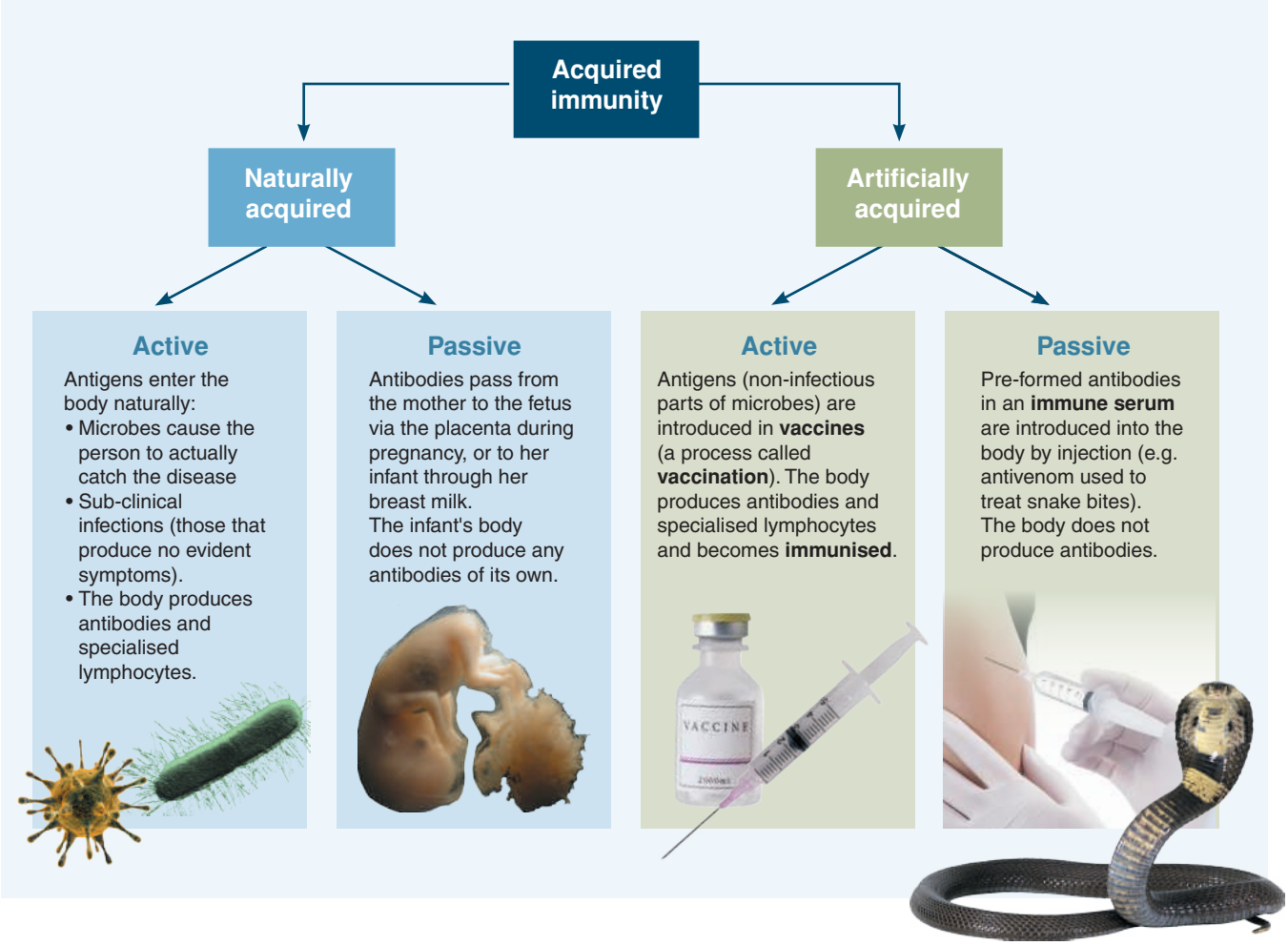
   (c)

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78 Acquired Immunity

Key Idea: Acquired immunity is a resistance to specific pathogens acquired over the life-time of an organism.

We are born with natural or innate resistance which provides non-specific immunity to certain illnesses. In contrast, **acquired immunity** is protection developed over time to specific antigens. **Active immunity** develops after the immune system responds to being exposed to microbes or foreign substances. **Passive immunity** is acquired from gaining preformed antibodies without exposure to the antigen. Immunity can be naturally acquired, through natural exposure to microbes, or artificially acquired as a result of medical treatment (below).



1. (a) What is passive immunity?

(b) Distinguish between naturally acquired passive immunity and artificially acquired passive immunity. Give examples:

2. (a) What is active immunity?

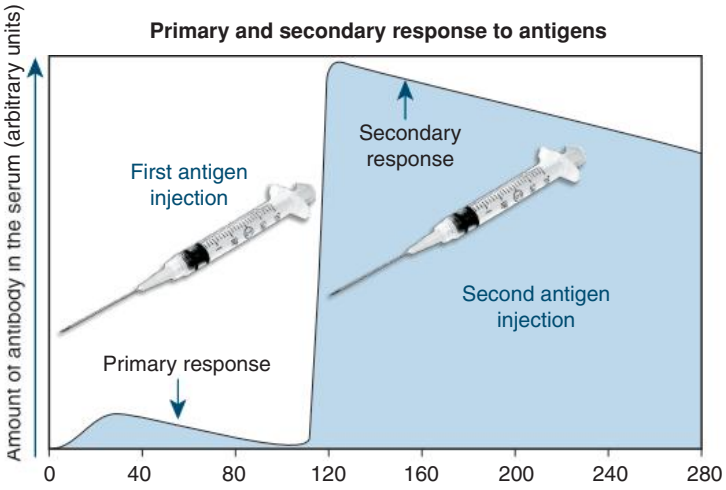
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Primary and secondary response to antigens

When the B cells encounter antigens and produce antibodies, the body develops active immunity against that antigen.

The initial response to antigenic stimulation, caused by the sudden increase in B cell clones, is called the **primary response**. Antibody levels as a result of the primary response peak a few weeks after the response begins and then decline. However, because the immune system develops an immunological memory of that antigen, it responds much more quickly and strongly when presented with the same antigen subsequently (the **secondary response**). This is shown on the right.

This process forms the basis of immunisation (vaccination) programmes, where a second injection (booster shot) is given following the initial vaccination.

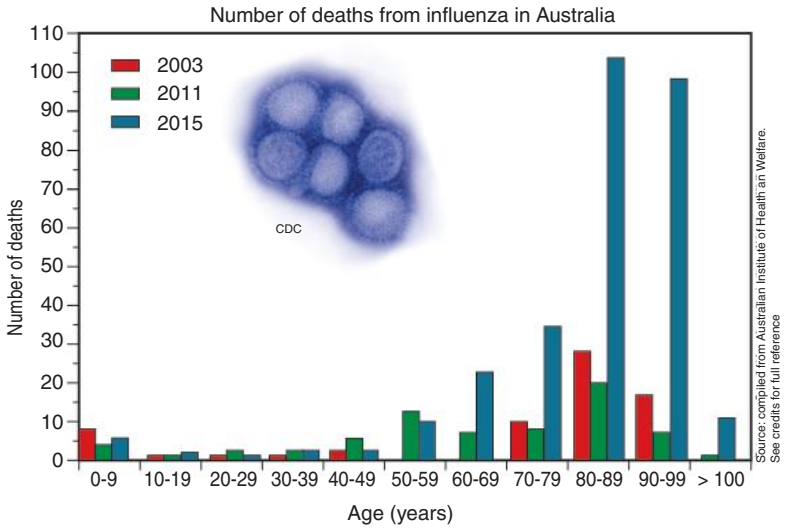


(b) Distinguish between naturally acquired active immunity and artificially acquired active immunity. Give examples:

3. (a) Describe the two main differences between the primary and secondary responses to presentation of an antigen:

(b) Why is the secondary response so different from the primary response?

4. Some diseases do not affect all members of a population equally. Socioeconomic factors, age, sex, ethnicity and where someone lives can influence how a disease affects a particular individual or population. The data (right) shows deaths from influenza (flu) in Australia by age over three years.



(a) Do you think the data shows an age related effect for influenza deaths? Explain your reasoning:

(b) Suggest what could be done to help reduce the number of influenza deaths:

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79 Chapter Review: Did You Get It?

1. Identify each of the following components of or processes in the adaptive immune system by the description provided:

   (a) The process by which a lymphocyte is stimulated to respond to a specific antigen: _____

   (b) This cell type secretes antibodies specific to a particular antigen: _____

   (c) This cell type processes and presents antigens to T helper cells: _____

   (d) These T cells destroy target cells on contact: _____

   (e) These cells are responsible for immunological memory: _____

   (f) This class of proteins are made by B cells and destroy specific antigens: _____

   (g) The chemicals activate T cells and cause them to multiply rapidly: _____

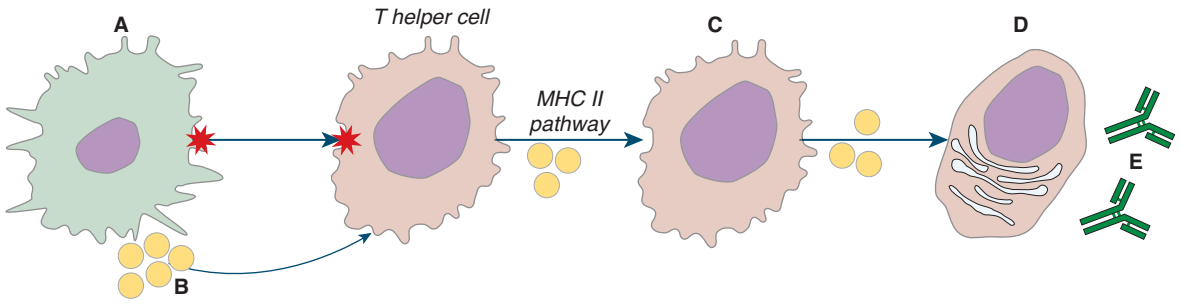
   (h) This immune system organ synthesises antibodies and removes antibody-coated material: _____

   (i) T cells mature in this organ: _____

   (j) Immunity developed over time to specific antigens: _____

2. Contrast the innate and the adaptive immune responses with reference to the basic action and the cells involved:

3. The diagram below shows part of the adaptive immune system reaction when an antigen is encountered.



   (a) What type of cell is A and what is its role? _____

   (b) Identify the structures labelled B and state their role in this part of the process: _____

   (c) What type of cell is C? _____

   (d) What type of cell is D? _____

   (e) Identify the structures labelled E and state their role: _____

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CHAPTER 9

Disease Challenges and Strategies

Key terms

autoimmune disease
biosecurity
emerging disease
endemic disease
epidemic
herd immunity
immunised
immunotherapy
immunity
isolation
monoclonal antibody
outbreak
pandemic
quarantine
re-emerging disease
transmission (of disease)
vaccination

The emergence of new pathogens

Key skills and knowledge

Activity number

- ☐ 1 Outline the history of pathogen introduction into naïve populations. Using examples, explain the effect novel pathogens have on naïve populations, e.g. smallpox and the impact of European arrival on Aboriginal peoples. How does global connectedness increase the vulnerability of populations to new diseases? **80**
- ☐ 2 Using examples, outline the factors involved in the emergence and re-emergence of diseases, e.g. the re-emergence of Hendra virus in Queensland the emergence of SARS-CoV-2 leading to the current 2020-2021 pandemic. **81**


USAF



CDC



Identifying and controlling the spread of pathogens

Key skills and knowledge

- ☐ 3 Describe strategies to identify pathogens and their sources (reservoirs) and track outbreaks, including molecular techniques and environmental testing. **82**
- ☐ 4 Explain what modes of transmission can tell us about how quickly a pathogen will spread in a population. What modes of transmission represent the greatest threat to controlling disease and why? **83**
- ☐ 5 Describe the methods by which countries control disease outbreaks, including the role of existing health infrastructure, vaccination programmes, and effective leadership and communication. **84 92**
- ☐ 6 Explain the role of quarantine and isolation in controlling the global spread of pathogen, including reference to the (current) Covid-19 pandemic. Describe the role of adequate sanitation, vaccination programmes, and safe behaviours in controlling disease on a local and global level. **85 87 92**
- ☐ 7 TEST Analyse and evaluate primary and secondary data relating to the effectiveness of recommended disease control methods. **86**
- ☐ 8 Describe the techniques scientists use to predict patterns of disease. Describe and explain the factors that influence the spread of pathogens through populations. **88 92**

Vaccination programmes

Key skills and knowledge

- ☐ 9 Explain what is meant by herd immunity and explain its importance to the resistance of the population to circulating pathogens. **89**
- ☐ 10 Recall the principles of vaccination, including reference to the primary and secondary response to infection and the role of these. **78 89**
- ☐ 11 Explain the role of vaccination programmes in maintaining herd immunity for a particular disease in the population and in (potentially) eliminating disease. **89 90 92**
- ☐ 12 PRAC Use a spreadsheet model to explore factors affecting spread of a disease. **91**

Immunotherapy

Key skills and knowledge

- ☐ 13 Describe the applications of monoclonal antibodies in treating cancer, including reference to principles and mode of action. **93 94**
- ☐ 14 Describe the applications of monoclonal antibodies in treating autoimmune diseases, including reference to principles and mode of action. **93 95**

80 Effect of Pathogens on Naïve Populations

Key Idea: Populations that have not encountered a specific pathogen before will likely suffer high infection rates and serious health consequences when exposed to it.

Populations in which a pathogen is endemic (naturally present all the time) are not always seriously affected by it. This may be because the population descended from survivors of an earlier epidemic and so have some immunity to the disease, gained by natural selection. It may also be a function of the pathogen being present all the time at a low level. With an endemic pathogen, there is always a certain level of immunity within the "host" population as a result of a certain level of infection and recovery. If an infectious pathogen does not exist in a population there is no selection pressure for immunity. If a new pathogen is introduced to a "immune naïve" population, a large proportion will be affected. There have been numerous examples of the effects of new pathogens on naïve populations throughout history and from all over the globe.

Global connectedness has spread pathogens

- Humans have travelled the globe since first migrating out of Africa. Sustained travel began around 4000 years ago with the beginning of trade routes across the Old World. Global travel began with European explorers reaching the New World. This global connectedness has helped spread pathogens across the world. It continues in modern times with pandemics such as the ongoing Covid-19 pandemic.
- As a result of travel, populations throughout history have often been exposed to new pathogens (e.g. the plague entering Europe via the Silk Road in the 1300s). Some of the best documented accounts of the effect of new diseases on naïve populations are those where previously isolated human populations have made contact with European explorers during the last 500 years. These populations include native Americans and the peoples of Australia and the Pacific.



Shaun Dunphy CC 2.0

A resting place for travellers on the Silk Road where plague could spread.

North America

- Smallpox, caused by *Variola major*, is a deadly disease with a mortality of ~35%. It did not reach the New World until European settlers arrived in the 16th century. Native Americans in both North and South America had no immunity to the disease and it caused enormous loss of life, wiping out half the population of some areas.
- The first smallpox epidemic among native Americans occurred in 1617 in Massachusetts. The epidemic spread across the continent, decimating native American populations between the time of the first epidemic and 1837. During the time of the American Revolution, the Piegan (right) lost half its people to smallpox. This level of mortality was typical for the native Americans exposed to the disease.



Public Domain

The Three Chiefs - Piegan. Image c1900 by Edward S. Curtis

Australia

- Like the Americas, Australia was populated with people naïve to the pathogens of Europe and Asia. One of the first and most deadly of the Old World diseases to affect Aboriginal Australians was, like the Native Americans, smallpox.
- Many European settlers had encountered the disease early in life (or been vaccinated from early smallpox vaccines or by variolation, an early inoculation method). However, its effect on Aboriginal Australians, who had never encountered the disease before European arrival, was devastating.
- Between 1788 and 1870 there were three smallpox epidemics (1789, 1829, and 1860). Coupled with many other new diseases, including measles and influenza, up to 70% of the Aboriginal population died.



Public Domain

The first smallpox epidemic began about 15 months after the arrival of the first fleet

1. What is a naïve population? _____

_____

2. Why were Pacific and American indigenous populations vulnerable to Old World diseases and pathogens?

_____

_____

_____

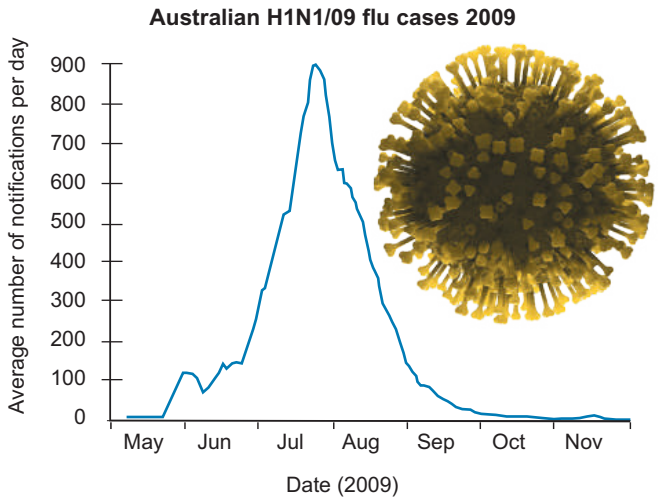
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Modern epidemics

- New pathogens appear constantly. Often these are slight variations of common diseases. As such, the human population generally has some level of immunity to them already. Occasionally, the variation is so great that the global population is essentially naïve. In these cases, the new disease can spread quickly and infect millions of people. Examples include the 1918 Spanish flu, the 2009 H1N1 influenza virus, and Covid-19 (2019).

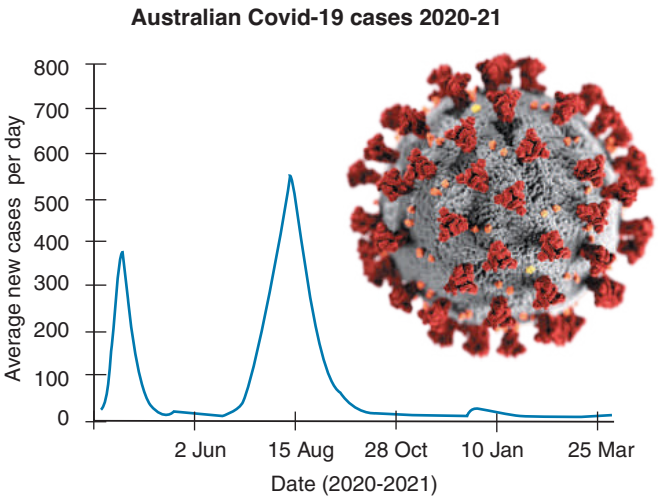
H1N1 Influenza

- The 2009 H1N1 influenza virus crossed the species barrier from pigs to humans in Mexico and from there spread to the rest of the world. Cases in Australia were first reported in May and quickly rose to a peak in late July 2009. Total cases for 2009 were over 37,000. The 2009 H1N1 influenza virus was the first influenza pandemic of the 21st century.
- The number of cases recorded in Australia showed a classic rapid increase as the virus spread through a naïve population (right).



Covid-19

- Covid-19 first appeared in China towards the end of 2019. By early 2020 it had swept across the globe infecting millions of people and killing tens of thousands. By April 2021 140 million people had been infected (75 million having recovered) and 3 million people had died worldwide.
- Covid-19 was first reported in Australia in January 2020. The spread of Covid-19 in Australia has been relatively limited due to State and Federal Government moves to severely restrict travel both within the country and into the country. Many Aboriginal communities have essentially isolated themselves from the rest of the country. As a result the Covid-19 infection rate in Aboriginal populations is six times lower than in the rest of the Australian population.
- Also infections and deaths from the virus in Australia are much lower per capita than elsewhere in the world where travel restrictions were not so strictly enforced. Relaxation of these measures resulted in a second wave (right) which again was controlled by resuming movement restrictions.



3. Using an example, explain why a disease entering a naïve population is likely to cause a severe epidemic:

4. How does global connectedness affect the spread of a disease? _____

5. Why has the spread of Covid-19 in Australia been relatively limited compared to many other countries?

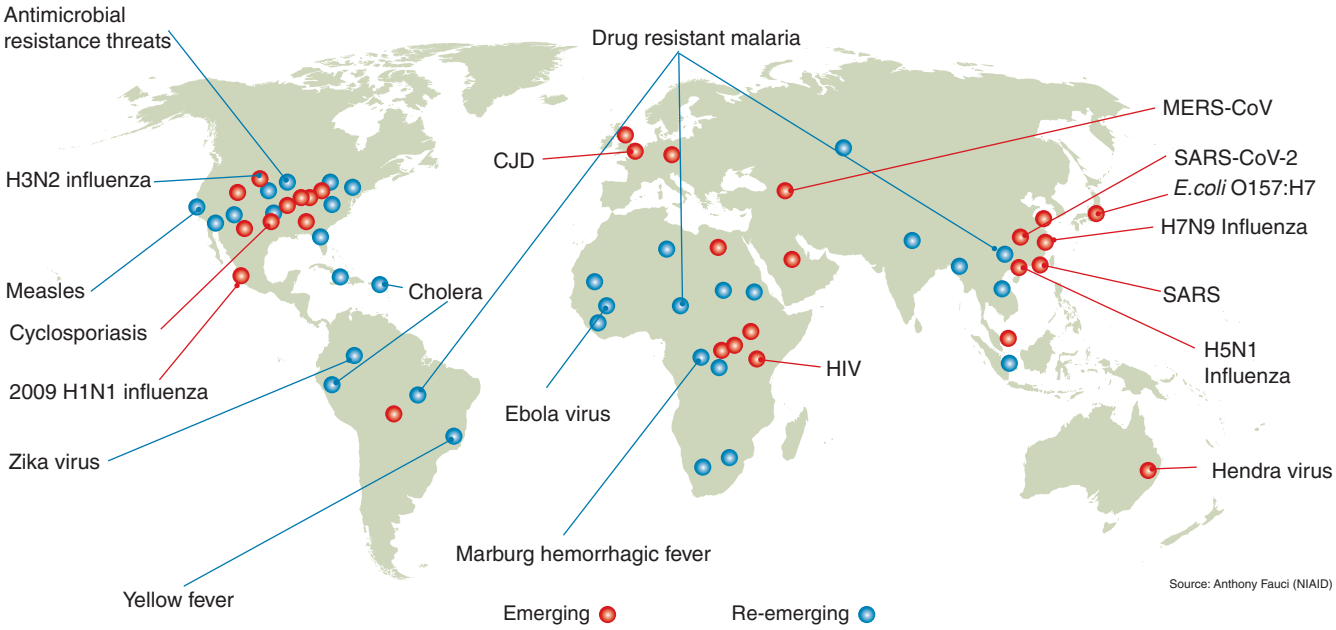
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81 Emergence of New Pathogens

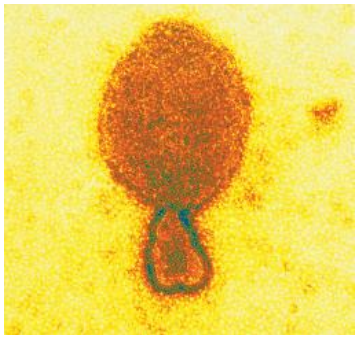
Key Idea: New pathogens are constantly being encountered. The presence of infectious disease never goes away. Some diseases are very difficult to eradicate and are always present, and some diseases are new (emerging). Diseases that are always present at low levels in a population or region are known as **endemic diseases**. Occasionally there may be a sudden increase in the prevalence of a particular disease. On a local level this is known as an **outbreak**. When an infectious disease spreads rapidly through a nation and affects large numbers of people it is called an **epidemic**. On rare occasions, a new kind of disease will appear and spread to other countries. The rapid spread of a disease throughout the world is a **pandemic**. Examples of pandemic diseases include HIV/AIDS, influenza, Zika virus, and Covid-19.

What are emerging and re-emerging diseases?

Emerging and re-emerging infectious disease since 1980



- Emerging infectious diseases are those that have never been encountered by humans (rare), have occurred previously but in small numbers and in isolated regions (e.g. Ebola), or have occurred throughout history but have only been recognised recently.
- Re-emerging infectious diseases are those that were once major health problems, then declined, but are now on the rise again. Examples include malaria and tuberculosis.
- The reasons for these emergences or re-emergences are varied and often particular to the disease. However some important factors include the movement of humans into previously uninhabited areas (e.g. by deforestation), greater movement of people from place to place and globally, or the failure of people to vaccinate or use antibiotics correctly (leading to antibiotic resistance).
- Although different countries have different abilities to identify new pathogens and diseases, the detection of new diseases over the last 40 years shows a uniform global pattern. No country or region appears to be more likely than any other to detect a new disease.



CSIRO cc 3.0

Hendra virus, which is carried by fruit bats and infects horses and humans, has emerged in Queensland.

1. What is the difference between emerging and re-emerging diseases? _____

2. (a) What factors are causing the emergence of new pathogens and diseases? _____

   (b) What factors are causing the re-emergence of once declining pathogens and diseases? _____

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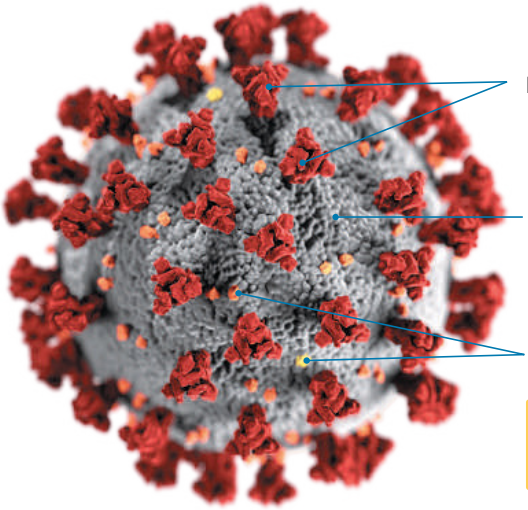
The emergence of Covid-19

- Reports of viral pneumonia (a lung infection) in Wuhan, China were reported on the 31st December 2019. Early in January 2020, a new coronavirus, SARS-CoV-2, was identified as the cause of the infections.

What is Covid-19?

- Covid-19 is the disease caused by infection with the SARS-CoV-2 virus (right).
- The virus affects the respiratory system.
- 80% of infected people recover without hospital care.
- 20% of infected people develop severe breathing problems and may require high level hospital care. The elderly and people with underlying medical problems are most at risk of becoming very sick.
- The virus is spread through the environment in small droplets from the nose and mouth (e.g. when a person speaks, sneezes, or coughs). People become infected when they breathe these droplets in, or when they touch a surface contaminated with the virus.
- Vaccines have now been developed.

A representation of the SARS-CoV-2 virus



CDC:Alissa Eckert & Dan Higgins

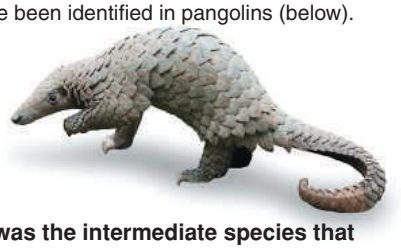
Where did SARS-CoV-2 come from?

- There are many questions over the SARS-CoV-2 virus that remain unanswered (below). Extensive investigations are ongoing.

Was the virus circulating earlier than the first reported case in December 2019?
This seems likely but researchers have not found antibodies in blood samples stored in blood banks before the first reported date of the virus. Very early circulation is therefore unlikely.

Did the virus come from bats?
While bats are a likely reservoir, no species has been found that carries viruses that completely match SARS-CoV-2. Also coronaviruses similar to SARS-CoV-2 have been identified in pangolins (below).





What was the intermediate species that passed the virus to humans?
The intermediate animal is still unknown. It is most likely a farmed animal brought to a wet market where live animals are sold is the direct source of the virus.

Did the virus originate in a "wet market?
Viral material has been found around wet markets in Wuhan. No firm conclusions have been drawn but wet markets provide the ideal conditions for viruses to jump between species.

To what extent was the virus circulating outside of China before November 2019?
Antibodies have been found in stored blood from Europe from November 2019 onwards. This is likely linked to travel from China. Early circulation could have been missed.


Wet market

Did frozen meat play a part in early infections?
Further analysis is needed, but it has been established that the virus jumped to people from live animals.

3. (a) What is the likely source (reservoir) of SARS-CoV-2? _____

   (b) How is the virus spread? _____

   _____

   _____

   (c) Why are investigations focusing are wet markets in Wuhan? _____

   _____

   _____

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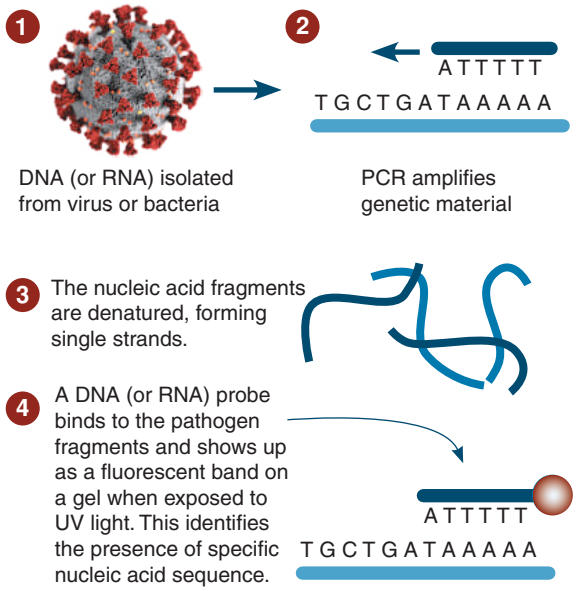
82 Identifying Pathogens and Their Sources

Key Idea: Pathogens can be identified by the symptoms they cause, by genomic studies, by environmental testing, and by tracking online reports and social media.

As technology has advanced it has provided a new range of tools that can be used to identify potential new pathogens, their origins, and to track their initial spread. While traditional disease surveillance relies on obtaining data through formal reporting systems, surveillance or online social networks (OSNs) can actually help researchers respond more quickly to potential disease outbreaks. Proactive testing of animal populations for potential pathogens is also helping us predict and plan for future epidemics.

Identifying novel pathogens

- Identifying pathogens is an important step in controlling or preventing the outbreak of disease. Because not all pathogens can be cultured and studied, modern molecular techniques are applied in identifying new pathogens. Most of these centre around analysing genetic material (DNA or RNA) and matching it to known sequences in global DNA databases.
- Genetic material is isolated and amplified using PCR. The exact method depends on whether the pathogen is viral or bacterial. Amplified material is then sequenced and the sequences matched to genome databases.
- Alternatively DNA or RNA probes designed to bind to specific DNA or RNA sequences are used to identify genetic material from potential pathogens (right).
- Novel pathogens will likely be related to known pathogens, so the new pathogen can be identified to a general degree relatively easily.
- For example, SARS-CoV-2 was originally identified by the analysis of its genome sequence from six affected patients. The genome sequence was found to have a 79.5% match with the SARS-CoV virus which was already known from a 2003 outbreak.
- Specific identification and categorisation of the pathogen are then carried out.



The hunt for new viruses

- PREDICT, a USAID collaborative program, takes a proactive approach in which researchers go into the field looking for new animal viruses.
- Bats are often a viral reservoir. The viruses causing SARS, MERS, and probably Covid-19, all originated in bats, so testing bats is logical when looking for new viruses.
- Blood samples are taken from bats (right) and analysed for viral genetic material. If the genetic material matches an entry in the database, the presence of a known virus can be confirmed. If there is no match in the database it means a new virus has been found.
- The database can also be used to diagnose patients and identify outbreaks. By testing a patient's blood it may be possible to identify the virus and develop a management plan. The detection of an unknown virus in a person's blood signals a new disease has emerged in humans.


USAF

1. Why are new pathogens found and identified using molecular techniques? _____

2. What are two ways of identifying new pathogen DNA? _____

3. What is the purpose of PREDICT and why is it a useful program? _____

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New ways to track a pandemic

- Traditional disease surveillance relies on obtaining data through formal reporting systems. The data is very good, but collating and analysing it takes time. Researchers are exploring faster, less formal ways to identify and track pandemics.
- Social media tags and internet search terms may help detect and map the spread of disease. In a disease outbreak, certain words (e.g. fever or cough) become more common on social media. People also do online searches for symptoms before visiting their doctor. Analysing the frequency and location of key terms could provide an early alert system. This type of analysis is not conclusive, but companies and health agencies are exploring it as a tool for early detection of disease outbreaks.
- Environmental testing can detect viruses in a population. Infected people shed SARS-CoV-2 in their faeces and into the sewage system. Analysing wastewater for the genetic material of a specific virus could detect outbreaks quickly, perhaps even before any symptoms are reported in the community. By sampling different parts of the sewage network, an outbreak can be pinpointed to a particular geographical area. This practice is being used in several countries to detect Covid-19. Several UK universities have reliable detection methods and are now looking at how to quantify the amount of virus in wastewater.



4. Why is surveillance of social media networks by health authorities a useful predictive tool? _____

5. The table below lists some infectious diseases. Complete the table identifying where and when the disease first appeared and how it came to infect humans (i.e. the source). You may need to do some extra research.

Disease	Where/when	Source (natural reservoir)
Zika virus		
Ebola		
HIV/AIDS		
Smallpox		
Measles		

6. Scientists are constantly trying to determine what the next potential disease epidemic will be and where it might start. Four families of viruses are likely candidates for causing the next epidemic or pandemic: picornaviruses, pneumoviruses, paramyxoviruses, and orthomyxoviruses. Work in groups of 4. Each person in the group should select one viral family to research. Which virus in your chosen family is a candidate for causing the next epidemic/pandemic and why (consider disease, hosts, transmission)? Report back to your group with an evidence-based argument for your choice. Based on the arguments of each person in the group, which virus ranks as the greatest threat? Justify your group's choice:

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83 Transmission of Disease

Key Idea: Infectious disease can spread rapidly within and between geographical regions given the right conditions.

The human body, like that of other large animals, is constantly exposed to a wide range of potential parasites and pathogens. Transmission and spread of a pathogen depends on its rate of growth, the density of the host population, the mobility of the host population, and the mode of transmission. The transmission of infectious diseases can be virtually eliminated by observing hygienic practices, providing adequate sanitation, and chlorinating drinking water.

Transmission and spread



Cough and fever are common symptoms of infection

Most pathogens, once inside the body, multiply rapidly, producing symptoms and making the host infectious within a few days. Others take longer to present symptoms. The infectious period can last from a few days to weeks, but in some cases the host may be infectious for long periods of time.



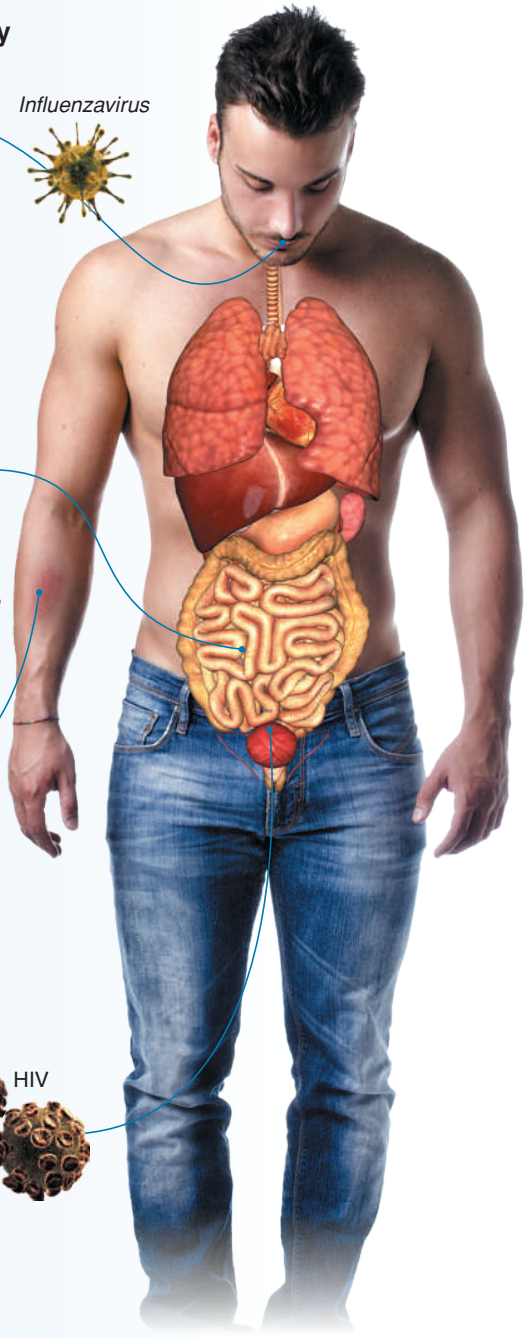
Human cities can contain millions of people, often living very closely together. In these congested conditions, infectious diseases can spread rapidly, especially if sanitation or personal hygiene is poor, or if seasonal weather produces conditions favourable to spread of the pathogen. High speed transport can help spread a pathogen around a region very quickly.



CDC Child with the measles rash

The type of transmission (direct, indirect, or vector) affects how quickly a pathogen can spread and also how easy (or difficult) it is to control its spread. Spread is also dictated by how infectious the pathogen is. Highly infectious pathogens spread much more rapidly than others. For examples measles is much more infectious than the flu.

Portals of entry



Respiratory tract

The mouth and nose are major entry points for pathogens, particularly airborne viruses, which are inhaled from the expelled mucus of infected people.
Examples: tuberculosis (TB), whooping cough, meningococcal meningitis, influenza, measles, rubella, chickenpox.

Influenzavirus

Salmonella typhi causes typhoid fever

Gastrointestinal tract

Food and water are often contaminated with microorganisms, but most of these are destroyed in the stomach.
Examples: cholera, typhoid fever, mumps, hepatitis A, poliomyelitis, salmonellosis.

Clostridium tetani causes tetanus

Breaking the skin surface

The skin provides an effective barrier to most pathogens, but cuts and abrasions allow pathogens to penetrate.
Examples: tetanus, gas gangrene, hepatitis B, rabies, malaria, and HIV.

Urinogenital openings

Urinogenital openings provide entry points for the pathogens responsible for sexually transmitted infections (STIs) and other opportunistic infections (i.e. thrush).
Examples: gonorrhoea, HIV.

HIV

1. Why can disease spread quickly in congested human cities? _____

_____

_____

2. Why would transmission by direct touch be slower than transmission by coughing or sneezing? _____

_____

_____

_____

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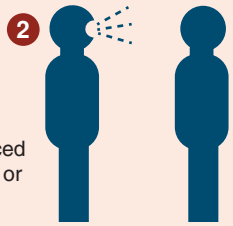
Modes of transmission

Direct

Direct person to person contact can occur when an infectious person touches or exchanges fluids with another.



Person to person transmission (e.g. touching, kissing).



Contact with droplets produced when couching or sneezing.

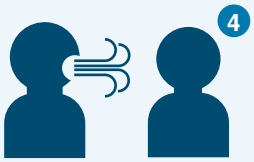
Indirect

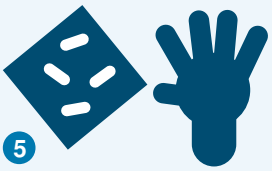
Indirect transmission occurs when an infected person infects another without having direct contact with that person.



Faecal-oral transmission. e.g. someone not washing hands properly after using the toilet.

In some cases the pathogen can become airborne. Transmission can occur by breathing or coughing.





Transmission by contact with a contaminated object touched by an infected person.

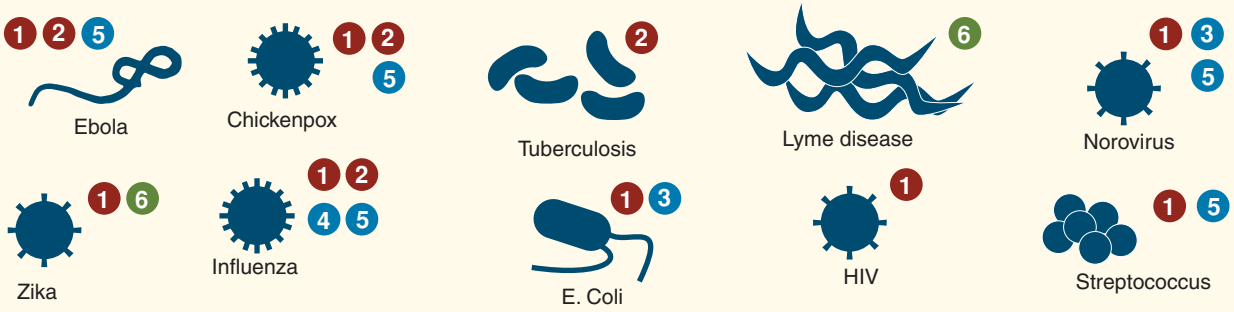
Vector

Vectors carry a pathogen from one person to another or from an animal to a human. Different vectors carry different pathogens.



Vector-borne pathogens are commonly carried by insects but sometimes other animals (e.g. ticks). They not only infect a person but can transfer the pathogen from person to person. The most well known example is malaria transmitted by the *Anopheles* mosquito.

Examples of transmission



Transmission of SARS-CoV-2

- The SARS-CoV-2 virus, which causes COVID-19, can be spread in the tiny liquid particles given off when an infected person coughs, sneezes, speaks, or breathes.
- Transmission can be by direct or indirect contact. Droplets may land on surfaces that are then touched by others. The virus needs to be breathed in or make contact with the mouth or nose. A person may touch a contaminated surface with their hands then touch their nose or use their hands to eat and so become infected by the virus.
- The modes of transmission of SARS-CoV-2 make wearing masks and washing hands before eating important factors in slowing the spread of the virus.



Coughing or sneezing into your hands is an ideal way of spreading viruses.

3. Why is coughing into your elbow more effective at stopping the spread of a virus than coughing into your hands?

4. How are vector-borne pathogens transmitted? _____

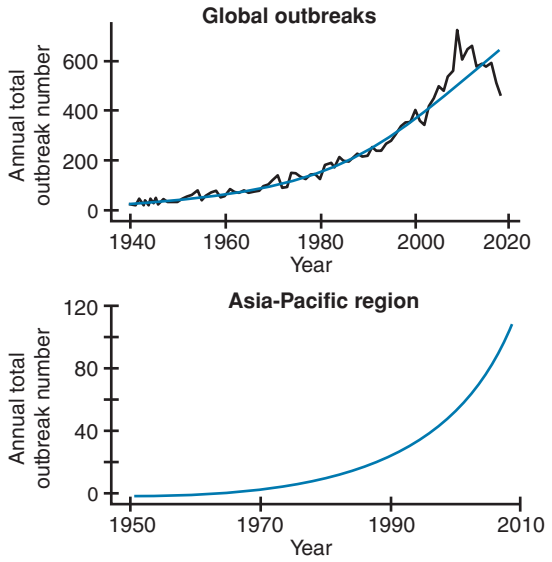
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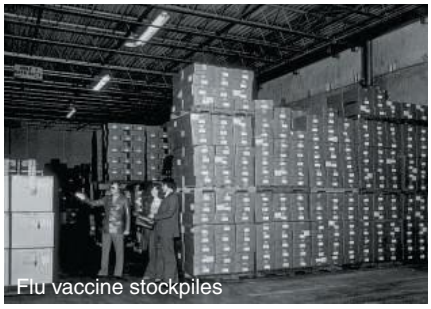
84 Managing Disease Outbreaks

Key Idea: High population densities and various socioeconomic factors throughout the world put many populations at risk of possible pandemics.

The vast range of countries, environments, beliefs, and political systems throughout the world makes it very difficult to produce a single actionable plan in the case of a pandemic. This has been seen in the various different approaches of different countries to the Covid-19 pandemic of 2020-21, some of which have not worked well. Exactly how an infectious disease is managed by a country depends on the type of disease, the size of the initial outbreak, and where the outbreak occurred.

- The total number of diseases with outbreaks and the total number of outbreaks has increased rapidly both throughout the world and in the Asia-Pacific region since the 1950s (right).
- Global disease outbreaks are not new. 2009 saw the H1N1 swine flu spread around the globe. Zika virus swept across the Pacific in 2013 and the Americas in 2015. In 2014, there was a large outbreak of Ebola in Africa. Although these diseases did spread far and relatively quickly, the outbreaks were also small enough to contain quickly. The relatively low rate of infection helped to keep them under control.
- Covid-19 first appeared in China at the end of 2019. By January 30 it had been declared a Public Health Emergency of International Concern (a pandemic). Within weeks the virus had spread so fast countries were beginning to close borders and to restrict the movements of citizens to try to contain the spread.
- How quickly countries did this and the severity to which they restricted movements made a big difference to how quickly internal outbreaks were contained.




Flu vaccine stockpiles

CDC

The cost of vaccines and health care, especially in poorer countries, is a major barrier to preventing outbreaks. A key part of containing outbreaks is to ensure developing countries have access to vaccines and treatments. But it is not reasonable to expect a nation with low financial resources to spend a large percentage of its income on medical equipment it might not need.


CDC

A lack of health infrastructure is another barrier to the prevention of outbreaks. For example, India spends just 3% of its income on health care whereas Australia spends more than 10% of its income on health care. This can mean that if a disease outbreak does occur in a vulnerable country, health care services may not be able to meet the demand for resources and treatments.


Panic buying is common in uncertain times

Danny Cho CC 2.0

Managing information is an important part of any pandemic management plan. People can now get up-to-date information on a disease from the internet. It is important for health authorities to maintain communication with the public. This can help to avoid speculation and panic, which may result in people taking inappropriate and ineffective actions.

1. Why are heavily populated countries with a low income more vulnerable to disease outbreaks than other countries?

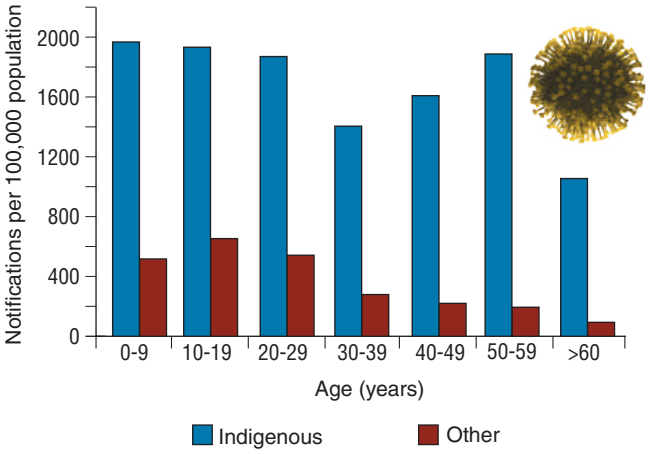
2. Why is maintaining communication with the public so important during a pandemic? _____

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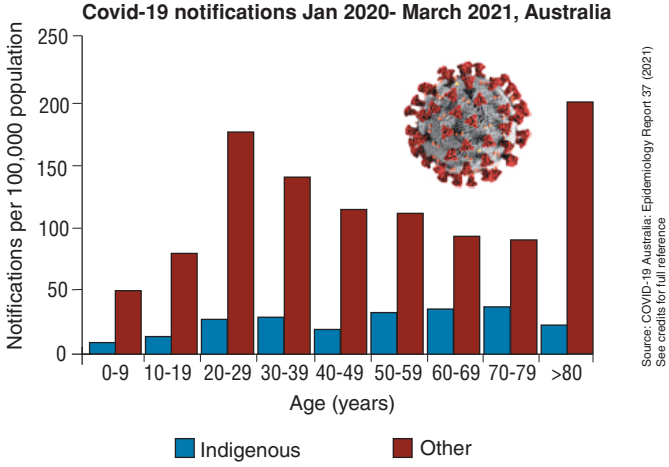
Responding to outbreaks

- How a country responds to a disease outbreak varies from outbreak to outbreak. It often depends on the type of disease, including how infectious it is and its mortality rate. It may also depend on a country's ideals and whether or not the new outbreak is perceived by the populace as a threat.
- It is informative to compare Australia's response to the 2009 outbreak of H1N1 influenza with the outbreak of Covid-19. In both cases, early efforts to delay the entry of the disease into Australia focussed on border control. However, while Australia's borders were never closed for the 2009 H1N1 flu, they were closed quickly for the Covid-19 outbreak. Movement restrictions (within and between states) were also used as necessary (e.g. following community transmission) to stop the spread of Covid-19. These are ongoing (as at May 2021). Many remote Indigenous communities isolated themselves from the greater populace.
- These differences in restrictions reflect the difference in severity of each disease. The SARS-CoV-2 virus is more infectious and more deadly that the 2009 H1N1 influenza virus. Infections require a much higher rate of hospitalisation and care.
- The rate of hospitalisation is key in the Covid-19 response. Closing the borders, restricting movements, and physical distancing measures were designed to reduce the strain on hospitals. If hospitals became crowded with Covid-19 patients they would be unable to perform their regular tasks (e.g. surgeries) and so death tolls could rise ever higher than from Covid-19 alone.
- The results of the different approaches to H1N1 flu and Covid-19 can be seen in the data below.

2009 H1N1 influenza notifications, 2009, Australia



Covid-19 notifications Jan 2020- March 2021, Australia



Source: COVID-19 Australia: Epidemiology Report 37 (2021)
See credits for full reference

Covid-19 cases in Australia

	Major cities	Locally acquired			Acquired interstate	Acquired overseas	Unknown	Total
		Inner regional	Outer regional	Remote Australia				
Indigenous peoples	91	15	6	1	2	34	1	150
Non-indigenous	20,706	921	221	16	125	6682	230	28,901

3. Why would restricting population movement help restrict the spread of a disease? _____

4. Study the data above. How did the different responses in Australia to H1N1 and Covid-19 affect the way the diseases spread through the population?

5. Why was the response to Covid-19 so much different to the H1N1 flu? _____

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85 Containing the Spread of Disease

Key Idea: Preventing the entry and spread of pathogens is important in protecting a country's population and industries from infectious diseases.

Many factors can influence the spread of disease, including the political and social climate, diet, general health, and access to medical care. Human intervention and modification of behaviour, including vaccination, can reduce the transmission rate of some diseases and inhibit their spread. Global air travel and international trade in commodities has increased the risk that diseases of humans, livestock, and crops will be spread between countries. Australia is fortunate in that its geographical isolation has helped to prevent the spread of disease from other parts of the world. Even so, pandemics such as H1N1 swine flu and Covid-19 still reached Australia.



Transmission of disease can be reduced by adopting 'safe' behaviours. Examples include using condoms to reduce the spread of STIs, **isolation** of people already infected, or establishing quarantine procedures for people who have been exposed to infection.



The environment can be made less suitable for the growth and transmission of pathogens. For example, spraying drainage ditches and draining swamps eliminates breeding habitats for mosquitoes carrying diseases such as Zika virus and malaria.



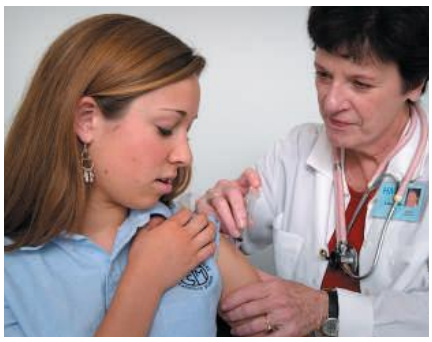
Disinfectants and sterilisation techniques, e.g. autoclaving (above), destroy pathogenic microbes before they have the opportunity to infect. The use of these techniques in medicine has significantly reduced post operative infections and associated deaths.



The development of effective sanitation, sewage treatment, and treatment of drinking water has virtually eliminated dangerous waterborne diseases from developed countries. These practices disrupt the normal infection cycle of pathogens such as cholera and giardia.



Appropriate personal hygiene practices reduce the risk of infection and transmission. Soap may not destroy the pathogens but washing will dilute and remove them from the skin. Although popular, antibacterial soaps encourage the development of strains resistant to antimicrobial drugs.



CDC

Vaccination schedules form part of public health programmes. Vaccination is one of the most effective ways of preventing transmission of contagious diseases. If most of the population is immunised, herd immunity limits outbreaks to sporadic cases and prevents epidemics.

1. (a) Identify three ways in which the environment can be made less suitable for establishment and transmission of diseases:

_____

_____

_____

(b) How is disease transmission reduced in medical care situations? _____

_____

(c) Why is sanitation important in preventing the spread of disease? _____

_____

_____

2. Why is reducing the prevalence of disease preferable to trying to contain an outbreak? _____

_____

_____

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Containing Covid-19

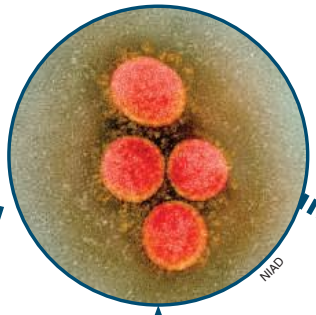
Breaking the chain of infection

Host susceptibility is an important link in the chain. Covid-19 spreads quickly in areas where people live close together and infectious people do not take steps to keep themselves away from others. Cultural differences between patients and medical workers, different countries, and in some cases mistrust of the authorities increase the risk of spread.

Susceptible host



Causative agent



The causative agent is the pathogen responsible for the infection, e.g. the SARS-CoV-2 virus. Eliminating this part of the chain from the environment is often the most difficult. It is likely the SARS-CoV-2 virus will never be fully eliminated.

The exact reservoir (primary host) of Covid-19 is still not known, although it is likely it is bats in the horseshoe bat family. Whether or not the virus is widespread or a novel strain is also not known.

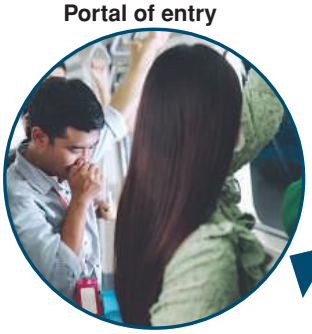
Reservoir



Covid-19 chain of infection

Targeting the transmission of the virus between people is one of the most effective ways to prevent infection. Preventing Covid-19 infection is based on good hygiene practices, which includes washing hands, disinfecting high touch surfaces (e.g. door handles), and wearing masks in public places.

Portal of entry



The portal of entry is how the organism enters a host. Covid-19 can enter the body through the nose and mouth.

Portal of exit



The portal of exit describes how the organism leaves the reservoir or host. It is still not known exactly how Covid-19 crossed from animals to humans, but it was possibly from sick animals in crowded markets.

Mode of transmission



3. Describe two important parts in the Covid-19 chain of infection that could result in continual infection of human populations:

4. Which part/parts of the Covid-19 chain of infection is/are likely the best to focus on in the current pandemic in order to break the chain of infection? Explain your answer?

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86 The Effectiveness of Hand Washing

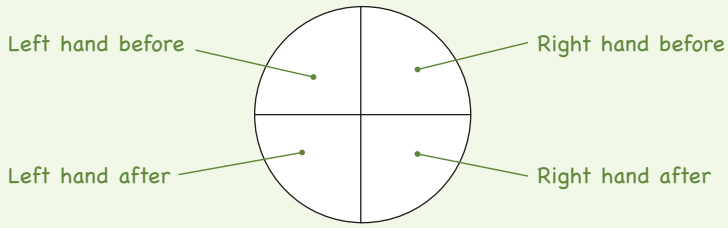
Assessment Task, Outcome 1: *Analysis and evaluation of primary and secondary data*

- We as humans spend much of our time manipulating objects with our hands, so it follows that our hands are covered with the microorganisms found in our environment. These microbes can then be easily transferred by touch to our mouths, such as when eating, or to other people, such as when we hand them an object. Hand washing after contact with potentially contaminated material reduces the chance of transmitting microbes to our internal environment or to others.
- In the practical below you will obtain data on the effectiveness of handwashing.

Investigation 9.1 Investigating the effectiveness of handwashing

See appendix for equipment list.

1. The class will be divided into thirds. One third will wash their hands with warm water. One third will wash their hands with soap and warm water and one third will use hand sanitiser. Your teacher will place you into one of these groups. **Do not wash your hands until step 5!**
2. Each person in the group should take a nutrient agar plate and use a marker pen to label the edge of the lid of the plate with name, the incubation temperature (e.g. 30°C), and which group you are in.
3. Then use the marker pen to divide the plate lid into quarters and label them as shown below:



4. Open the lid and press the tips of your middle and fore fingers from your left hand in the "Left hand before" quarter. Hold them there for 5 seconds. Then press the tips of your middle and fore fingers from your right hand in the "Right hand before" quarter. Hold them there for 5 seconds. Close the lid.
5. Now wash your hands using the regime assigned to your group (water, soap and water, hand sanitiser). Dry your hands if needed with a clean paper towel.
6. Open the lid of the agar plate again and press the tips of your middle and fore fingers from your left hand in the "Left hand after" quarter. Hold them there for 5 seconds. Then press the tips of your middle and fore fingers from your right hand in the "Right hand after" quarter. Hold them there for 5 seconds. Close the lid and seal it with clear tape.
7. Incubate the plate at your chosen incubation temperature, lid down, for 24 hours.
8. Retrieve the agar plates and observe the four different quarters. Count and record the number of bacterial colonies on the plate in each half (before and after). Do this for all the plates in your assigned group. If you only have a small number in your group, just enter the data you have. Calculate the mean number the colonies before and after (below).
9. Compare your means with means from the other groups in the class.

1. (a)

Your technique: _____	Plate number										Mean
Number of colonies before washing hands											
Number of colonies after washing hands											

(b) Handwashing technique: _____ Mean colonies before: _____ Mean colonies after: _____

(c) Handwashing technique: _____ Mean colonies before: _____ Mean colonies after: _____

2. Which technique appears to have the greater ability to remove bacteria from your hands? Explain why:

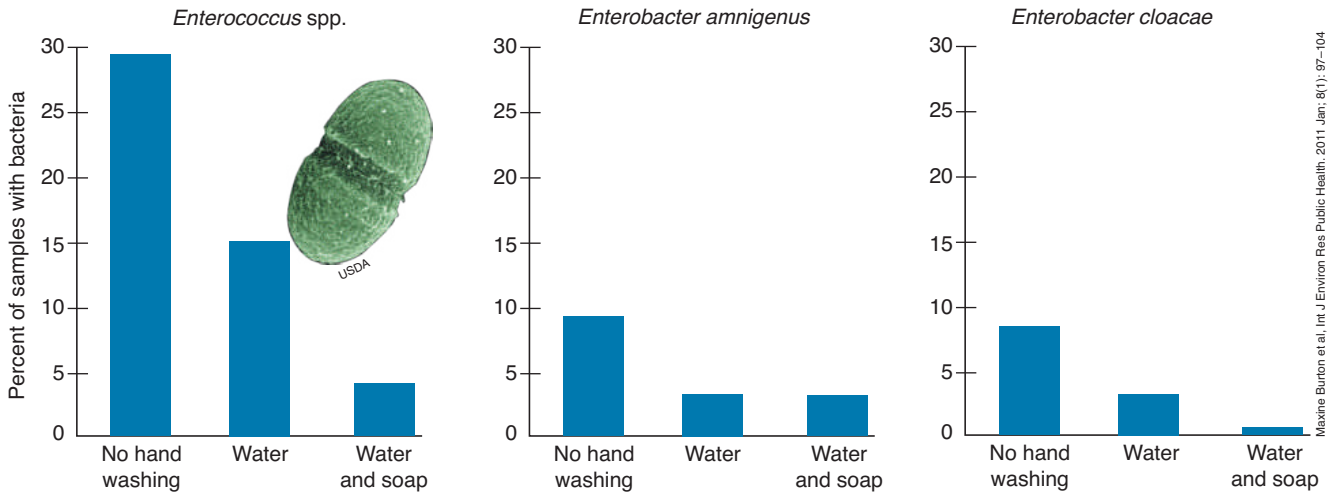
_____

_____

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Testing the effectiveness of hand washing

- A 2011 study performed the following experiment on the effectiveness of hand washing.
- Twenty volunteers deliberately contaminated their hands by contact with hard surfaces such as hand rails and door knobs in public areas.
- They were then randomly allocated to one of three groups: no hand washing, hand washing with tap water, or handwashing with soap and tap water. No instructions were given as to how to hand wash or for what length of time. Volunteers simply washed their hands as they would normally.
- Swabs were then taken from the volunteers' hands and transferred to agar plates. These were incubated at 35°C for 48 hours. This procedure was carried out 24 times for each volunteer for a total of 480 samples.
- The results are shown below. The bacteria found all occur in the intestines of animals (collectively called faecal coliforms).



Maxine Burton et al, Int J Environ Res Public Health. 2011 Jan; 8(1): 97–104

Questions 3-5 are with reference to the study above:

3. (a) Was hand washing an effective way to remove bacteria from the hands? _____

   (b) Which was the most effective method of removing bacteria from the hands? _____

4. Which bacterium was most common on the hands? _____

5. Why do you think the researchers gave no handwashing instructions to the volunteers?_____

6. Use your data and the experiment above to explain why hand washing is an important part of controlling the spread of disease, especially in a epidemic or pandemic situation (e.g. the Covid-19 pandemic).

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87 Quarantine and Biosecurity

Key Idea: Quarantine is a way of ensuring diseases do not enter a new area. It is an important part of biosecurity.

Biosecurity is important for Australia which, because of its relative isolation, has managed to exclude many of the pathogens that infect plants and animals in other countries. Precautions such as **quarantine**, which isolates exposed individuals that may be infected, as well as screening international travellers and imported produce, help to limit the entry of diseases into Australia. Quarantine is distinct from **isolation**, which aims to contain disease by isolating an already infected person. Quarantine played an important role in reducing the number of cases of Covid-19 entering the Australian population and thus reduced the incidence of community transmission.

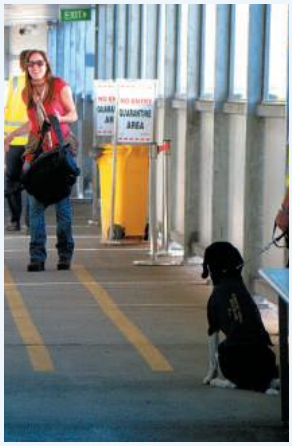
Biosecurity in Australia

Australia has strict biosecurity rules and measures in place to prevent the entry of pests and diseases into the country. For most people, this is most visible in airports when entering Australia from overseas. Passengers disembarking from aircraft are repeatedly reminded of what can't be brought into the country and instant fines are given to those who ignore the warnings. Inspection officers use X-ray machines and detector dogs to check' luggage for prohibited goods, especially fresh food or animal and plant materials.

Biosecurity inspections are also made on goods entering on cargo ships. Shipping containers are inspected for unwanted plants or animals that may have entered the container when loading. This is common in fresh food containers (e.g. fruits). If pests are found, the containers may be turned away. Inspection of many goods occurs at the home port before they are loaded on to cargo ships.

Goods PROHIBITED entry into Australia without authorisation

- Milk and dairy products
- Seeds and beans
- Popping corn and raw nuts
- Eggs and egg products
- Fresh fruit and vegetables
- Live animals
- Meat and fish products
- Live plants
- Biological materials (e.g. human/animal vaccines)
- Deer horn/velvet
- Soil and sand



Bjorn Christian Tørrissen CC3.0

The role of quarantine

When organisms are brought into Australia they must undergo a quarantine period to monitor health and ensure no pests or diseases are in or on the organism. Quarantine may also apply to travellers who have been in contact with infected persons or have returned from places experiencing disease outbreaks. These procedures were used during the SARS epidemic in 2003, the swine flu pandemic in 2009, and the Covid-19 pandemic.

The equine industry is an important part of the Australian economy. Live horses entering the country are quarantined to check for diseases that may affect the industry. In August 2007, equine influenza was found in horses at the Eastern Creek Quarantine Station, the first time it had entered Australia. Somehow, it escaped quarantine and spread throughout NSW and into Queensland. As a result of strict non-movement orders, the outbreak was contained by February 2008.



Equine influenza is easily spread.

1. Why is biosecurity important for Australia? _____

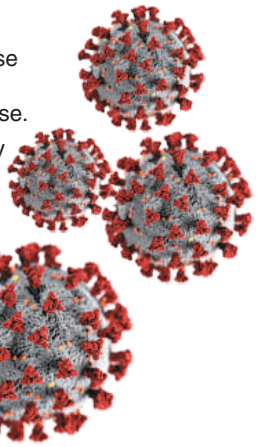
2. Why is pre-inspecting goods at the home port a useful biosecurity measure? _____

3. How does quarantine prevent the spread of disease? _____

4. How does Australia's geographic position help prevent the entry and spread of disease in Australia? _____

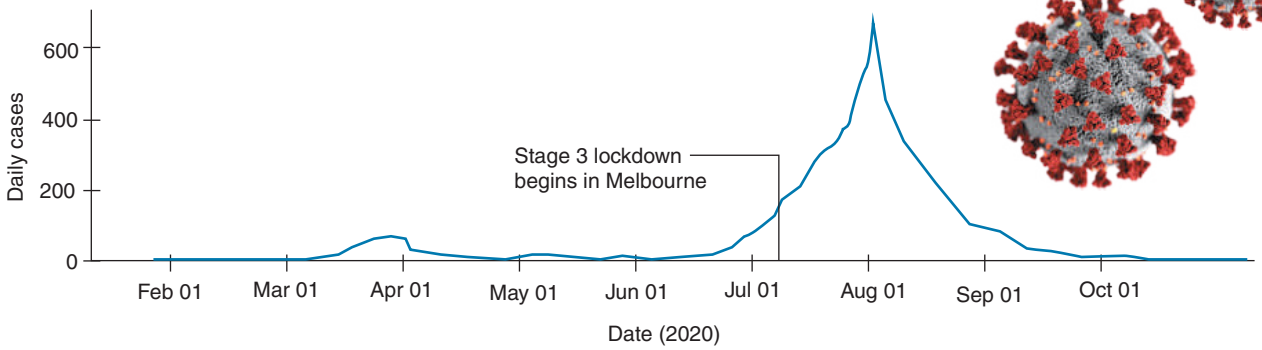
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The effectiveness of lock-downs in containing Covid-19



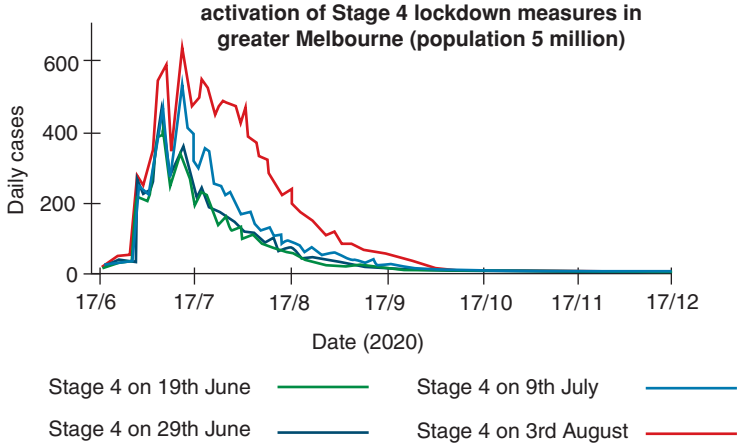
- Early in 2020 Australia implemented strict measures to keep infections of SARS-CoV-2 under control. These included closing the border to all but returning Australian citizens. Some states implemented lockdown measures where citizens were required to stay home and all but essential businesses were required to close.
- These measures were especially important in Melbourne, which experienced a second wave of community cases much greater than when Covid-19 was first detected.

Daily cases Covid-19 Victoria



- On August 3rd, the Melbourne metropolitan area began a Stage 4 lockdown. All non essential businesses were closed and citizens were required to stay within 5 kilometres of their home. An 8 p.m. to 5 a.m. curfew was put in place. Face coverings were made mandatory shortly after. The lockdown ended on 8 November when all restrictions were lifted.
- The lockdown eliminated the virus from the community, with zero cases being recorded at the end of the lockdown. Authorities went into the lockdown with the goal of eliminating the virus, saying it was important a clear goal was set for Melburnians to obtain.
- Subsequent modelling (right) showed the reduction in case numbers that could have been achieved by earlier activation of a level 4 lockdown compared to lockdown on August 3rd (red curve).

Predicted Covid-19 case numbers for earlier activation of Stage 4 lockdown measures in greater Melbourne (population 5 million)



Source: GJ Milne *et al.* 2020, see credits for full reference.

5. Why did Covid-19 cases continue to rise even after Melbourne entered a strict lockdown? _____

6. Melbourne entered a level 4 lockdown on 3rd August 2020. Describe the effect on Covid-19 cases if the level 4 lockdown had been activated earlier:

7. Why would setting a clear goal for a lockdown play an important part in reducing cases of Covid-19 (or any other disease) and maintaining moral?

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88 Predicting Future Patterns of Disease

Key Idea: Predicting future disease outbreaks relies on monitoring current disease episodes and using population statistics to identify potentially vulnerable groups of people.

Disease outbreaks have occurred throughout history. The plague that spread through Europe in the 1400s (the Black Death) and again in the mid 1600s (the Great Plague) were some of the most devastating outbreaks ever. Before Covid-19, the Spanish flu was the last great worldwide pandemic. In most cases of modern pandemics, the improved global cooperation and more effective action by health authorities have improved disease containment and most pandemics rarely last longer than a few months. However, Covid-19 has shown the world how quickly a new virus can get out of control if people do not follow health advice.



Predicting future disease is often a case of identifying diseases in animals that could cross over to humans. Many infectious diseases have an animal origin including various strains of influenza (e.g. avian flu H5N1 and swine flu H1N1). Identifying these pathogens in animals, especially livestock and poultry living in close proximity to humans, can help prepare for possible outbreaks.



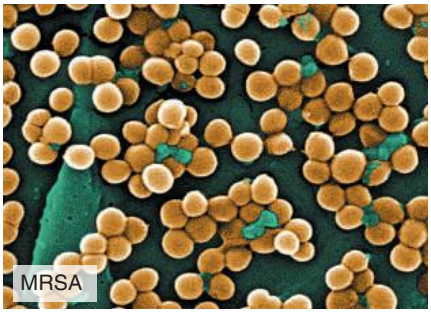
Population density is important to how quickly an infectious disease can spread. Cities generally have very high population densities, with some reaching densities of over 20,000 per km^2. Disease spreads most quickly in areas with poor living conditions, poor sanitation, and low levels of immunity. For example, the 1918 Spanish flu initially spread quickly due to the cramped confines of military camps and hospital wards.



How quickly an infectious disease spreads also depends of the population's mobility. The 1918 Spanish flu spread around the world due to infected troops returning home and taking the disease with them. Part of predicting where and when diseases will occur is being able to predict the movements of groups of people. For example people moving from rural areas to cities may transport potential pathogens from livestock.



The spread of disease also depends on the mode of transmission. Is it spread by air borne particles or by touch? The most feared scenario is an airborne pathogen that is highly contagious, has a long infectious period, and is ultimately deadly.



Resistance to antibiotics is becoming a greater problem with many bacterial strains becoming extremely difficult to treat. Plans need to be in place for if (or when?) a highly resistant pathogenic bacteria begins to spread through the general population.



Possible climate change is already being taken into account to predict where possible outbreaks may occur. For example, malaria may spread further north and south from the tropics as the climate becomes more favourable for its mosquito vectors.

All photos this row: CDC

1. Explain how each of the following are important in predicting where the next epidemic may originate:

   (a) Pathogen in livestock related to human diseases: _____

   _____

   (b) Population density: _____

   _____

   (c) Global travel networks: _____

   _____

   (d) Resistance to antibiotics: _____

   _____

   (e) Climate change: _____

   _____

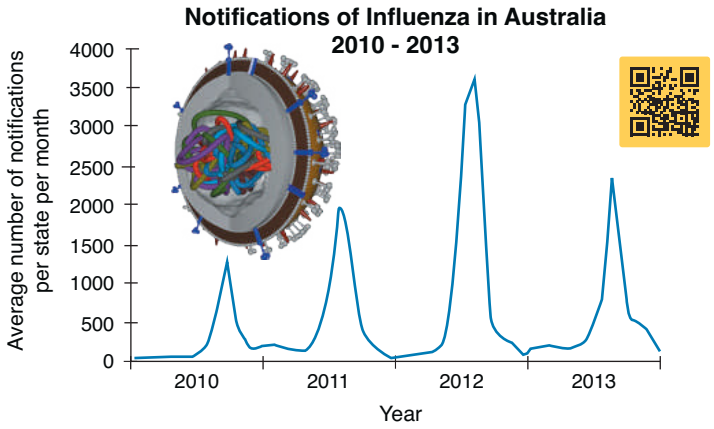
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Seasonal patterns

- Some diseases are seasonal, showing patterns of increased or decreased prevalence at specific times of the year. Influenza (the flu) commonly becomes more prevalent in the winter of both the Southern and Northern Hemispheres. In Australia, Ross River fever becomes more common during the summer/autumn rainy season (January to March). The seasonal patterns of these diseases allow for simple predictions of when most cases will occur. For example, health authorities prepare for increased influenza cases in winter by offering the latest vaccines.

Influenza

Although it is often looked upon as simply a bad cold, influenza (commonly called "the flu") has arguably caused more human deaths than any other disease throughout history. Influenza occurs in seasonal epidemics, usually infecting around five million people globally, and causing the death of between 250,000 and 500,000 people annually. The influenza virus continually changes in two ways. **Antigenic drift** involves small cumulative genetic changes over time. Occasionally the virus will also undergo **antigenic shift**, producing an entirely new strain that causes a pandemic. The latest of these is the H1N1/09 strain, commonly referred to as swine flu. This strain caused around 14,000 deaths globally. Compare this the Spanish flu pandemic of 1918-1920, during which possibly up to 100 million people died globally (more than all deaths during World War I and possibly as many as World War II).

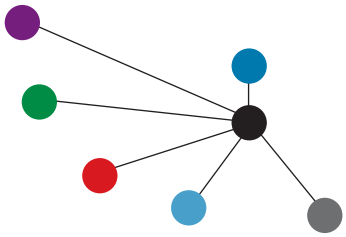


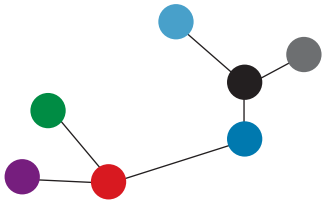
Predicting global spread

- In ancient times the quickest way to reach a destination was to travel on land - usually walking. Distance thus predicted how quickly a disease would spread. A disease often spread in a circular pattern from a central point or followed common trade routes.
- Today it is not always distance that dictates the spread of a disease but time. Global air travel routes make it often faster and easier to reach a destination by flying from a small hub to a larger one then on to another small hub, rather than flying directly between two small hubs. For example, one does not fly direct from Melbourne to Rio de Janeiro. Instead one flies to Sydney then to San Francisco, then to Houston, then to Rio de Janeiro.
- Thus the spread of a disease can be modelled based on the origin and where the major travel hubs are located and the time taken to get between those hubs.


A few central flight hubs (large airports) service the rest of the world. This has a big impact on how a disease spreads.

Jpatokal CC 3.0


Based on distance, a travel map may look like this.


Based on flight times, a travel map may look like this.

2. (a) What time of the year is the influenza virus most prevalent in Australia? _____

   (b) Why does Influenza show the seasonal pattern it does? _____

   _____

   _____

   _____

3. In the boxes below draw a diagram to show how a disease might spread based solely on distance from the source, and then based on modern travel times from the source, as shown above:

Travel distance

Travel time

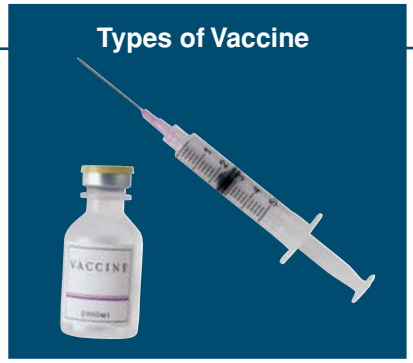
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89 Vaccines and Vaccination

Key Idea: A vaccine is a suspension of antigens that is deliberately introduced into the body to protect against disease. If enough of the population are vaccinated, herd immunity provides protection to unvaccinated individuals.

A **vaccine** is a preparation of a harmless foreign antigen that is deliberately introduced into the body to protect against a specific disease. The antigen in the vaccine is usually some part of the pathogen and it triggers the immune system to produce antibodies against the antigen, but it does not cause the disease. The immune system remembers its response and will produce the same antibodies if it encounters the antigen again. If enough of the population are vaccinated, herd immunity (indirect protection) provides unvaccinated individuals in the population with a measure of protection against the disease. There are two basic types of vaccine, subunit vaccines and whole-agent vaccines (below).

Types of Vaccine



Whole-agent vaccine

Contains whole, non-virulent microorganisms

Inactivated (killed)

Viruses for vaccines may be inactivated with formalin or other chemicals. They present almost no risk of infection, e.g. most influenza vaccines, Salk polio vaccine.



Attenuated (weakened)

Attenuated viruses are usually strains in which mutations have accumulated during culture. These live viruses can back-mutate to a virulent form, e.g. MMR vaccine.



Subunit vaccine

Contains some part or product of microbes that can produce an immune response. Includes vaccines made using genetic engineering, inactivated toxins, and conjugated and **acellular vaccines**, e.g. the diphtheria-tetanus-pertussis vaccine and the vaccine against bacterial meningitis.



Why are vaccinations given?



CDC

Vaccines against common diseases are given at various stages during childhood according to an immunisation schedule. Vaccination has been behind the decline of some once-common childhood diseases, such as mumps and measles.



Most vaccinations are given in childhood, but adults may be vaccinated against a disease (e.g. TB, tetanus) if they are in a high risk group (e.g. the elderly or farmers) or to provide protection against seasonal diseases such as influenza.



Tourists may need specific vaccines if the country they are visiting has a high incidence of a certain disease. For example, travellers to South America should be immunised against yellow fever, a disease that does not occur in Australia.

1. (a) What is a vaccine? _____

_____

(b) Provide some examples of when vaccinations are needed: _____

_____

_____

_____

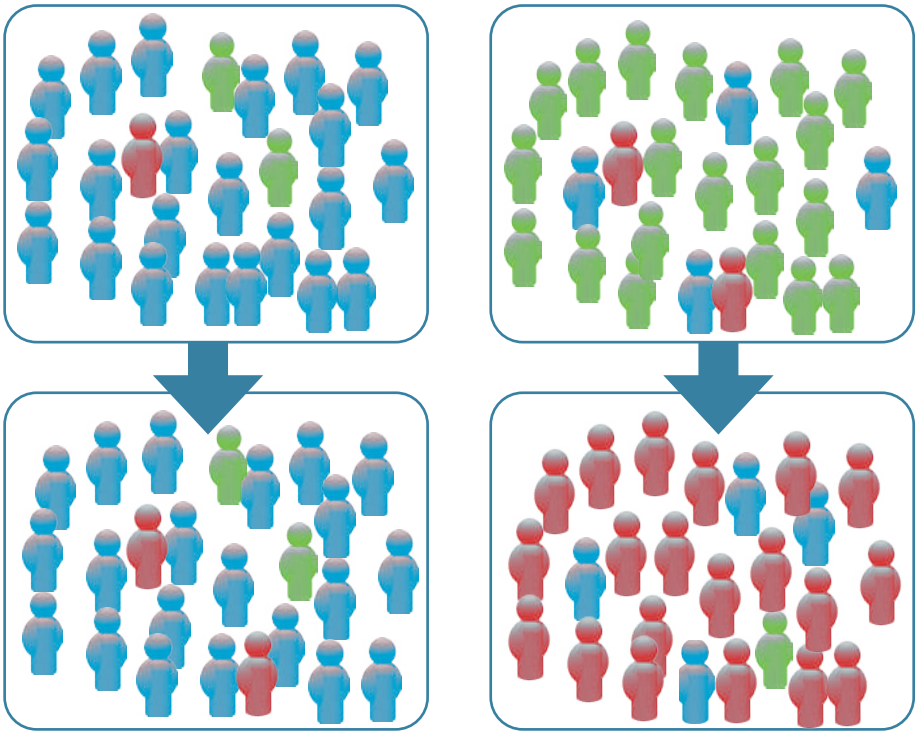
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Vaccination can provide herd immunity

Herd immunity occurs when the vaccination of a significant portion of a population provides some protection for individuals who have not developed immunity (e.g. have not been vaccinated and are not immunised). In order to be effective for any particular disease, a high percentage of the population needs to be vaccinated against that disease. High vaccination rates make it difficult for the disease to spread because there are very few susceptible people in the population. Herd immunity is important for people who cannot be vaccinated (e.g. the very young, people with immune system disorders, or people who are very sick, such as cancer patients).

High herd immunity: Most of the population is immunised. The spread of the disease is limited. Only a few people are susceptible and become infected.

Low herd immunity: Only a small proportion of the population is immunised. The disease spreads more readily through the population infecting many more people.



Immunised and healthy

Not immunised and healthy

Not immunised, sick and contagious

DID YOU KNOW?

The level of vaccination coverage to obtain herd immunity differs for each disease. Highly contagious diseases (e.g. measles) need a much higher vaccine uptake (95%) than a less contagious disease such as polio (80-85%).

2. Attenuated viruses provide long term immunity to their recipients and generally do not require booster shots. Why do you think attenuated viruses provide such effective long-term immunity when inactivated viruses do not?

3. (a) What is herd immunity?

(b) Why are health authorities concerned when the vaccination rates for an infectious disease fall?

4. Some members of the population are unable to be vaccinated. Give an example and explain why herd immunity is very important to them?

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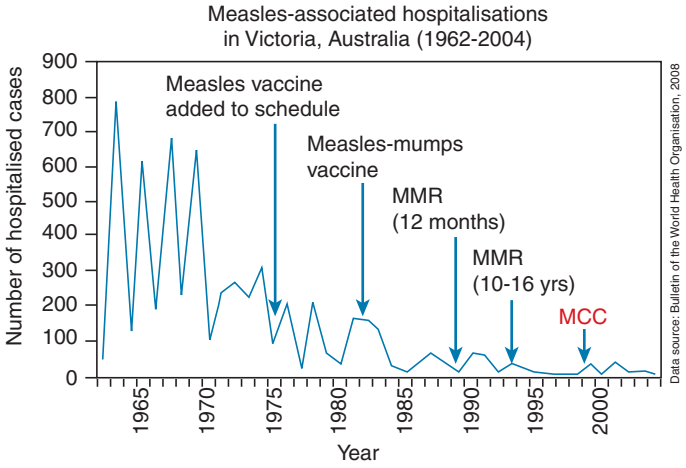
90 Vaccines Can Eliminate Infectious Disease

Key Idea: Vaccination programmes have been successful in the global eradication of smallpox, but other diseases (such as influenza) are more difficult to eradicate.

To date, the only infectious disease globally eradicated has been smallpox. Several factors lead to this success. Smallpox is easily identifiable by its characteristic rash making surveillance and containment of infected patients easier. It has no other natural carriers so once immunisation rates reached a critical level, its spread through the population was limited. Other diseases can be more difficult to eradicate. This is especially true for diseases that have a long period between infection and the symptoms showing (e.g. TB) or diseases caused by pathogens with high rates of mutation (e.g. *influenzavirus* or HIV).

Measles elimination in Australia

Measles is a highly contagious disease, one infected person can infect 12-18 people during their infectious period. In 2014, the World Health Organisation (WHO) announced measles had been eliminated from Australia. High vaccination rates contributed to its elimination. However, measles still occurs in other countries so it could be reintroduced if an infected traveller entered Australia. Maintaining high levels of vaccination is important in preventing its reintroduction.



The graph above shows the role of vaccination in reducing measles hospitalisations in the state of Victoria.
MMR is the introduction of the measles/mumps/rubella vaccine.
MCC (measles control campaign) was an extensive mass vaccination and monitoring campaign.


CDC

Whooping cough (above) is a respiratory disease caused by the bacterium *Bordetella pertussis*. Despite high vaccination rates, whooping cough is increasing in Australia. Several factors may be contributing to this.

- Until 1997, a whole vaccine was used. It contained hundreds of different antigens and provided protection against many strains of the pertussis pathogen. In 1999, an acellular vaccine, which does not contain the whole pathogen, was introduced (inset above). It only contains five antigens and so provides less protection.
- New strains of *B. pertussis* are evolving, and the new vaccine is not effective against them.
- More adults who were vaccinated against whooping cough in childhood are contracting the disease. This suggests the effectiveness of the vaccine declines over time.

1. The graph above provides long term immunity data for measles in Victoria. Use this data to provide evidence for the role of vaccination programmes in eliminating measles from Australia:

_____

_____

_____

_____

2. What could happen if vaccination rates for measles fell too low? _____

_____

3. (a) Why could the change to a new vaccine have affected the rates of whooping cough in Australia? _____

_____

_____

_____

(b) Why do you think a new vaccine was introduced? _____

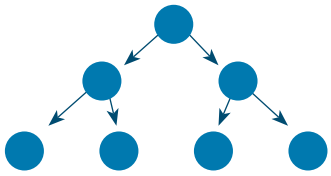
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91 Modelling Disease Outbreak and Spread

Key Idea: Being able to model the spread of a disease can help predict where, when, and how it will spread.
Modelling how a disease spreads can help preparation for an eventual outbreak. Elements of the model must account for how infectious a pathogen is and for how long, the density and mobility of the population, and even the level of mortality of infected people. These models can be used to test the effectiveness of public health measures.

Modelling a disease

- A spreadsheet can be used to model the spread of disease. There are also numerous online models that can be used.
- In the most simple model (right) whenever an infected person meets another, a new infection occurs. The number of interactions at each infection cycle affects the spread of the disease.
- Using a spreadsheet, you will first model an infected person meeting (and infecting) two other people. In this model, once the infected person has infected two people they are no longer infectious.



A simple infection model. One person infects two, who infect two more...

Investigation 9.2 Modelling disease outbreak and spread

See appendix for equipment list.

1. Working in pairs, enter the following into a spreadsheet:

	A	B
1	New infections	Total Infections
2	1	=SUM($A$2:A2)
3	=A2*2	=SUM($A$2:A3)
4	=A3*2	=SUM($A$2:A4)
5	=A4*2	=SUM($A$2:A5)
6	=A5*2	=SUM($A$2:A6)
7	=A6*2	=SUM($A$2:A7)
8	=A7*2	=SUM($A$2:A8)
9	=A8*2	=SUM($A$2:A9)

One infection cycle. Copy this down to row 12 (10 cycles of interactions).

1. How many new infections are there per infection cycle after 10 infection cycles? _____
2. How many infected people are there in total after 10 infection cycles? _____

2. Now set the interactions per infected person to 3 (A2*3) and reset the model.

3. How many new infections are there per cycle of infection after 10 infection cycles? _____
4. How many infected people are there after 10 cycles of infection? _____

3. We can now extend the model by adding in a little randomness. The number of people interacting with each infected person may not always be the same. In our extended model, we shall randomise the number of people interacting to between 1 and 4.

	A	B	C
1	New infections	People interacted with per person	Total infected people
2	1	=RANDBETWEEN(1,4)	=SUM($A$2:A2)
3	=A2*B2	=RANDBETWEEN(1,4)	=SUM($A$2:A3)
4	=A3*B3	=RANDBETWEEN(1,4)	=SUM($A$2:A4)
5	=A4*B4	=RANDBETWEEN(1,4)	=SUM($A$2:A5)
6	=A5*B5	=RANDBETWEEN(1,4)	=SUM($A$2:A6)
7	=A6*B6	=RANDBETWEEN(1,4)	=SUM($A$2:A7)
8	=A7*B7	=RANDBETWEEN(1,4)	=SUM($A$2:A8)
9	=A8*B8	=RANDBETWEEN(1,4)	=SUM($A$2:A9)
10	=A9*B9	=RANDBETWEEN(1,4)	=SUM($A$2:A10)

Add new infections to total from previous row (cycle)

Generates a random number between 1 and 4

Calculates the total number of people infected

5. Run the model five times by recalculating the spreadsheet using the **recalculate** or **calculate now** option (depending on your spreadsheet). On average, how many people in total have been infected after ten cycles?

_____

_____

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4. Not all interactions will result in an infection. The pathogen may not be highly infectious or the correct mode of transmission may not have occurred (for example, a person with a cold may have been careful where and how they coughed).

5. First we need to decide the probability of each interacting person being infected. For this model we will say there is a 50% chance that any interacting person will be infected. We shall first produce a random number between 0 and 1 (see * below). We can now use this block of infected (1) or not infected (0) cells in our model. Once the formula is set up, you can recalculate the spreadsheet to obtain different infection scenarios.

	A	B	C	D
1	New infections	People interacted with per person	Infected people	Total infected people
2	1	=RANDBETWEEN(1,4)	=IF(B2=1,$B$15,IF(B2=2,$B$15+$B$16,IF(B2=3,$B$15+$B$16+$B$17,IF(B2=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A2)
3	=A2*C2	=RANDBETWEEN(1,4)	=IF(B3=1,$B$15,IF(B3=2,$B$15+$B$16,IF(B3=3,$B$15+$B$16+$B$17,IF(B3=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A3)
4	=A3*C3	=RANDBETWEEN(1,4)	=IF(B4=1,$B$15,IF(B4=2,$B$15+$B$16,IF(B4=3,$B$15+$B$16+$B$17,IF(B4=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A4)
5	=A4*C4	=RANDBETWEEN(1,4)	=IF(B5=1,$B$15,IF(B5=2,$B$15+$B$16,IF(B5=3,$B$15+$B$16+$B$17,IF(B5=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A5)
6	=A5*C5	=RANDBETWEEN(1,4)	=IF(B6=1,$B$15,IF(B6=2,$B$15+$B$16,IF(B6=3,$B$15+$B$16+$B$17,IF(B6=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A6)
7	=A6*C6	=RANDBETWEEN(1,4)	=IF(B7=1,$B$15,IF(B7=2,$B$15+$B$16,IF(B7=3,$B$15+$B$16+$B$17,IF(B7=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A7)
8	=A7*C7	=RANDBETWEEN(1,4)	=IF(B8=1,$B$15,IF(B8=2,$B$15+$B$16,IF(B8=3,$B$15+$B$16+$B$17,IF(B8=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A8)
9	=A8*C8	=RANDBETWEEN(1,4)	=IF(B9=1,$B$15,IF(B9=2,$B$15+$B$16,IF(B9=3,$B$15+$B$16+$B$17,IF(B9=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A9)
10	=A9*C9	=RANDBETWEEN(1,4)	=IF(B10=1,$B$15,IF(B10=2,$B$15+$B$16,IF(B10=3,$B$15+$B$16+$B$17,IF(B10=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A10)
11	=A10*C10	=RANDBETWEEN(1,4)	=IF(B11=1,$B$15,IF(B11=2,$B$15+$B$16,IF(B11=3,$B$15+$B$16+$B$17,IF(B11=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A11)
12	=A11*C11	=RANDBETWEEN(1,4)	=IF(B12=1,$B$15,IF(B12=2,$B$15+$B$16,IF(B12=3,$B$15+$B$16+$B$17,IF(B12=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A12)
13				
14				
15	= RAND()	=IF(A15>0.5,1,0)		
16	= RAND()	=IF(A16>0.5,1,0)		
17	= RAND()	=IF(A17>0.5,1,0)		
18	= RAND()	=IF(A18>0.5,1,0)		
19				
20				
21				

* Produces a 50% probability of infection

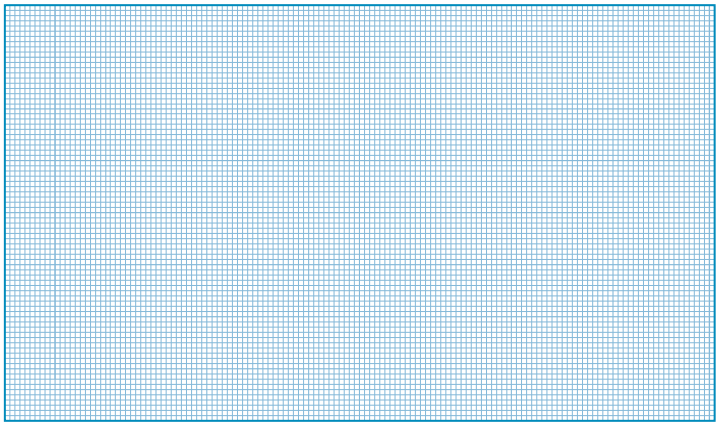
The "IF" statement incorporates the number of interactions and the probability of infection into the model

6. Run the model five times by recalculating the spreadsheet as before. On average, how many people in total have been infected after ten cycles now?

7. The third model above is much more realistic than the first, but still lacks many factors that would affect the model outcome. List at least three factors that could be added to the model to make it even more realistic:

Modelling with S, I, and R

- A more advanced predictive mathematical model than your spreadsheet called SIR can be used to show the transmission of infectious diseases. In this model there are three compartments: **S** (the number of susceptible individuals), **I** (the number of infected individuals), and **R** the number removed (those who have been removed through recovery or death).
- The data in the table (below right) is a theoretical example. It assumes a closed system (e.g. a single state with no travel), no prior immunity (everyone is susceptible), no vaccine, and no physical distancing or other precautionary measures in place.



Week	S	I	R
0	7,000,000	2	0
1	6,999,986	15	1
2	6,999,881	113	8
3	6,999,090	847	65
4	6,993,162	6352	488
5	6,948,741	47,597	3664
6	6,618,002	354,538	27,462
7	4,271,669	2,523,602	204,731
8	0	5,533,470	1,466,532
9	0	2,766,735	4,233,267
10	0	1,383,368	5,616,634
11	0	691,684	6,308,318
12	0	345,842	6,654,160
13	0	172,921	6,827,081
14	0	86,460	6,913,542

8. Plot the tabulated SIR data left on the grid provided. Plot all three data sets on one axis with a key

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9. Describe the relationship between the three compartments (S,I,and R) over time: _____

Mathematical models and vaccination

Mathematical models of the effect of vaccination on populations have been used since the mids 1700s. In 1760, Swiss mathematician Daniel Bernoulli published a study on the effect of immunisation with cowpox (against smallpox) on the life expectancy of the immunised population. Around the time of the First World War, Ronald Ross produced mathematical models to show that malaria could be controlled without removing every last mosquito. These mathematical models are the basis for many vaccination programmes and show why herd immunity is an important aim of public health programmes.

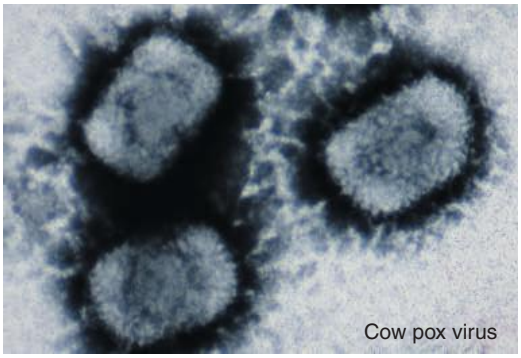
- All that is needed for the incidence of disease to decline is that every case or primary infection should generate less than one other case or secondary infection (on average).
- The number of secondary infections caused by an infectious individual is denoted as R. $\mathbf{R_0}$ (R nought) is the basic reproductive number of the pathogen. It is the number of secondary infections caused by a primary infection introduced into a wholly susceptible population.
- $\mathbf{R_{0p}}$ is the basic reproductive number under vaccination. It is the number of secondary infections caused by a primary infection introduced into a population where a proportion (*p*) of the population is vaccinated. For a perfect vaccination that confers life long immunity:

$$\mathbf{R_{0p} = (1 - p)\, R_0}$$

- p_c is the critical vaccination proportion that will achieve eradication. To achieve this proportion, the basic reproductive number under vaccination (R_{0p}) must be just less than 1, so:

$$\mathbf{p_c = 1 - \frac{1}{R_0}}$$

Calculating p_c requires estimates of R_0 (right)



Cow pox virus

Estimates of R_0 for populations and dates

Infection	Location	Date	R_0
Measles	Senegal	1964	18
Smallpox	West Africa	1960	2.3
Mumps	UK	1987	8
Rubella	USA	1967	6
Influenza	UK	2010	1.5
Covid-19	Global	2019	~2.5

10. How are mathematical and computer models useful in controlling disease? _____

11. What is the critical vaccination proportion for each of the following diseases:

    (a) Measles: _____

    (b) Influenza: _____

    (c) Covid-19: _____

12. Why is the p_c of measles so high? _____

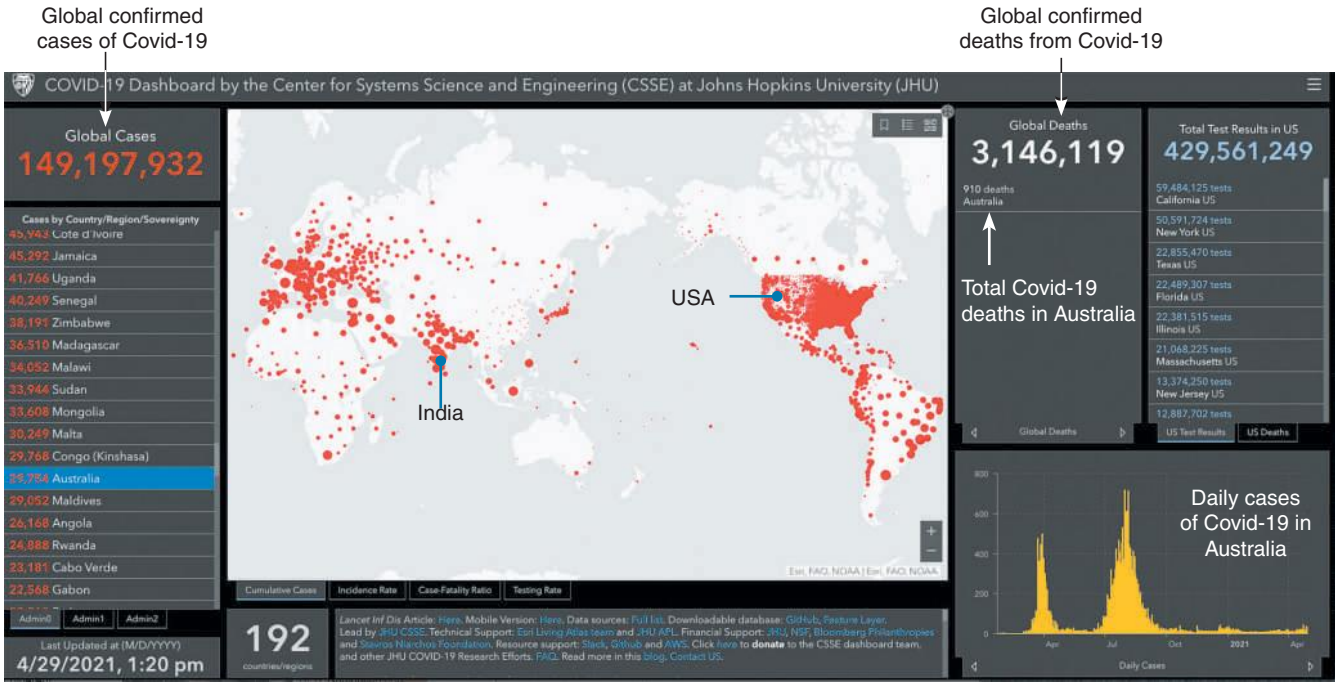
13. Calculating the R_0 of Covid-19 was a critical step in controlling the spread of the virus. What does the R_0 of Covid-19 tell us? Could the disease have been controlled without a vaccine, or is vaccination the only way to stop its spread?

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92 The Covid Pandemic: Where Are We Now?

Key Idea: How successful has Australia's Covid-19 response been compared with other countries?

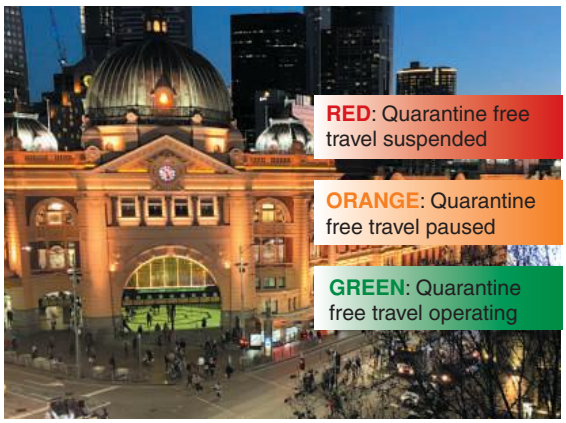
The World Health Organisation (WHO) declared the SARS-CoV-2 pandemic in March 2020. Some countries have had more success limiting the spread of Covid-19 than others. The differences come down to a number of factors including geographical location, government interventions, travel restrictions, health department responses, access to medical resources, and the willingness of the population to support the containment measures. Development of a Covid-19 vaccine has been fast-tracked, and the vaccines are being used in many countries to help control Covid-19 infection.



For the most recent information on Covid-19 visit the WHO Covid-19 Interactive Dashboard or the Johns Hopkins University of Medicine Covid-19 Dashboard (above). Find the details for both sites on **BIOZONE's Resource Hub**. The graphic above provides a snapshot of Covid19 infections at the time of printing. Red dots in the graphic above show cumulative rates of Covid-19. A high density of red indicates high levels of Covid-19 infection. Note the relatively low levels of cases in Australia and New Zealand, and high cases in the Americas, Western Europe, and India.

How is Australia restricting the spread of Covid-19?

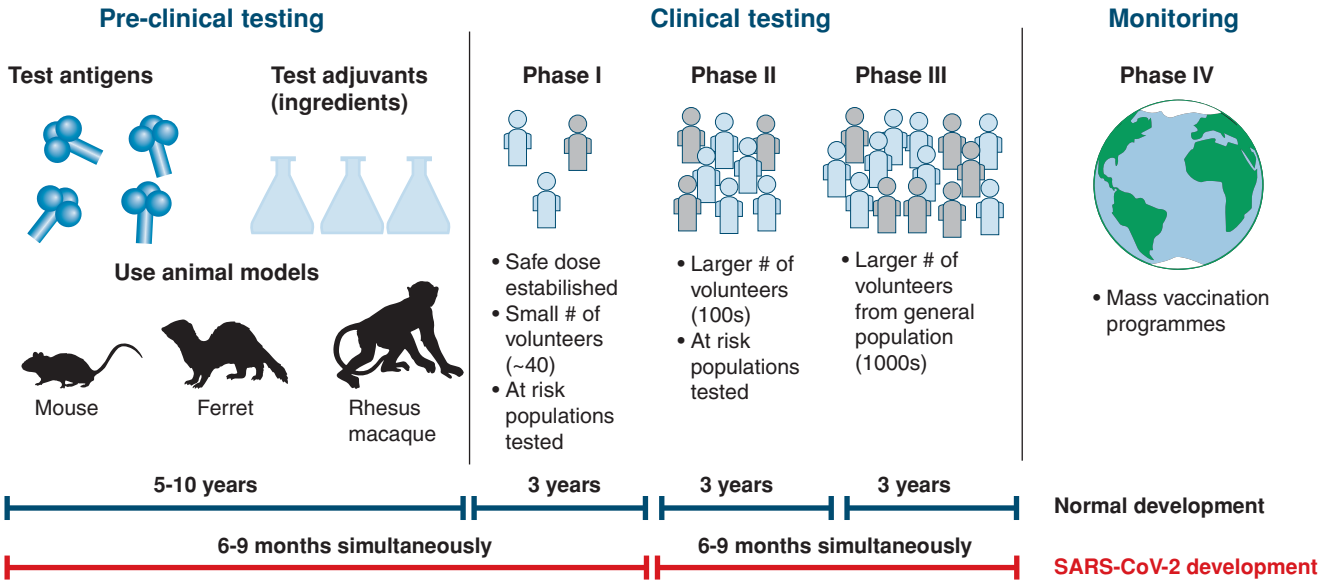
- Many countries use a traffic light system to categorise countries based on their Covid-19 risk. Governments may apply different restrictions and requirements to travellers depending on where they are travelling from. In Australia these may include limiting entry to Australian citizens and permanent residents only, and Covid testing or quarantine measures. Australia applies a traffic light system to manage quarantined travel between its own states and also between New Zealand (right).
- Currently, the Australian government discourages overseas travel except with a limited number of green zone countries. Interstate travel is closely monitored, and state borders can shut down if a community outbreak occurs. An outbreak may trigger a circuit breaker lockdown. This is where specific areas enter into a short lockdown period to stop Covid-19 spreading. Longer lockdowns may be needed if the virus has not been contained. Wearing masks in public, limiting numbers at gatherings, social distancing, and staying home if sick are other strategies the Australian government have used to control Covid-19.
- The development of a Covid vaccine has been fast-tracked (opposite page). Covid vaccines have undergone the same rigorous development and testing process as other vaccines, but over a shorter period. A number have been approved as safe to use. In Australia, vulnerable people (including older people, those with serious health issues, and Aboriginal and Torres Strait Islander peoples) are prioritised for the vaccine because they are more at risk of developing health complications if they are infected. The vaccination uptake to achieve herd immunity varies with each vaccine, and it is not yet known how long protection lasts. The full list of vaccine side effects has not yet been determined, but the benefits of having the vaccine outweigh the known side effects. New strains are evolving and the effectiveness of the vaccines against these still needs to be assessed.





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Developing a Covid-19 vaccine



1. (a) Study the map of global Covid-19 cases on the previous page. How successful has Australia's been in controlling Covid-19 infections compared with the USA and India?

(b) What role do you think Australia's geographical location has played in controlling Australia's number of Covid cases?

2. (a) How have international and domestic travel restrictions helped control the spread of Covid-19 in Australia?

(b) What other steps has Australia taken to minimise the virus spreading?

3. (a) Why have vulnerable people have been prioritised in Australia's Covid-19 vaccination programme?

(b) Some people say the Covid-19 vaccine has not been tested and should not be used. Why is this untrue?

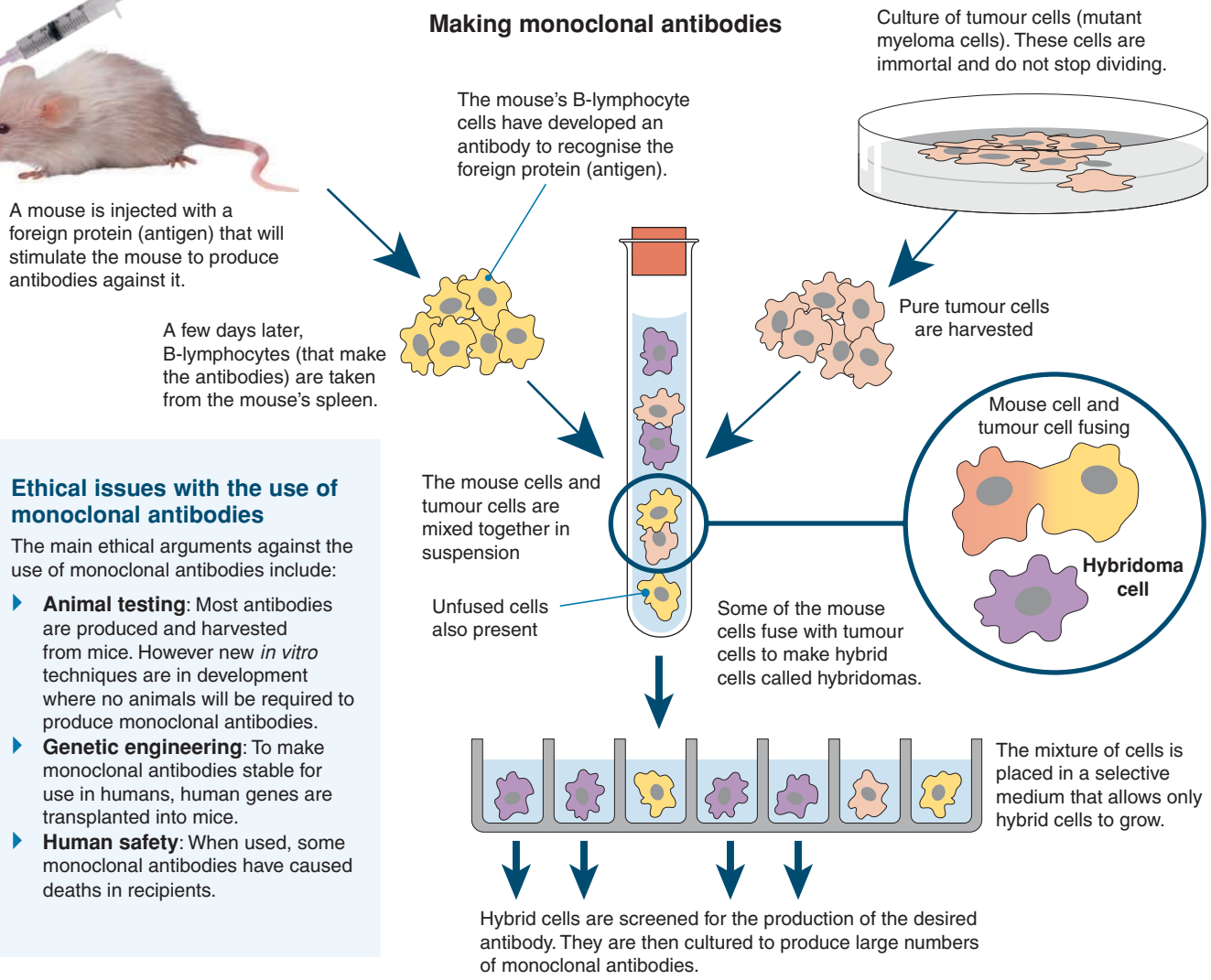
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93 What Are Monoclonal Antibodies?

Key Idea: Monoclonal antibodies are artificially produced antibodies that neutralise specific antigens. They have many diagnostic and therapeutic applications.

A **monoclonal antibody** is an artificially produced antibody that binds to and neutralises one specific type of antigen. A monoclonal antibody binds an antigen in the same way that a normally produced antibody does. Monoclonal antibodies are useful because they are identical (i.e. clones), they can be produced in large quantities, and they are highly specific for a particular antigen. Most monoclonal antibodies are produced in mice, and in some people the foreign mouse proteins can cause an unwanted immune response. Monoclonal antibodies have wide applications in diagnosing and treating disease, in detecting pregnancy, and in food safety tests.





Ethical issues with the use of monoclonal antibodies

The main ethical arguments against the use of monoclonal antibodies include:

- **Animal testing**: Most antibodies are produced and harvested from mice. However new *in vitro* techniques are in development where no animals will be required to produce monoclonal antibodies.
- **Genetic engineering**: To make monoclonal antibodies stable for use in humans, human genes are transplanted into mice.
- **Human safety**: When used, some monoclonal antibodies have caused deaths in recipients.

1. (a) What is a monoclonal antibody? _____

_____

_____

(b) In what way are monoclonal antibodies the same as a regular antibody? _____

_____

2. (a) Which mouse cells are used to produce monoclonal antibodies? _____

(b) What potential health problem is associated with the use of mice to produce monoclonal antibodies? _____

_____

_____

3. Which characteristic of tumour cells allows an ongoing culture of antibody-producing lymphocytes to be made? _____

_____

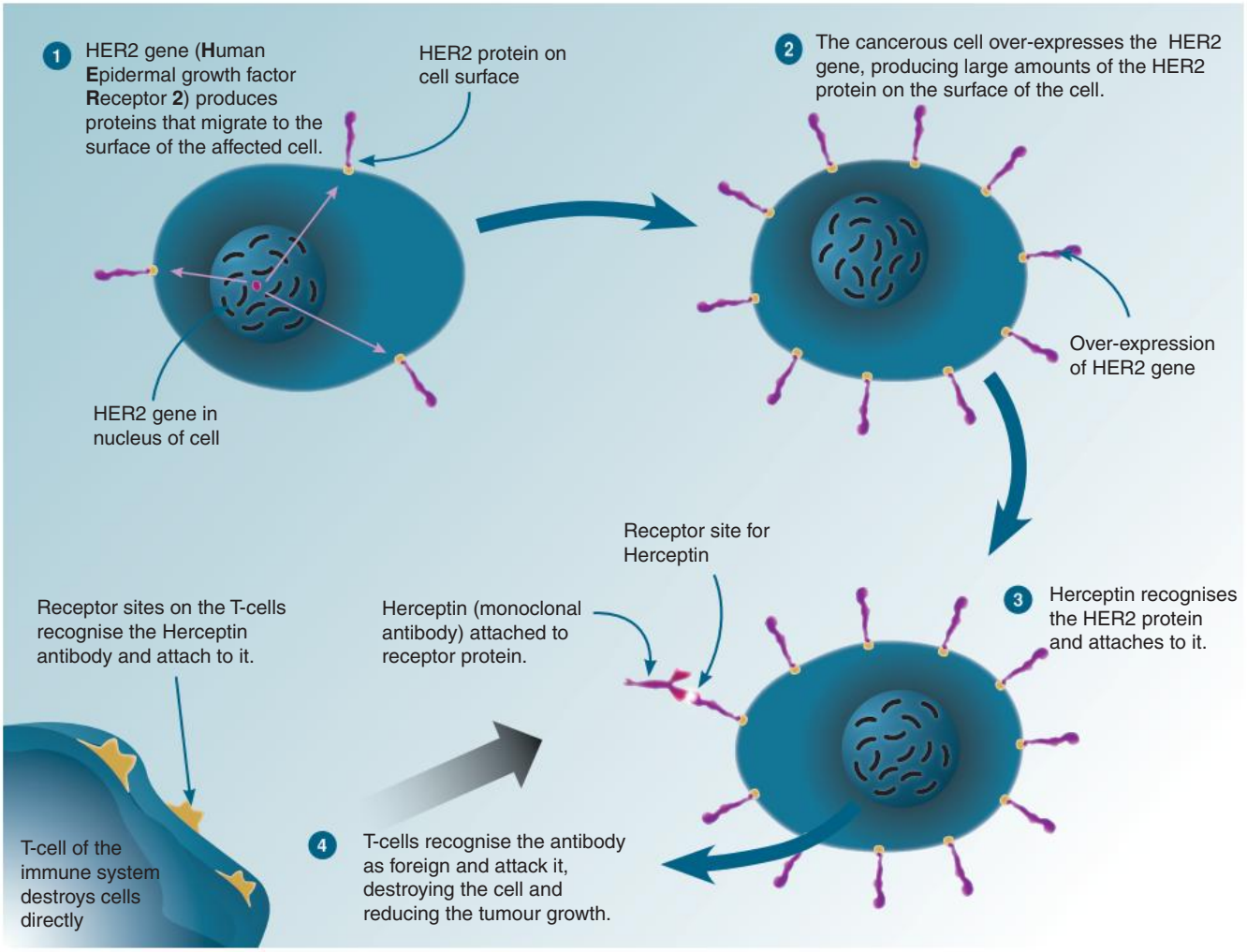
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94 Monoclonal Antibodies to Treat Cancer

Key Idea: Herceptin is a monoclonal antibody that attaches to the HER2 receptor protein on cells to help T-cells target them for destruction.

Herceptin is the patented name of a monoclonal antibody for the treatment of breast cancer. It targets the HER2 receptor proteins on cancerous cells that signal to the cell when it should divide. The proteins are produced by the proto-oncogene HER2. Cancerous cells contain 20-30% more of the HER2 gene than normal cells and this causes over-expression of HER2, and large amounts of HER2 protein. The over-expression causes the cell to divide more often than normal, producing a tumour. HER2 protein is not a foreign protein, so cancerous (**HER2⁺**) cells are not recognised by the immune system as abnormal. Herceptin binds to the HER2 protein on the surface of the cancerous cell so that the immune system can recognise it as foreign and destroy it.

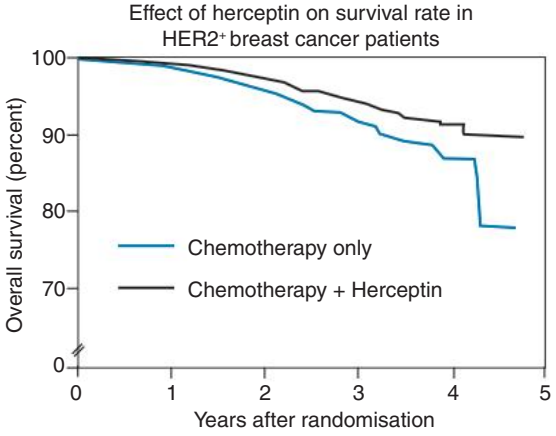
Herceptin targeted destruction of cancer cells



1. (a) Why does the immune system not detect HER2⁺ cells as abnormal and destroy them? _____

(b) How does Herceptin detect and destroy HER2⁺ cells?

(c) Study the graph (right). What effect does Herceptin have on survival rates of women treated for HER2⁺?



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95 Monoclonals Against Autoimmune Disease

Key Idea: Monoclonal antibodies can help reduce the effects of autoimmune diseases by blocking specific cell receptors.

Many autoimmune diseases result in part from the excessive production of, or hyper-sensitivity to, inflammation-promoting molecules. These kinds of inflammatory autoimmune diseases include rheumatoid arthritis and multiple sclerosis. One of the most import molecules in these diseases is the protein interleukin 6. This is a cytokine (signal molecule) that promotes the inflammatory response. Monoclonal antibodies block the cell receptors for interleukin 6, reducing its effect.

IL-6 and inflammatory autoimmune disorders

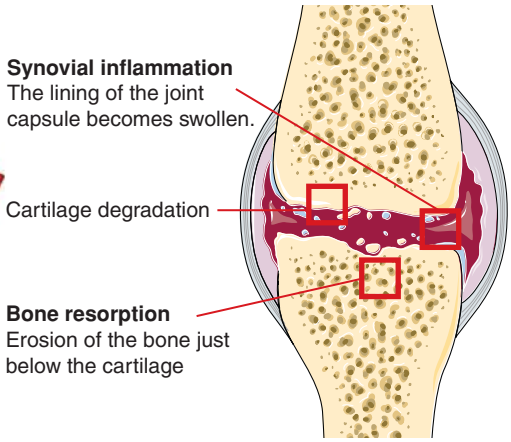


Rheumatoid arthritis in the hands

- Interleukin 6 (IL-6) promotes inflammation. It is produced by immune cells and is involved in cell signalling, including stimulating T and B cells.
- In particular, IL-6 increases the number of pro-inflammatory T cells relative to regulatory T cells. This can result in inflammatory autoimmune diseases such as rheumatoid arthritis.
- Effects of IL-6 on joint inflammation are shown right.

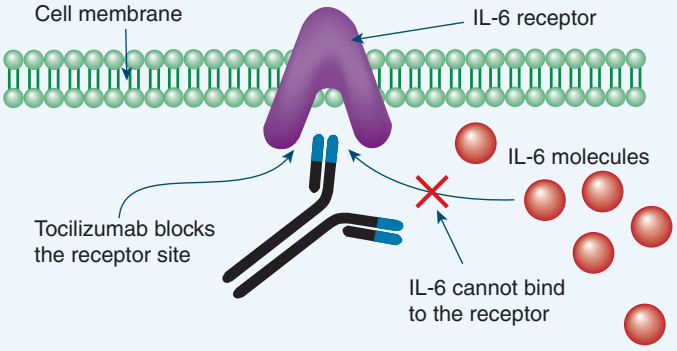


IL-6 protein



- Rheumatoid arthritis is an autoimmune disease that primarily affects the joints. Thickening of the synovial membrane invades and damages adjacent cartilage and bone.
- It results in swollen and painful joints, but can include numerous other issues including low red blood cell count, and inflammation of the lungs and heart.

- In affected patients, IL-6 binds to the IL-6 receptor in the cell membrane. This triggers processes in the cell resulting in the autoimmune effects of IL-6. Receptors can also be found in soluble form outside the cell. These bind with IL-6 and then dock with the cell membrane.
- **Tocilizumab** is a monoclonal antibody that binds to the IL-6 receptor and blocks IL-6 from binding. This then reduces the effect of IL-6 on the immune response.



1. What is the role of IL-6? _____

2. How does it cause autoimmune diseases such as rheumatoid arthritis? _____

3. How does Tocilizumab reduce the symptoms of autoimmune diseases like rheumatoid arthritis? _____

4. Why is using a monoclonal antibody useful to target specific receptors? _____

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96 Chapter Review: Did You Get It?

1. Match each term to its definition, as identified by its preceding letter code.

epidemic .....

herd immunity .....

incidence .....

immunised .....

pandemic .....

quarantine .....

vaccination .....

A Indirect protection from an infectious disease provided to susceptible individuals because most of the population has been immunised against a specific pathogen.

B The occurrence of a disease on an international scale.

C The status of a person with resistance against a particular pathogen as a result of vaccination.

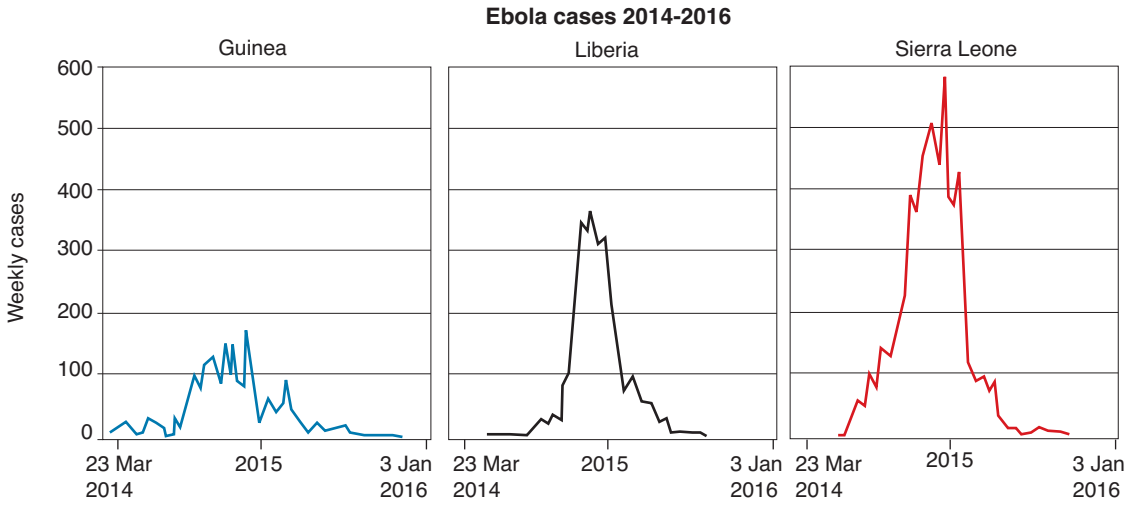
D The delivery of antigenic material to produce immunity to a disease (produce an immunised individual).

E A period or place of isolation in which an individual who may have been exposed to a disease is placed in order to prevent them from possibly spreading a disease.

F The widespread occurrence of a disease within a community or population.

G The rate of occurrence or frequency of a disease in a population.

2. Study the graphs of the 2014-2015 West Africa Ebola outbreak below:



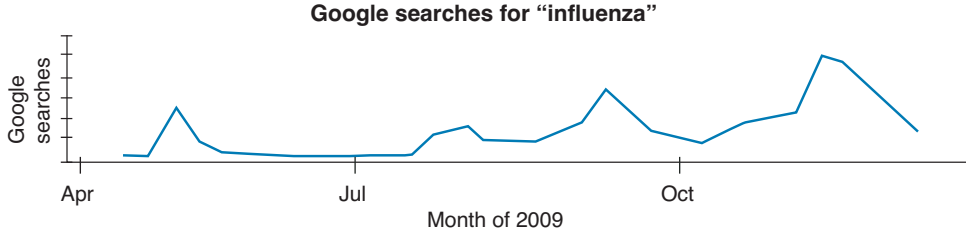
(a) In which country did Ebola first appear? _____

(b) Which country had the greatest number of cases of Ebola? _____

(c) What was the highest number of new cases reported per week? _____

(d) When and where did this occur? _____

3. Various health intelligence networks e.g. the Global Public Health Intelligence Network, monitor internet searches in order to determine if a disease outbreak is imminent. For example, the graph below shows the number of Google searches including the word "influenza" for 2009:



How would monitoring the number of internet searches about diseases or symptoms help identify and locate potential outbreaks?

_____

_____

_____

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97 Synoptic Questions: Unit 4, Area of Study 1

1. Over their lifetime, a person can develop resistance to specific pathogens. This is called acquired immunity. Acquired immunity can be obtained through natural or artificial means, and by active or passive processes.
   - Use examples to describe the differences between naturally and artificially acquired immunity.
   - Discuss the differences and similarities between passive immunity and active immunity.

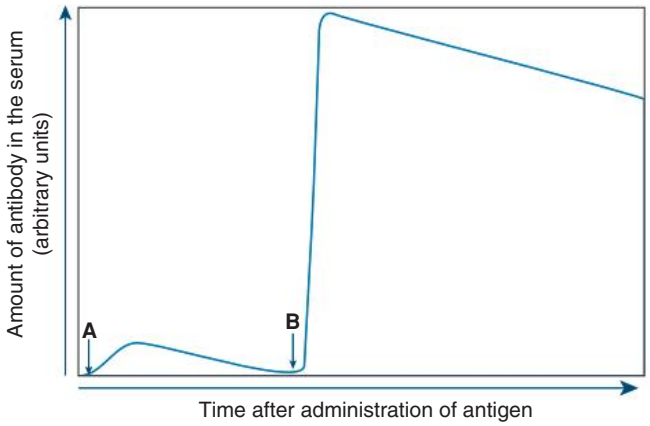
2. The graph right shows a primary and secondary immune response to an artificially introduced antigen.

   (a) What type of immunity is this? _____

   (b) What has occurred at point A? _____

   (c) What has occurred at point B? _____

   (d) Describe and explain the differences in the amount of antibody detected after each of the two events:



3. Pertussis, commonly known as whooping cough, is a highly contagious respiratory infection caused by the bacterium *Bordetella pertussis*. In Australia, the vaccine is given at two, four and six months of age. Booster doses are given at 18 months, four years and between 10-15 years. Epidemics occur in Australia every 3-4 years, but between 2008-2012 there has been a significant increase in cases of whooping cough. Most of the people contacting whooping cough have been adults who had been immunised in childhood.

   (a) Suggest why many adults are contracting whooping cough (even though they were vaccinated as children):

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(b) What effect could the high number of whooping cough cases have on young children who have not yet completed their vaccination schedule?

(c) How could the rates of whooping cough be reduced in Australia?

4. It is important to maintain high standards of hygiene in medical environments (e.g. hospitals or aged care facilities) to prevent the spread of pathogens. Handwashing with soap and water reduces the number of bacteria present, but it is not always convenient or possible to do this. The use of alcohol-based sanitisers has become a common alternative.
The data right shows the effect of handwashing or alcohol sanitiser on reducing bacterial load on the fingers of a group of intensive care nurses. 204 samples were taken from the nurses' fingers to determine the base level of contamination (shown by growth of bacterial colonies on agar). The nurses were then split into two groups (soap or alcohol rub). After they had cleaned and dried their hands, the fingers were pressed onto agar to determine the remaining bacterial load.

The effect of hand wash versus alcoholic hand rub on the disinfection of hands.

Bacterial growth on agar plates	Untreated hands (*n* = 204)	After soap & water wash (*n* =102)	After alcohol rub (*n* =102)
No growth or scanty growth (< 20 colonies)	16	51	91
Moderate growth (20-100 colonies)	136	44	5
Heavy growth (> 100 colonies)	52	7	0

Data source: Maliekal, M et al (2005) Indian J Crit Care Med. Vol 9(3).

(a) Did the two treatments reduce bacterial contamination?

(b) Which treatment was most effective:

(c) What evidence supports your choice?

5. Hong Kong is a densely packed region, with 7 million people within 1,104 km^2. In 1997, there was an outbreak of avian influenzavirus (bird flu) in Hong Kong. All 18 humans infected had been in recent contact with live domestic fowl (e.g. chickens) in markets. Six of the 18 infected people died. Authorities ordered the slaughter of all live chickens within Hong Kong (1.6 million birds) and stopped the import of more birds. No further cases of bird flu in humans were reported.

(a) Health authorities suspected that the chickens were the source of the virus. Based on the information above decide if you think they were correct in their hypothesis and explain your reasoning:

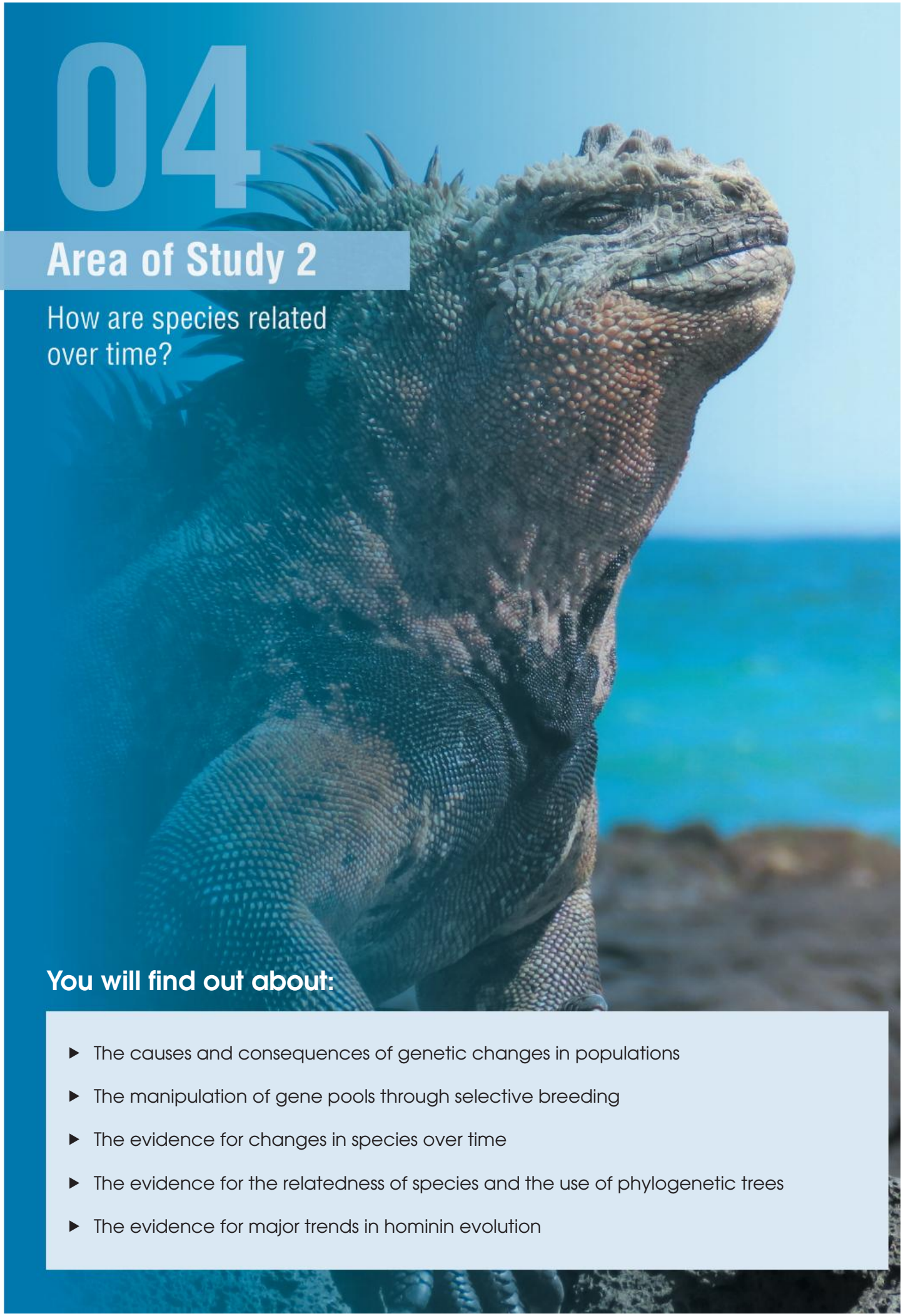
(b) Based on the evidence, do you think that the large scale slaughter of chickens and the ban on bird imports were justified? Explain your reasoning:

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04

Area of Study 2

How are species related over time?



You will find out about:

- The causes and consequences of genetic changes in populations
- The manipulation of gene pools through selective breeding
- The evidence for changes in species over time
- The evidence for the relatedness of species and the use of phylogenetic trees
- The evidence for major trends in hominin evolution

CHAPTER 10

Genetic Changes in a Population Over Time

Key terms

adaptation
allele frequency
antibiotic resistance
antigenic drift
antigenic shift
differential survival
directional selection
disruptive selection
evolution
fitness
fixation (of alleles)
founder effect
gene flow
gene pool
genetic bottleneck
genetic drift
genetic equilibrium
genotype
heterozygous advantage
microevolution
mutation
natural selection
phenotype
polyploidy
population
selection pressure
selective breeding (=artificial selection)
stabilising selection

Causes of changing allele frequencies

Key skills and knowledge

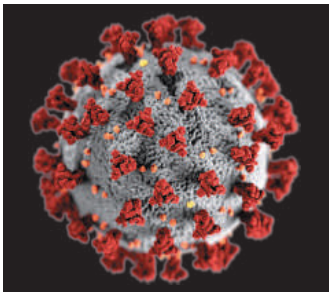
Activity number

- ☐ 1 Recall the role of mutation in creating new alleles and sexual reproduction in producing new variants. Summarise how genotype, the environment, and epigenetic influences interact to produce phenotypic variation in populations. — 98
- ☐ 2 Recognise microevolution as changes in allele frequencies in a population. Explain the effect of microevolutionary processes (mutation, gene flow (migration), natural selection, and genetic drift) on the allele frequencies of populations. — 100 103
- ☐ 3 Explain the mechanism of natural selection and understand its role in sorting the variability within a gene pool and establishing adaptive genotypes. Using examples, distinguish between stabilising, disruptive, and directional selection. — 99-101
- ☐ 4 Understand what is meant by (evolutionary) fitness. Explain how evolution, through adaptation, equips species for survival. — 98 102
- ☐ 5 PRAC Use a spreadsheet model to explore how natural selection affects gene pools. — 104





J. Podos



Consequences of changes in allele frequencies

Key skills and knowledge

- ☐ 6 Describe the biological consequences of changing allele frequencies. Which processes increase genetic diversity? Which decrease genetic diversity? — 100 101
- ☐ 7 Explain the genetic and evolutionary consequences of the founder effect. — 105
- ☐ 8 Explain the genetic and evolutionary consequences of the bottleneck effect. — 107
- ☐ 9 Recognise genetic drift as an important process in evolution. Describe its consequences and the conditions under which it is important (see #6 and 7). — 106
- ☐ 10 PRAC Use a spreadsheet model to explore how genetic drift affects gene pools. — 106
- ☐ 11 Explain the biological consequences of different selection pressures on a phenotypic characteristic such as skin colour. How is human skin colour a consequence of a balanced response to opposing selection pressures? — 108
- ☐ 12 TEST Analyse and evaluate heterozygous advantage in the distribution of human haemoglobin disorders. Why don't deleterious alleles disappear? — 109

Manipulating gene pools through selective breeding

Key skills and knowledge

- ☐ 13 Explain how selective breeding (artificial selection) can manipulate and alter the allele frequencies of a gene pool and cause phenotypic and genotypic change. — 110-114
- ☐ 14 Describe examples to show how selective breeding has created phenotypic and genotypic change in populations. Examples include the development of modern domestic livestock breeds and crop varieties from their wild ancestors. — 110-114

Ongoing challenges from pathogens

Key skills and knowledge

- ☐ 15 Explain how antibiotic resistance can arise and spread in bacterial populations. Describe the challenges bacterial resistance presents to the treatment of disease. — 115
- ☐ 16 Distinguish between antigenic drift and antigenic shift in viruses. Describe the challenges these forms of viral evolution pose for vaccination programmes, prevention of viral disease, and protection of public health. — 116

98 Sources of Variation

Key Idea: The characteristics of sexually reproducing organisms show variation. This phenotypic variation is the result of genes, the environment, and the contribution of epigenetic factors. Natural selection acts on the available phenotypic variation to favour the best suited phenotypes.

Variation refers to the diversity of genotypes (allele combinations) and phenotypes (appearances) in a population. Variation in phenotypic characteristics, such as flower colour or birth weight, is a feature of sexually reproducing populations. Some characteristics are controlled by many genes at different loci and show continuous variation over a wide range of phenotypic variants. Some are controlled by a small number of genes and there are a limited number of phenotypic variants in the population. Both genotype, environment, and epigenetic factors determine, to different degrees, the final phenotype. Variation is the raw material for natural selection. Individuals with variations that contribute to greater fitness are more likely to pass on their genes.

Mutations

gene mutations, chromosome mutations

Mutations are the source of all new alleles. Existing genes are modified by mutations to form new alleles. Neutral mutations, which are neither detrimental nor beneficial, may escape selection pressure until conditions change.



Sexual reproduction

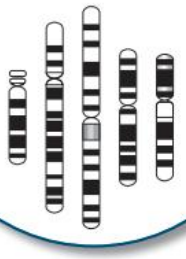
independent assortment, crossing over and recombination, fertilisation, mate selection

Sexual reproduction rearranges and reshuffles the genetic material into new combinations in the offspring. Variation arises during meiosis and also as a result of random fertilisation of gametes.



Genotype

Determines the **genetic potential** of an individual



Dominant, recessive, codominant, and multiple alleles, epigenetic modifications (such as methylation), and interactions among genes, combine in their effects.

Phenotype

An individual's phenotype is the result of the interactions among genetic, environmental, and epigenetic factors during its lifetime. The expression of genes in an organism can be influenced by both the internal and external environment during and after development.

Environmental factors

The external and internal environments can influence the expression of the genotype. The external environment includes physical factors such as temperature, or biotic factors such as competition. The internal environment, e.g. presence or absence of hormones, growth factors, or epigenetic tags during development, may also affect genotypic expression.



Variation is the essential raw material for natural selection. Different phenotypic variants will have different relative survival and reproductive success (**fitness**) in the prevailing environment and only the most successful variants will increase in number.

1. (a) What is the basis of the genetic variation of sexually reproducing organisms? _____

_____

_____

_____

_____

(b) How does the environment contribute to the phenotype we see: _____

_____

_____

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99 Variation and Natural Selection

Key Idea: Natural selection is the evolutionary mechanism by which organisms that are better adapted to their environment survive to produce a greater number of offspring.

Evolution is simply the change in inherited characteristics in a population over generations. Darwin recognised this as the consequence of four interacting factors: (1) the capacity of populations to increase in numbers, (2) the phenotypic variation of individuals, (3) that there is competition for resources, and (4) proliferation of individuals with better survival and reproduction.

Natural selection is the varying survival and reproduction of individuals due to differences in phenotype. Organisms with more favourable phenotypes will survive in greater numbers to produce a greater number of viable offspring. The proportion of their alleles in subsequent generations will therefore increase. This is the basis of Darwin's theory of evolution by natural selection. Natural selection is one of the most important microevolutionary processes.

We can demonstrate the basic principles of evolution using the analogy of a 'population' of M&M's candy.



In a bag of M&M's, there are many colours, which represents the variation in a population. As you and a friend eat through the bag of candy, you both leave the blue ones, which you both dislike, and return them to bag.



The blue candy becomes more common...



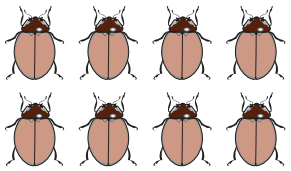
Eventually, you are left with a bag of blue M&M's. Your selective preference for the other colours changed the make-up of the M&M's population. This is the basic principle of selection that drives evolution in natural populations.

Darwin's theory of evolution by natural selection

Darwin's theory of evolution by natural selection is outlined below. It is widely accepted by the scientific community today and is one of founding principles of modern science.

Overproduction

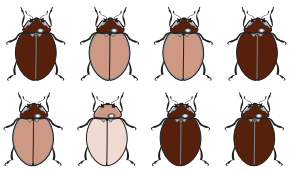
Populations produce too many young: many must die



Populations generally produce more offspring than are needed to replace the parents. Natural populations normally maintain constant numbers. A certain number will die without reproducing.

Variation

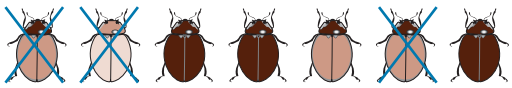
Individuals show variation: some variations more favourable than others



Individuals in a population have different phenotypes and therefore, genotypes. Some traits are better suited to the environment, and individuals with these have better survival and reproductive success.

Competition

Individuals compete for limited resources. Not all survive.



Natural selection favours the individuals best suited to the environment at the time. Those with favourable variations are more likely to survive and breed. Relatively more of those with less favourable variations will die.

Relative increase in favourable variants

Variations are inherited: the best suited variants leave more offspring



The variations (both favourable and unfavourable) are passed on to offspring. Each generation will contain proportionally more descendants of individuals with favourable characteristics.

1. Identify the four factors that interact to bring about evolution in populations: _____

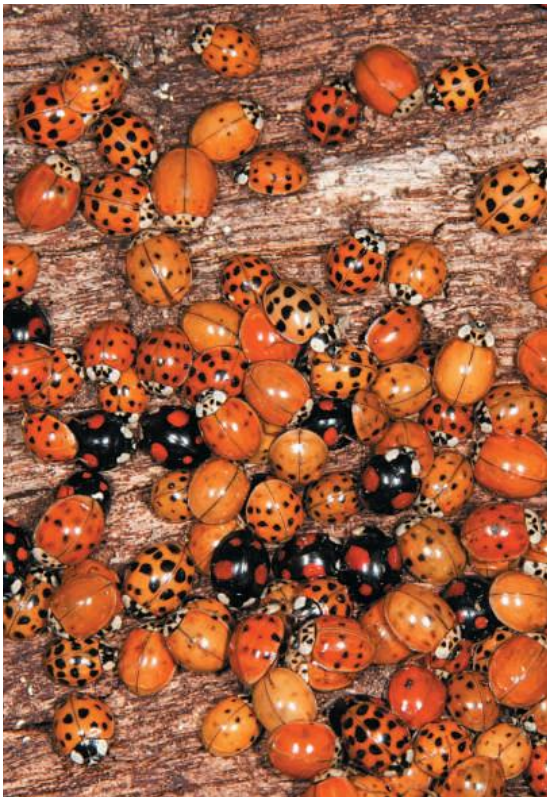
_____

_____

_____

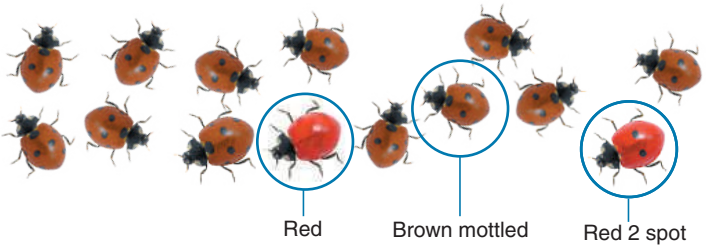
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Variation, selection, and population change



Natural populations, like the ladybird population above, show genotypic (and therefore phenotypic) variation. This is a result of **mutation** (which creates new alleles) and **sexual reproduction** (which produces new combinations of alleles). Some phenotypic variants are more suited to the environment of the time than others. These variants will leave more offspring, as described for the hypothetical population (right).

1. Variation through mutation and sexual reproduction:
In a population of brown beetles, mutations independently produce red colouration and 2 spot marking on the wings. The individuals in the population compete for limited resources.



2. Selective predation:
Brown mottled beetles are eaten by birds but red ones are avoided.



3. Change in the genetics of the population:
Red beetles have better survival and fitness and become more numerous with each generation. Brown beetles have poor fitness and become rare.



2. What process creates new alleles? _____

3. Give your own definition of evolution: _____

_____

4. Explain how the genetic make-up of a population can change over time: _____

_____

_____

_____

_____

_____

_____

5. Complete the table below by calculating the percentage of beetles in the example above right.

Beetle population	% Brown beetles	% Red beetles	% Red beetles with spots
1	(a)	(b)	(c)
2	(d)	(e)	(f)
3	(g)	(h)	(i)

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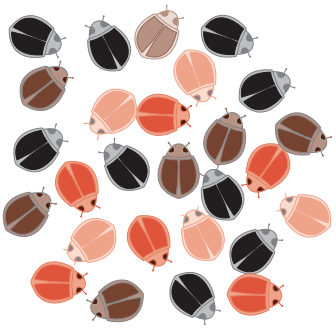
100 Microevolutionary Processes in Gene Pools

Key Idea: Mutations, gene flow, genetic drift, and natural selection all contribute to changes in the genetic makeup (frequency of different alleles) of a population.

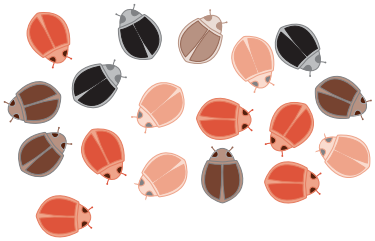
A population can be regarded as a collection of all its alleles (the **gene pool**). Changes in the frequency of these alleles in the population over time is what we call **evolution**. As we have seen, mutation and sexual reproduction create genotypic (and therefore phenotypic) variation and phenotypic variation is the raw material for natural selection. Four microevolutionary processes can contribute to genetic change in populations. **Mutation** creates new alleles. Migration creates **gene flow** as alleles enter or leave a population. **Natural selection** sorts variation and establishes adaptive phenotypes and is a major agent of evolution. **Genetic drift** alters alleles frequencies randomly and its effects are due to chance events. Increasingly, genetic drift is being recognised as an important agent of change, especially in small, isolated populations (e.g. island colonisers).

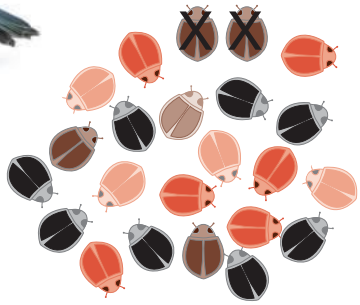
Genetic variation

Genetic variation refers to the number of different types of alleles in a population. Genetic variation produces **phenotypic variation** (e.g. colour of ladybirds). It is this phenotypic variation that is the raw material for natural selection. This ladybird population has five different phenotypes (black, dark brown, tan, brick red, and pale).



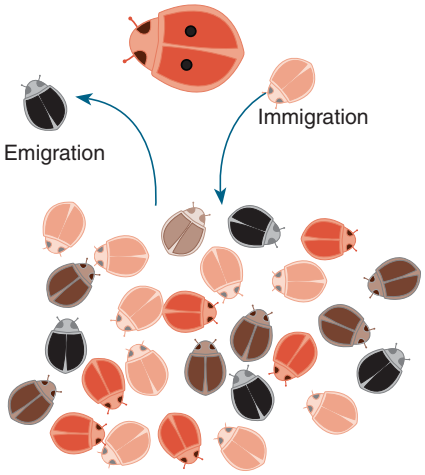






As we have seen in earlier activities, genetic variation arises through mutations and the recombination of alleles through sexual reproduction.

For example, a **mutation** produces a ladybird with a new spotted phenotype (below).



Natural selection

Natural selection acts on populations to maintain favourable phenotypes and eliminate unfavourable phenotypes. Over time, favourable phenotypes become more common in the population because those individuals reproduce more.

For example, black ladybirds are more easily seen by birds and are eaten more often than the other phenotypes. The lighter phenotypes become more common in the next generation.

Genetic drift

Genetic drift is the change in a population's allele frequency due to random events. Genetic drift has a more pronounced effect in small populations.

For example, falling rocks kill a number of ladybirds, but more of the dark brown ladybirds are crushed than any other phenotype. The proportion of dark brown ladybirds remaining in the population is drastically reduced, and their representation in the next generation is also reduced.

Migration (gene flow)

Migration is the movement of individuals into and out of a population. Through immigration or emigration, alleles can enter or leave the population. Gene flow tends to decrease the genetic differences between populations because alleles are being exchanged.

In the example above, several black lady birds have left and some very pale lady birds have arrived changing the proportion of remaining phenotypes in the population.

1. Clearly explain what is meant by the following terms:

(a) Gene flow: _____

_____

_____

(b) Genetic drift: _____

_____

_____

(c) Natural selection: _____

_____

_____

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2. One of the important theoretical concepts in population genetics is that of **genetic equilibrium**, which states that "***for a large, randomly mating population, allele frequencies do not change from generation to generation***". If allele frequencies in a population are to remain unchanged, all of the following criteria must be met: the population must be large, there must be no mutation or gene flow, mating must be random, and there must be no natural selection. Evolution is a consequence of few if any of these conditions ever being met in natural populations. For each of the five factors (a-e) below, describe how and why each would affect the allele frequency in a gene pool. Use the diagrams to help you.

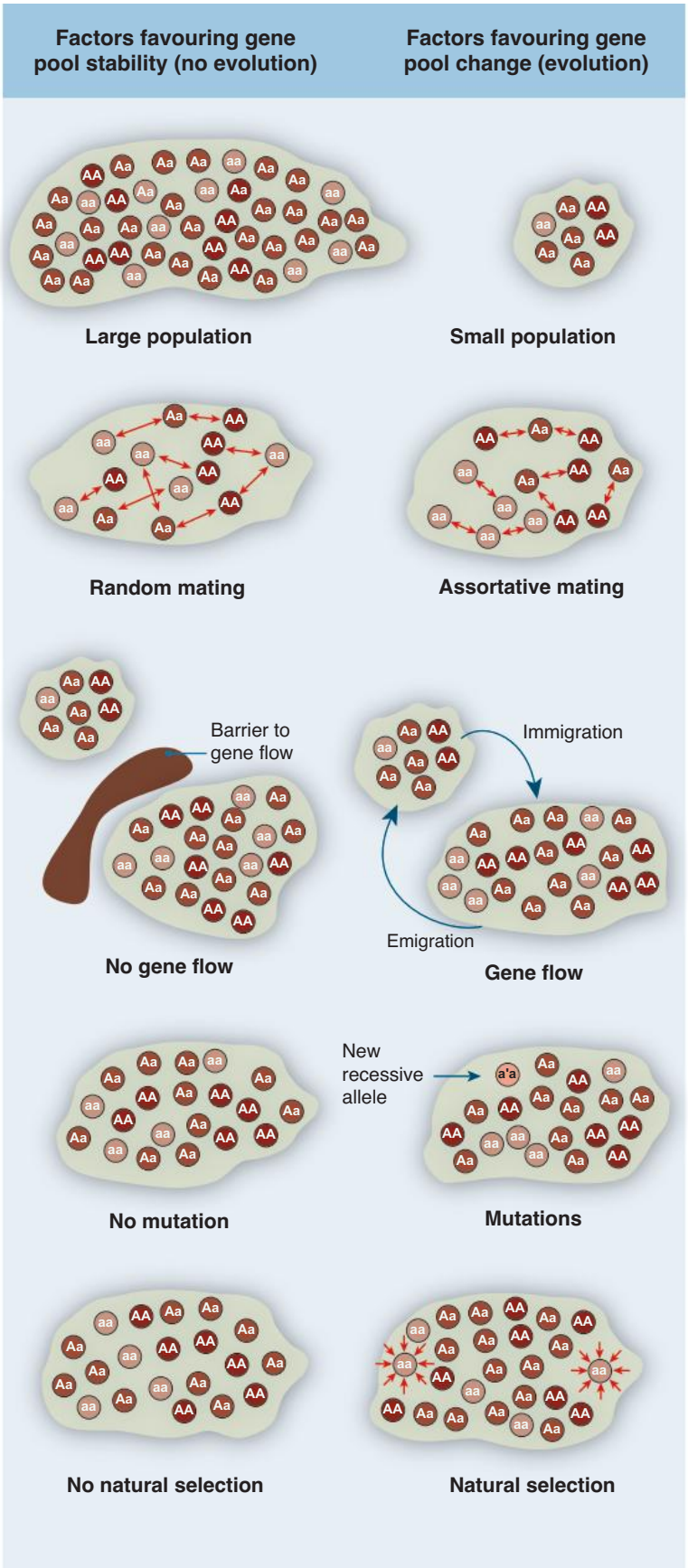
(a) Population size: _____

(b) Mate selection: _____

(c) Gene flow: _____

(d) Mutation: _____

(e) Natural selection: _____



3. Identify a factor that tends to:

(a) Increase genetic variation in populations:

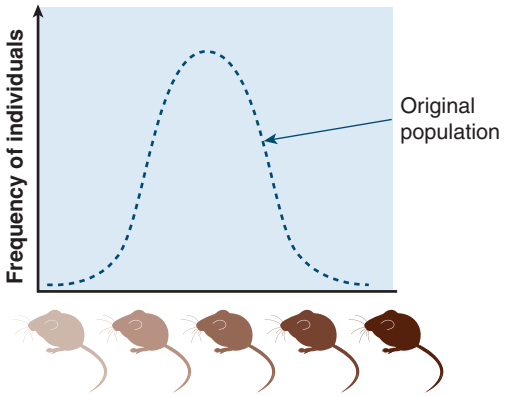
(b) Decrease genetic variation in populations:

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101 Types of Natural Selection

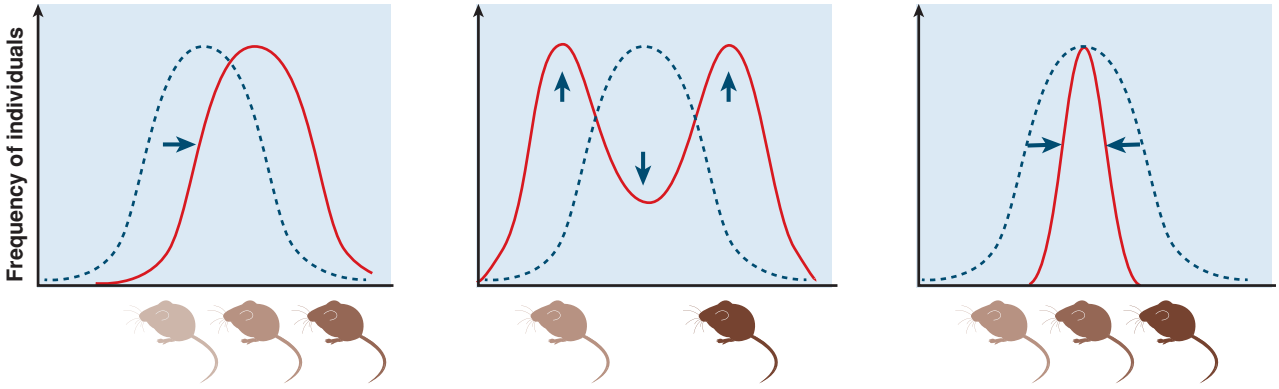
Key Idea: Natural selection is responsible for the differential survival of some phenotypes (and genotypes) over others. It is an important cause of genetic change in populations.

Natural selection operates on the phenotypes of individuals, produced by their particular combinations of alleles in the particular environment. It results in the differential survival of some phenotypes over others. Individuals with phenotypes conferring greater fitness in the environment at the time will become relatively more numerous in the population. Over time, natural selection may lead to a permanent change in the genetic makeup of a population. Natural selection is always linked to phenotypic suitability in the prevailing environment so it is a dynamic process. It may favour existing phenotypes or shift the phenotypic median, as is shown below.





Natural selection acts on phenotypic variation. Even slight variations may be enough for selection to occur. The white streak on the mouse on the right may make it stand out to predators. The darker mouse may be able to more easily hide in the shadows.



Directional selection

An environmental pressure, e.g. predation, or higher temperatures, selects against one of the phenotypic extremes. The adaptive phenotype is shifted in one direction and one phenotype is favoured over others.

Disruptive selection

Disruptive selection favours two phenotypic extremes at the expense of intermediate forms. Disruptive selection may occur when environments or resources are fluctuating or distinctly divergent.

Stabilising selection

Extreme variations are selected against and the middle range (most common) phenotypes are retained in greater numbers. Stabilising selection decreases variation for the phenotypic character involved.

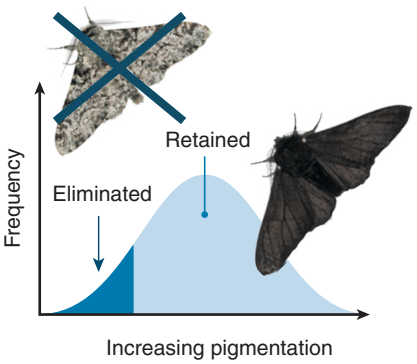
1. Explain why fluctuating (as opposed to stable) environments favour disruptive (diversifying) selection:

2. What would be the likely effect of rapid environmental change on a population with very low phenotypic variation?

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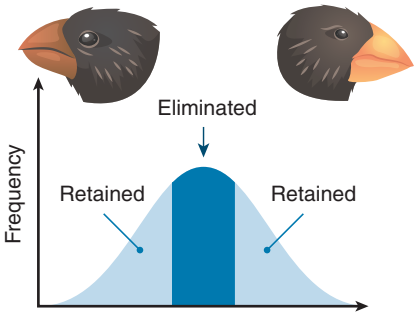
Examples of selection

Directional selection



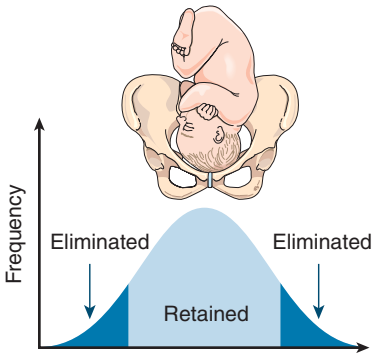
Directional selection was observed in peppered moths in England during the Industrial Revolution when soot-covered trees were common. In England's current environment, the selection pressures on the moths are more balanced, although lighter morphs predominate.

Disruptive selection



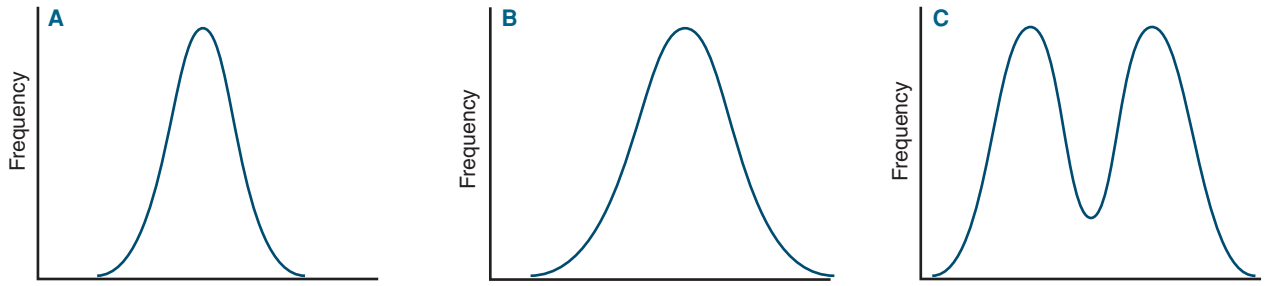
During a prolonged drought on Santa Cruz Island in the Galápagos, it resulted in a population of ground finches that was bimodal for beak size. Competition for the usual medium-sized seed sources was so intense that selection favoured birds able to exploit either small or large seeds.

Stabilising selection



Stabilising selection operates most of the time in most populations and acts to prevent divergence from the adaptive phenotype, e.g. birth weight of human infants or number of eggs laid in a nest.

3. Which of the graphs below relate to the examples above:



A: _____

B: _____

C: _____

4. Disruptive selection can be important in the formation of new species:

   (a) Describe the evidence from the ground finches on Santa Cruz Island that provides support for this statement:

   _____

   _____

   (b) The ground finches on Santa Cruz Island are one interbreeding population with a strongly bimodal distribution for the phenotypic character beak size. Suggest what conditions could lead to the two phenotypic extremes diverging further:

   _____

   (c) Predict the consequences of the end of the drought and an increased abundance of medium size seeds as food:

   _____

5. Explain why the number of eggs in a bird nest is most likely governed by stabilising selection:

   _____

   _____

   _____

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102 Adaptation and Fitness

Key Idea: Adaptive features enhance an individual's fitness.

Adaptations are heritable traits that suit organisms to their functional role in the environment. Adaptations increase **fitness**, which is mathematical measure of the contribution an organism makes to the next generation. Adaptations are the result of the genotype of the organism. The more an adaptation contributes to survival and successful reproduction in the environment, the more likely it is that the organism will pass on the genes responsible for that adaptation. In this way, natural selection shapes adaptations over time.

Number of horns in rhinoceroses

Not all differences between species can be convincingly interpreted as adaptations to particular environments. Rhinoceroses charge rival males and predators, and the horn(s), when combined with the head-down posture, add effectiveness to this behaviour. Horns are obviously adaptive, but it is not clear that the possession of one (Indian rhino) or two (black rhino) horns is necessarily related directly to the environment in which those animals live.



Krish Dulal CC 3.0



Ear length in rabbits and hares

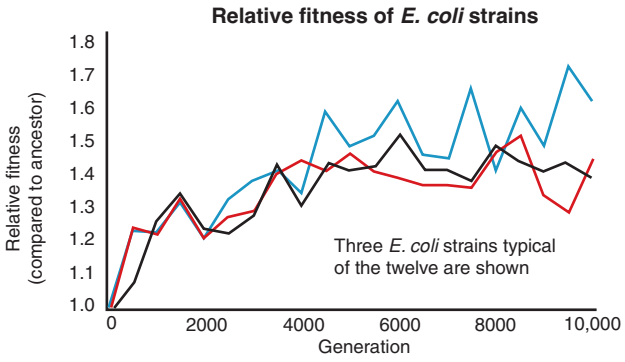
The external ears of many mammals are used as important organs to assist in thermoregulation (controlling loss and gain of body heat). The ears of rabbits and hares native to hot, dry climates, such as the jack rabbit of south-western USA and northern Mexico, are relatively large. The Arctic hare lives in the tundra zone of Alaska, northern Canada and Greenland, and has ears that are relatively short. This reduction in the size of the extremities (ears, limbs, and noses) is typical of cold adapted species.





Fitness and adaptation in *E. coli*

The Long Term *E. Coli* Experiment has been running since 1988. Its goal is to track the evolution or 12 populations of *E. coli* kept in a minimal glucose growth medium. The fitness of the 12 populations relative to the original 12 populations has been measured every 500 generations. The graph on the right shows how the fitness of the populations changed over the first 10,000 generations. Note that the features evolved by the *E. coli* provide them with greater fitness only in the low glucose environment. Placed in a different environment, their relative fitness is actually lower than the original population's.



1. How is fitness related to genetics? _____

_____

_____

_____

2. How is fitness related to the environment? _____

_____

_____

_____

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103 Changes in a Gene Pool

Key Idea: Natural selection and migration can alter the allele frequencies in gene pools.

The diagram below shows an hypothetical population of beetles undergoing changes as it is subjected to two 'events'. The three phases represent a progression in time (i.e. the same gene pool, undergoing change). The beetles have two phenotypes (dark and pale) determined by the amount of pigment deposited in the cuticle. The gene controlling this character is represented by two alleles **A** and **a**. Your task is to analyse the gene pool as it undergoes changes.

1. For each phase in the gene pool below fill in the following tables (the first has been done for you):

   (a) Count the number of A and a alleles separately. Enter the count into the top row of the table (left hand columns).
   (b) Count the number of each type of allele combination (AA, Aa and aa) in the gene pool. Enter the count into the top row of the table (right hand columns).
   (c) For each of the above, work out the frequencies as percentages (bottom row of table):

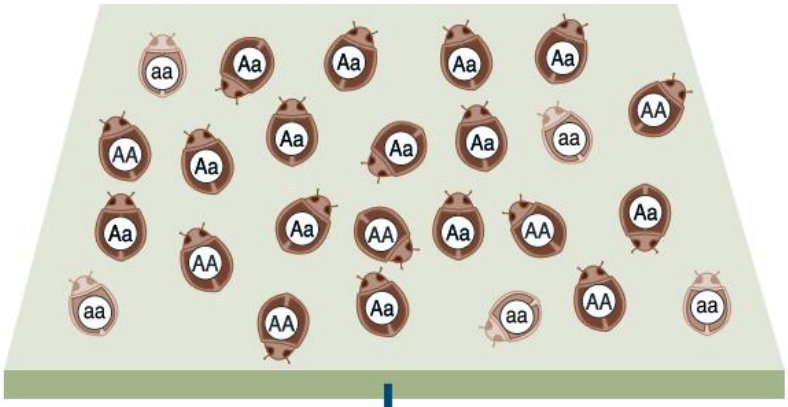
Allele frequency = No. counted alleles ÷ Total no. of alleles x 100

Phase 1: Initial gene pool



	A	a	AA	Aa	aa
No.	27		7		
%	54		28		

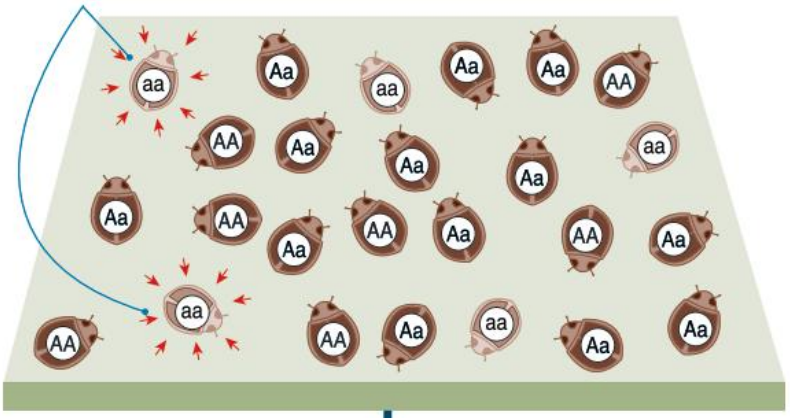
Allele types *Allele combinations*



Two pale individuals died. Their alleles are removed from the gene pool.

Phase 2: Natural selection

In the same gene pool at a later time there was a change in the allele frequencies. This was due to the loss of certain allele combinations due to natural selection. Some of those with a genotype of aa were eliminated (poor fitness).
These individuals (surrounded by small white arrows) are not counted for allele frequencies; they are dead!



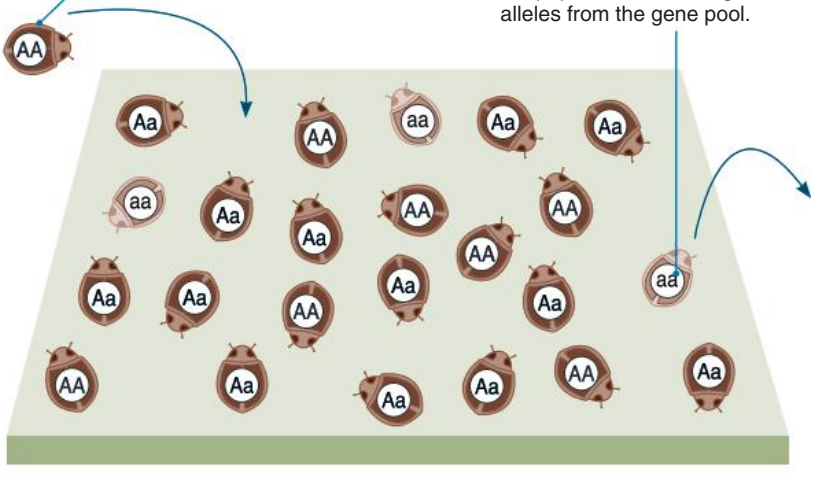
	A	a	AA	Aa	aa
No.					
%					

This individual is entering the population and will add its alleles to the gene pool.

This individual is leaving the population, removing its alleles from the gene pool.

Phase 3: Immigration and emigration

This particular kind of beetle exhibits wandering behaviour. The allele frequencies change again due to the introduction and departure of individual beetles, each carrying certain allele combinations.
Individuals coming into the gene pool (AA) are counted for allele frequencies, but those leaving (aa) are not.



	A	a	AA	Aa	aa
No.					
%					

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104 Computational Analysis of Genotypic Changes

Key Idea: The process of natural selection can be modelled using a simple series of steps on a spreadsheet.

Changes in gene pools are often modelled using physical representations of the genotypes in a population. This type of modelling is tedious and subject to human error. Modelling genotypic changes using a spreadsheet is quicker and allows the model to be changed to simulate different scenarios occurring in the gene pool.

Investigation 10.1 Investigating natural selection

Some natural selection labs work by manually placing tokens representing alleles into a bag and withdrawing them randomly to make genotypes. A certain genotype is then selected against by not returning it to the bag and the next generation is drawn from the remaining alleles. This exercise is long and difficult to manipulate, and it reduces the population numbers over time so that an accurate simulation is not entirely possible.

These problems can be solved by using a spreadsheet to compute allele changes over time. Once the formulae are in place, the spreadsheet can be manipulated in different ways to produce a more accurate (yet still simple) simulation. This spreadsheet can also be used to simulate genetic drift in Activity 106.

Download the spreadsheet from the BIOZONE Resource Hub or use the notes and screenshots below to recreate the spreadsheet yourself.

PART 1: Setting Up the spreadsheet

1. Open a new spreadsheet. The first thing to do is to switch off automatic calculation. This makes calculation of future allele frequencies simpler and under manual control, so that you can calculate them when you're ready. Each spreadsheet program will have slightly different ways of doing this. For Microsoft Excel click on the **Formulas** tab then on the **Calculation Options** menu and click **Manual**. Calculations can then be made using the **Calculate Now** button beside the Calculation Options menu or using the **F9** button.

2. The headings A and B represent the alleles A and a. This is necessary because the COUNTIF formula used later in the spreadsheet is not case sensitive (it does not recognise the difference between A and a).

3. 0.5 is the frequency of the A allele in the initial population (generation 0). The frequency of the B allele in the population is equal to 1-A. In our initial population, 50% of the alleles will be A and 50% will be B (A and a in the population).

	A	B	C	D
1	Allele frequency			
2	A	B		
3	0.5	=1-A3		
4				
5				
6	Gamete 1	Gamete 2		Zygote
7	=IF(RAND()<=$A$3,"A","B")	=IF(RAND()<=$A$3,"A","B")		=CONCATENATE(A7,B7)
8	=IF(RAND()<=$A$3,"A","B")	=IF(RAND()<=$A$3,"A","B")		=CONCATENATE(A8,B8)
9	=IF(RAND()<=$A$3,"A","B")	=IF(RAND()<=$A$3,"A","B")		=CONCATENATE(A9,B9)
10	=IF(RAND()<=$A$3,"A","B")	=IF(RAND()<=$A$3,"A","B")		=CONCATENATE(A10,B10)
11	=IF(RAND()<=$A$3,"A","B")	=IF(RAND()<=$A$3,"A","B")		=CONCATENATE(A11,B11)

4. The RAND formula produces a random number between 0 and 1 and compares it to the number in cell A3. If the random number is less than or equal to the number in cell A3 then an A is displayed in the cell. If the random number is greater, a B is displayed. The $ symbol tells the spreadsheet that cell A3 is a reference cell and must not change.

5. The CONCATENATE formula takes gametes A and B and puts them together to make the zygote.

6. Highlight cells and copy down all formulas to row 56 to produce 100 random gametes containing alleles A or B and 100 zygotes.

7. You now need to count up the number of AA, AB, and BB genotypes

	F	G	H	I	J
1	AA	AB	BB		Total Genotypes
2	=COUNTIF($D$7:$D$56,"AA")	=COUNTIF($D$7:$D$56,"AB")+COUNTIF($D$7:$D$56,"BA")	=COUNTIF($D$7:$D$56,"BB")		=SUM(F2:H2)
3					
4					

8. The COUNTIF formula counts up the number of AA, AB, and BB genotypes.

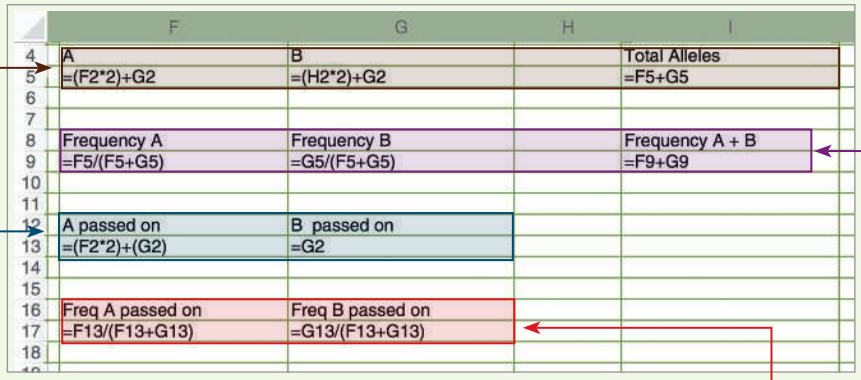
9. The SUM formula adds up the number of genotypes. It should add up to 50.

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10. Now you must calculate the number of A and B alleles present in this generation (Generation 1).In cell **F4** type the heading **A** and in cell **G4** type the heading **B**. In cell **I4**, type the heading **Total Alleles**.

11. Cell F5 adds up all the alleles from the AA genotype and the A alleles from the AB genotype. Cell **G5** adds up all the B alleles. Cell I5 adds up all the alleles. Click **Calculate Now** and you should see the number 100 appear.

12. Cells F9 and G9 calculate the frequency of As and Bs in Generation 1. Cell I9 adds up cell F9 and G9. This should add to 1.



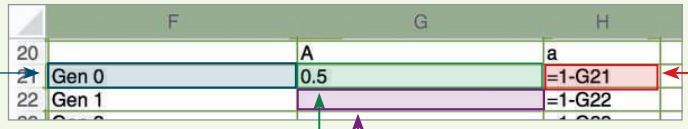
	F	G	H	I
4	A	B		Total Alleles
5	=(F2*2)+G2	=(H2*2)+G2		=F5+G5
6				
7				
8	Frequency A	Frequency B		Frequency A + B
9	=F5/(F5+G5)	=G5/(F5+G5)		=F9+G9
10				
11				
12	A passed on	B passed on		
13	=(F2*2)+(G2)	=G2		
14				
15				
16	Freq A passed on	Freq B passed on		
17	=F13/(F13+G13)	=G13/(F13+G13)		
18				

13. The **selection pressure** is be against the recessive phenotype. The alleles in the recessive genotype (BB) will not be passed on, so the frequency of alleles in the population is different to the frequency of alleles that will be passed on. These cells calculate the **number** of alleles that will be passed on (excluding those in genotype BB).

14. These cells calculate the frequency of alleles that will be available to the next generation.

15. Finally you must keep a record of each generation's allele frequencies before mating (i.e. before BB is excluded).

16. Gen 0 was your starting population. Copy down to cell **F31** to get ten generations.



	F	G	H
20		A	a
21	Gen 0	0.5	=1-G21
22	Gen 1		=1-G22

18. The frequency of a is simply 1-A. Copy down to **H31**.

17. The frequency of A in Gen 0 was 0.5

19. Click **Calculate Now**. Note the numbers that appear in cells **F9** and **G9**. Type the number in **F9** into **G22**. This is the frequency of the A allele in the first generation.

20. Now type the number in cell **F17** into cell **A3** and click **Calculate Now** to produce the second generation of alleles in cells F9 and G9. Again enter the number in **F9** into **G23** and the number in **F17** into **A3** before clicking **Calculate Now**.

21. Each time you do this, the spreadsheet calculates a new generation of genotypes and their alleles based in the number you enter into A3.

22. Save your spreadsheet.

PART 2: Natural Selection lab

Now that you have built the spreadsheet and are familiar with it, you can begin the natural selection lab.

1. To do this you will select against the recessive phenotype (and hence the aa genotype, represented as BB in the spreadsheet). In this scenario any BB individuals never get to breed (it is irrelevant what the phenotype is, what is important is that no BB individuals will enter their alleles into the next generation).

2. To start the lab, make sure **0.5** is entered into cell **A3**. Enter **0.5** into cell **G21** and make sure the cells below them are clear. Highlight cells **F17** and **G17** and under the **Format** menu click **Cells**, then click the **Number** category and set it to **2** decimal places. Click **OK**.

3. It is also worth tracking the numbers of AA, Aa and aa individuals before breeding. You can do this by simply recording the numbers on a new part of the spreadsheet, the same way as recording the A and a allele frequencies. Theoretically, Generation 0 will start as 12.5 AA, 25 Aa, and 12.5 aa, but because only whole numbers of individuals are allowed these will need to be rounded to the nearest whole number that still

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produces a total of 50 (12, 26, 12). In cell **F35**, type the heading **Gen0**. **Highlight** the cell and copy it down to cell **F45**. In cell **G34**, type the heading **AA**, Cell **H34** type Aa and in **I34** type **aa**. Into cell **G35** type **12**, in **H35** type **26**, and in **I35** type **12**.

	F	G	H	I
35	Gen 0	12	26	12
36	Gen 1			
37	Gen 2			

4. Click **Calculate Now**.
5. Enter the results in cell **F9** into Generation 1 A (cell **G22**). Enter the numbers in **F2**, **G3,** and **H2** into **G36, H36**, and **I36**. Enter the number from **F17** into **A3** and click **Calculate Now** again.
6. Repeat this until you have ten generations of alleles.

PART 3: Graphing the data

1. You can now produce a graph of the results. Highlight the cells **F20** to **H31** and click **Insert** then click on a **line graph** with markers.
2. The graph should automatically produce two lines for A and a. Give the graph appropriate titles and axes labels by clicking **Add Chart Element** (depending on your spreadsheet programme) and selecting **title** and **axes** labels.
3. Repeat this for the AA, Aa, and aa individuals.
4. Print the graphs and staple them to this page.

1. (a) What happens to the frequency of the a alleles over ten generations when the aa genotype is totally excluded from passing its alleles to the next generation?

(b) What happens to the frequency of the A alleles over ten generations when the aa genotype is totally excluded from passing its alleles to the next generation?

(c) Why do your observations from (a) and (b) happen? _____

(d) What is the effect on the phenotypes over time? (Assume AA and Aa produce the same dominant phenotype and aa is the recessive phenotype).

(e) Predict what might happen if some of the aa genotype were able to breed: _____

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PART 4: Changing the number of aa genotypes that can breed

1. What about if some of the aa genotype get to breed (i.e their fitness is not 0)? You can modify the spreadsheet to account for this. Instead of cell **G13** using just the number from the Aa genotype, you can add in a specific proportion of the aa genotype by entering the formula **=G2+((H2*2)*0.4)** into G13. (0.4 is the proportion of a alleles entering the next generation from the aa genotype (or you can use any other proportion of aa you want to pass to the next generation e.g. 0.7). This calculates the total number of aa alleles, then reduces the number by a predetermined proportion and adds to it to the a alleles from the genotype Aa.
2. Produce ten generations of alleles starting at frequencies of 0.5 for each allele A and a as in part 2 but change the proportion of aa genotypes than can breed to some new proportion (i.e not 0) as above. Produce graphs for allele frequencies and genotypes as in part 3 print them and attach them to this page.

2. (a) What difference occurs when some of the aa genotype is able to pass a proportion of alleles to the next generation as opposed to none?

(b) Try a proportion higher than you chose in part 4, and a proportion lower than you chose in part 4. Carry out the simulations again. Explain the results of these simulations.

3. Imagine a change happens in the environment so that now the dominant phenotype instead of the recessive phenotype is excluded from breeding. What would happen to the proportion and A and a alleles in the population (assuming the aa hadn't become extinct before then)?

4. Describe a situation or situations where natural selection against a specific genotype occurs in the real world and what the effect of the selection could be:

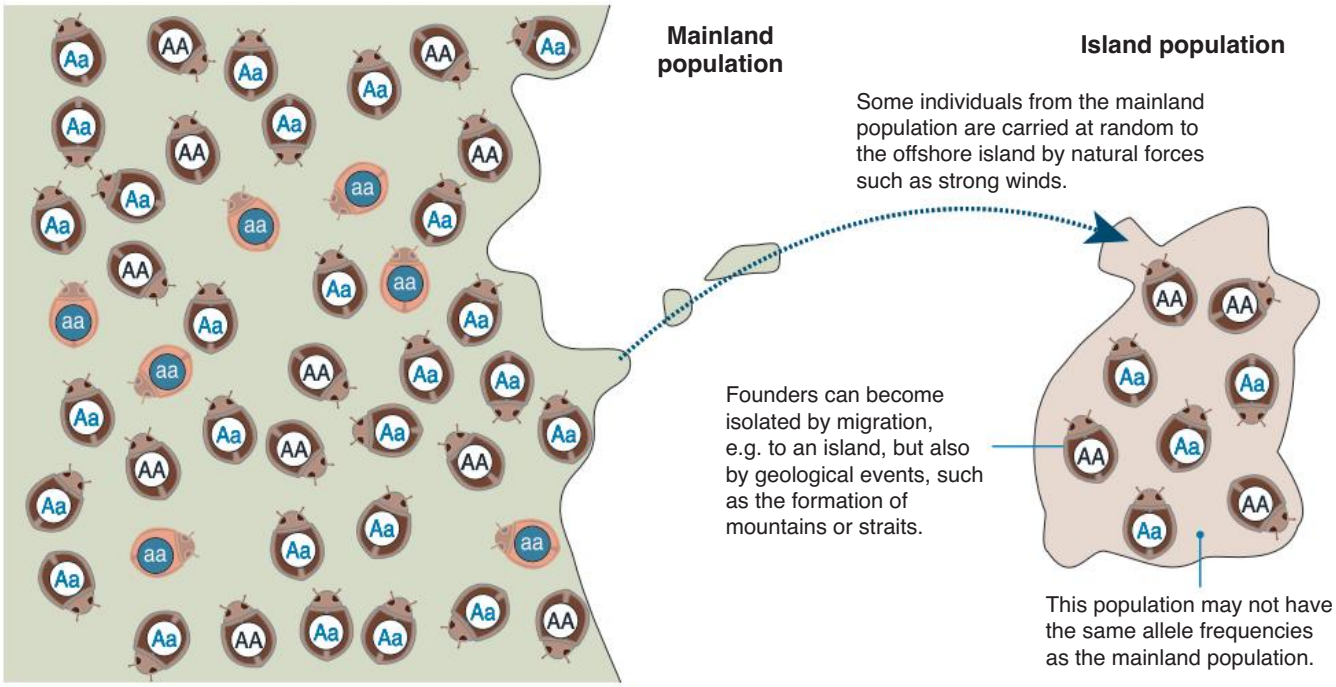
5. Imagine the genotypes AA, Aa, and aa all have different phenotypes (e.g. Aa is co-dominant or incompletely dominant). What might happen to the frequencies of A and a if the Aa phenotype (and hence genotype) was selected against?

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105 The Founder Effect

Key Idea: The founder effect can result in differences in allele frequencies between a parent and founder populations.

If a small number of individuals from a large population becomes isolated from their original parent population, their sample of alleles is unlikely to represent the allele proportions of the parent population. This phenomenon is called the **founder effect** and it can result in the colonising (founder) population evolving in a different direction to the parent population. This is particularly the case if the founder population is subjected to different selection pressures in a new environment and if the population is missing alleles that are present in the parent population.



Mainland population

	Allele frequencies		Phenotype frequencies	
	Actual numbers	Calculate %	Dark	Pale
Allele A				
Allele a				
Total				

Colonising island population

	Allele frequencies		Phenotype frequencies	
	Actual numbers	Calculate %	Dark	Pale
Allele A				
Allele a				
Total				

1. Compare the mainland population to the population which ended up on the island (use the spaces in the tables above):
   (a) Count the phenotype numbers for the two populations (i.e. the number of black and pale beetles).
   (b) Count the allele numbers for the two populations: the number of dominant alleles (A) and recessive alleles (a). Calculate these as a percentage of the total number of alleles for each population.

2. How are the allele frequencies of the two populations different? _____

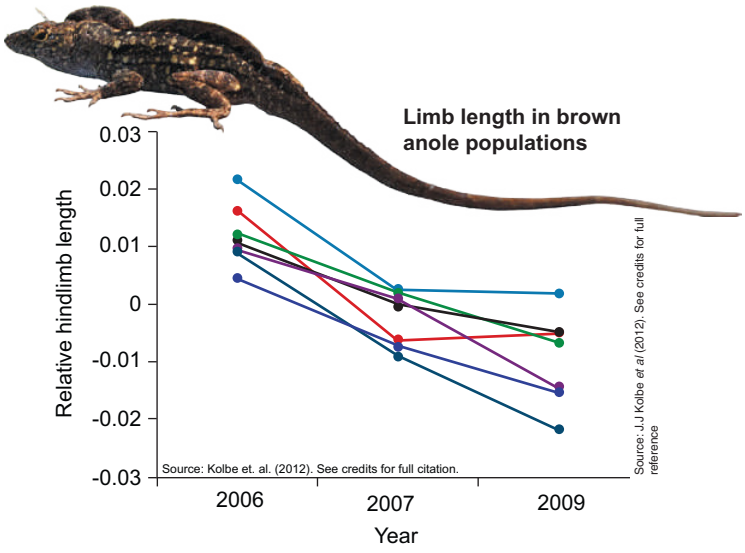
3. (a) What changes are likely when a founder population is isolated in a new environment? _____

   (b) What factors might influence the end result or the speed of the changes? _____

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Founder effect in brown anole lizards

- In 2004 Hurricane Francis wiped out the brown anole lizard (*Anolis sagrei*) populations on several cays (small sandy islands) around the Bahamas. Scientists used this as a chance to study the founder effect. They took pairs of lizards from the mainland and placed them on different cays.
- The vegetation on the cays is much smaller and scrub-like with thin branches or twigs compared to the much larger trees of the mainland. On the mainland, scientists noted that the lizards use their long limbs to climb around the trees. They hypothesised that the populations isolated on the cays would eventually evolve shorter limbs to adapt to the scrub-like, less supportive vegetation. They measured the limb length over several years.
- It was found that limb length indeed became shorter over successive generations in all the populations. Importantly, populations founded by lizards with the longest legs still had the longest legs and populations founded by lizards with the shortest legs still had the shortest legs. The characteristics of the founder populations influenced the descendant populations.

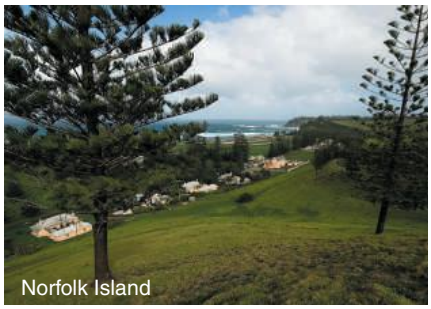


Founder effect in human populations



thinboyfatter CC 2.0

Due to the many episodes of human migration around the world there are many instances of the founder effect in human populations. In 1790, nine mutineers from the ship HMS Bounty along with six Tahitian men, eleven Tahitian women, and a baby girl settled on Pitcairn island. The population eventually grew to 193 by 1856.



In 1856 the entire population of Pitcairn Island resettled on Norfolk Island after it was decided Pitcairn was over populated. The effect of this can still be seen in genetic studies of the Norfolk Island population. In 1859, 16 people returned to Pitcairn Island and founded a new population, that eventually reached 250 people by 1936. The population is now around 56.



Brian Gratwicke CC 2.0

Tristan da Cunha sits 2,400 km from Africa and more than 3,500 km from South America. The current settlement of Tristan da Cunha was founded by the English in 1817. In 1961, a genetic study traced 14% of all genes in the population of 300 to one founding couple. Around 47% of the population are affected by asthma. From the 15 original settlers at least three had asthma.

4. (a) Why were conditions good for setting up an experiment on the founder effect on the cays around the Bahamas?

(b) Describe how the founder effect was demonstrated in the brown anole lizards:

5. (a) The rate of asthma in the UK is about 8%. Calculate the rate of asthma in the original Tristan da Cunha settlers:

(b) How has this affected the current population of Tristan da Cuhna?

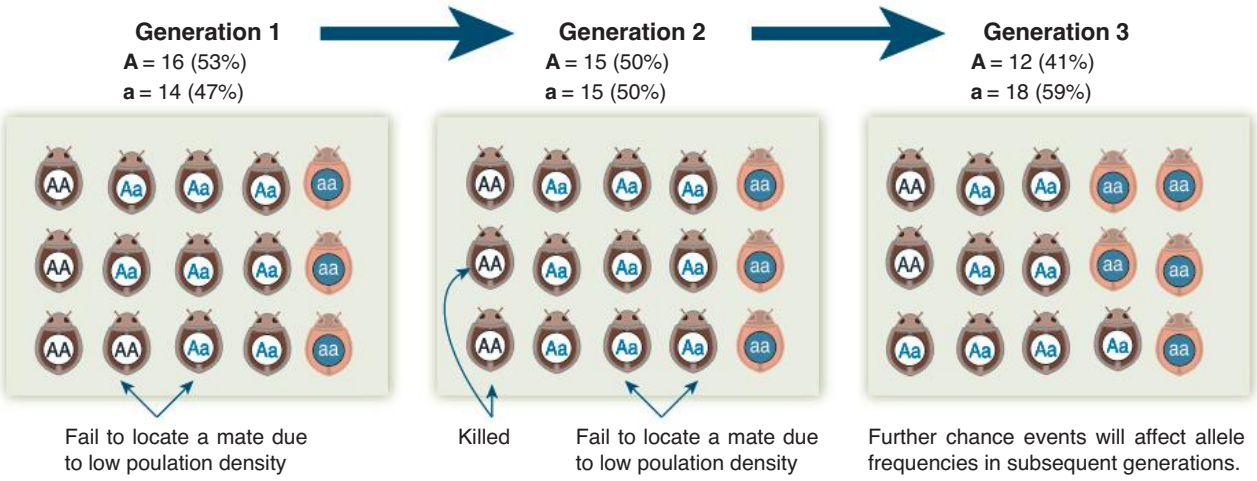
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106 Genetic Drift

Key Idea: Genetic drift describes the random changes in allele frequency that occur in all populations. It has a more pronounced effect in small populations.

Not all individuals, for various reasons, will be able to contribute their genes to the next generation. This random change in allele frequencies is called **genetic drift**. It is the result of "sampling error" in the selection of alleles from the current gene pool for the next generation (not an error of how we might observe or record breeding events). The effect of genetic drift on a gene pool are more pronounced in small populations. Alleles may become **lost** from the gene pool (frequency = 0%) or **fixed** as the only allele present for the gene (frequency = 100%). Recall that in your natural selection spreadsheet, even starting with 0.5 *A* and 0.5 *a* the next generation of alleles were not 0.5 *A* and 0.5 *a* (even before selection had occurred). Due to the random mating set in the simulation, sometimes more or less than 0.5 *A* alleles were passed on. This directly affects the frequencies in the next generations's gene pool. Even without any selection, random changes in the gene pool can have important effects.

The genetic makeup (allele frequencies) of the population changes randomly over a period of time

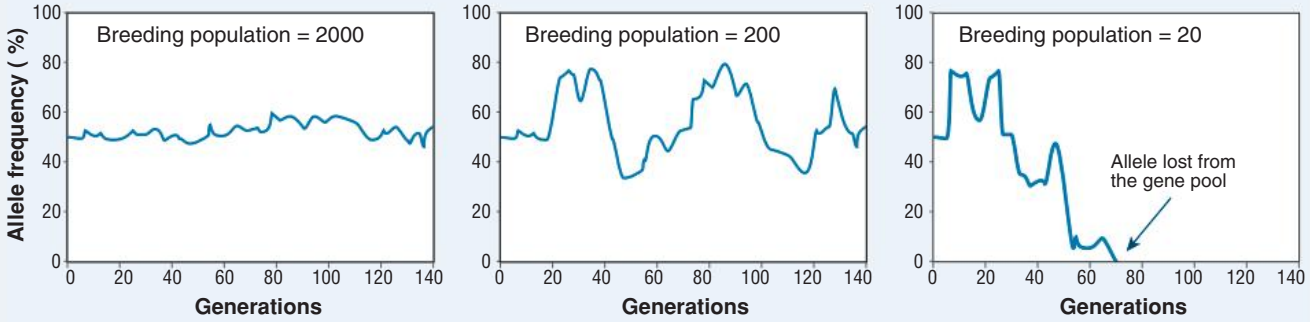


This diagram shows the gene pool of a hypothetical small population over three generations. For various reasons, not all individuals contribute alleles to the next generation. With the random loss of the alleles carried by these individuals, the allele frequency changes from one generation to the next. The change in frequency is directionless as there is no selecting force. The allele combinations for each successive generation are determined by how many alleles of each type are passed on from the preceding one.

Computer simulations of genetic drift

- Computer simulations are used to carry out population experiments which are impractical to fully observe in wild populations. For instance, the generation times may be too long or obtaining genetic samples impossible.

Below are displayed the change in allele frequencies in a computer simulation showing random genetic drift. The breeding population progressively gets smaller from left to right. Each simulation was run for 140 generations.



Large breeding population
Fluctuations are minimal because large numbers buffer the population against random loss of alleles. On average, losses for each allele type will be similar in frequency and little change occurs.

Small breeding population
Fluctuations are more severe in smaller breeding populations because random changes in a few alleles cause a greater percentage change in allele frequencies.

Very small breeding population
Fluctuations in very small breeding populations are so extreme that the allele can become fixed (frequency of 100%) or lost from the gene pool altogether (frequency of 0%).

- You can use the spreadsheet you made in Activity 104 to investigate the effect of population size on allele frequency changes due to genetic drift (see next page). You will need to modify the spreadsheet to make random changes to the frequency with which alleles are passed on to the next generation. This simulates random events removing alleles from the gene pool (as above).

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Investigation 10.2 Modelling genetic drift

Part 1

1. Modify the spreadsheet you made for Activity 104 to complete this simulation. Save the spreadsheet under a new name.
2. Open the spreadsheet. In cell **F13** (under the heading A passed on) change the formula to **=(F2*2*RAND())+(G2*RAND())**. This randomly selects a number of A alleles from the total available to pass to the next generation.
3. In cell **G13**, change the formula to **=(H2*2*RAND())+(G2*RAND())**. This randomly selects B alleles.

F	G
AA	AB
=COUNTIF($D$7:$D$56,"AA")	=COUNTIF($D$7:$D$56,"AB")+COUNTIF($D$7:$D$56,"BA")
A	B
=(F2*2)+G2	=(H2*2)+G2
Frequency A	Frequency B
=F5/(F5+G5)	=G5/(F5+G5)
A passed on	B passed on
=(F2*2*RAND())+(G2*RAND())	=(H2*2*RAND())+(G2*RAND())
Freq A passed on	Freq A passed on
=F13/(F13+G13)	=G13/(F13+G13)

4. Set cell **A3** to **0.5** and click **Calculate Now**. Record the A and a frequency for each generation, the numbers of AA, Aa, and aa individuals and produce graphs for twenty generations (stop if an allele becomes fixed) as in part 2 of Activity 102. Note Gen 0 AA = 13, Aa = 26 and aa = 13.

Part 2

1. You can now change the population number and see how genetic drift affects allele frequencies. In cell **F2**, change the COUNTIF formula to **=COUNTIF($D$7:$D$26,"AA")** to count only the first 20 individuals.
2. In cell **G2**, change the formula to **=COUNTIF($D$7:$D$26,"AB")+COUNTIF($D$7:$D$26,"BA")** and in cell **H2** change the formula to **=COUNTIF($D$7:$D$26,"BB")**. Click **Calculate Now** and cell J2 should show 20.
3. Set cell A3 to **0.5** and click **Calculate Now**. Record the A and a frequency for each generation, the numbers of AA, Aa, and aa individuals and produce graphs for twenty generations (stop if an allele becomes fixed). Note Gen 0 AA = 5, Aa = 10 and aa = 5.
4. Now repeat the above but set the COUNTIF formulae to count to row **106**. Highlight cells **A56** to **D56** and copy them down to row **106**. Click **Calculate Now** and cell J2 should show 100.
5. Set cell **A3** to **0.5** and click **Calculate Now**. Record the A and a frequency for each generation, the numbers of AA, Aa, and aa individuals and produce graphs for twenty generations (stop if an allele becomes fixed) Note Gen 0 AA = 25, Aa = 50 and aa = 25.

1. What is genetic drift and why are its effects more pronounced in smaller populations?_____

2. Which of the simulations you did above appears to have the most fluctuations in the A and a alleles? Explain why:

3. Suggest why genetic drift is regarded as an important process in the evolution of small populations. You can use your simulation as evidence if you wish:

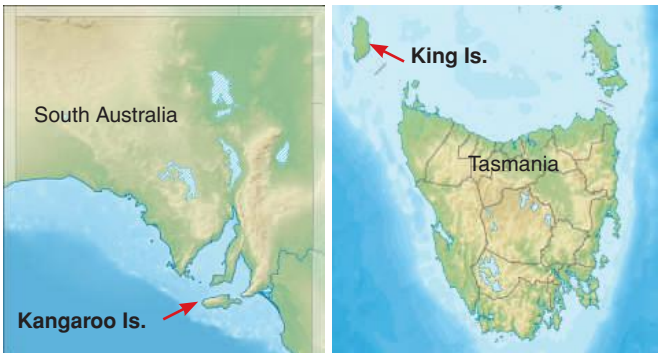
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Island platypus and the perils of genetic drift

- A 2012 study of genetic diversity in platypus populations on mainland Australia, Tasmania, King Island, and Kangaroo Island has revealed very low immunological diversity in the island populations. The study (Lillie *et al.*) looked at the diversity of the MHC DZB gene and three MHC associated markers, all of which are involved in immune function. High allelic diversity in immune genes is important because it provides the variation necessary to resist different kinds of diseases. Without genetic variation, the population is likely to have low resistance to new diseases and environmental change.
- The study found that populations on the Australian mainland and in Tasmania have high levels of genetic diversity within their populations, with 57 DZB alleles identified in 70 individuals. However platypuses on King Island and Kangaroo Island (see maps), had very low levels of genetic diversity. For the King Island populations, there was no variation at all (only one allele at the DZB locus).
- Why is the genetic diversity of these island populations so low compared to the mainland populations? The Kangaroo Island population was founded from an introduction of around 20 animals in the 1930s and 1940s. The population on King Island is endemic, separated for some 14,000 years since the last ice age.
- Inbreeding in a small population and genetic drift have resulted in the loss of alleles and a dangerously low diversity in immune genes. These island populations will now need careful management to protect them from disease risk.



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Nzeemin cc 3.0

Source: Mette Lillie *et. al.* (2012) See credits for full reference

4. What factors have contributed to the low genetic diversity of the platypus populations on King Island and Kangaroo Island and explain their effect(s)?

5. Why would genetic drift have more impact on the genetic diversity of these populations than those on the mainland?

6. (a) Describe why a population with low MHC diversity is more likely to be affected by a new disease than a population with high MHC diversity:

   (b) Why is it important to try to keep small isolated populations free of new diseases?

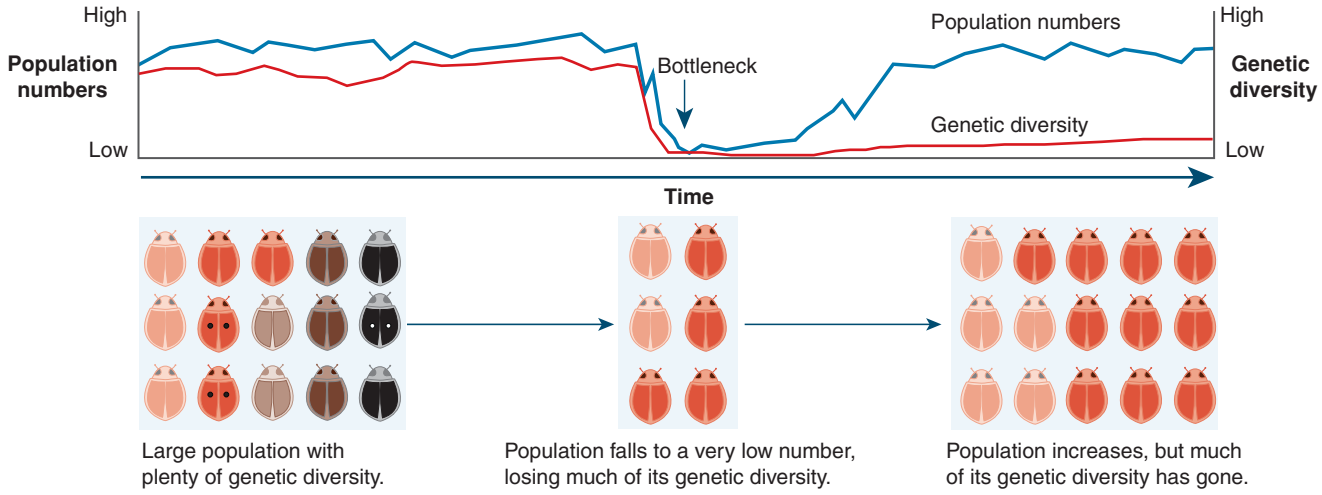
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107 Genetic Bottlenecks

Key Idea: Genetic bottlenecks occur when population numbers and diversity fall dramatically. Although a population's numbers may recover, its genetic diversity often does not.

Populations may sometimes be reduced to low numbers by predation, disease, or periods of climatic change. These large scale reductions are called genetic (or population) bottlenecks. The sudden population decline is not necessarily selective and it may affect all phenotypes equally. Large scale catastrophic events, such as fire or volcanic eruptions, are examples of such non-selective events. Affected populations may later recover, having squeezed through a 'bottleneck' of low numbers. The diagram below illustrates how population numbers may be reduced as a result of a catastrophic event. Following such an event, the gene pool of the surviving remnant population may be markedly different to that of the original gene pool. Genetic drift may cause further changes to allele frequencies. The small population may return to previous levels but with a reduced genetic diversity.

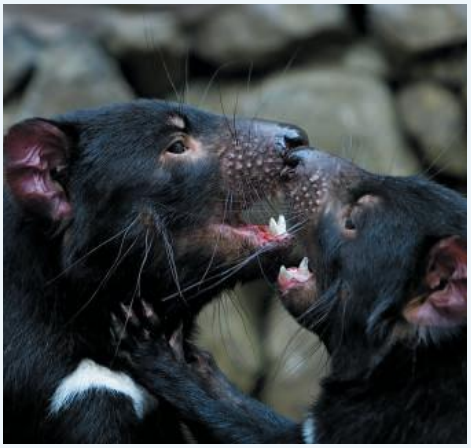
Change in population numbers and diversity



Bottlenecks and low allelic diversity in Tasmanian devils

Tasmanian devils are the largest surviving marsupial carnivore. Devils were once found throughout mainland Australia, but became locally extinct about 3000 years ago. They are now restricted to Tasmania*. Genetic evidence suggests that the devils went through at least two historic population crashes, one ~30,000 years ago and another ~3000 years ago. There have also been modern declines (1850 to 1950) as a result of trapping and disease. These historic population crashes are likely to be responsible for the very low diversity in the MHC I and II genes in devils.

The MHC genes are important in immunity and the body's self recognition system. Low allelic diversity for MHC is implicated in the spread of devil facial tumour disease (DFTD), a contagious cancer that appeared in populations in the mid 1990s and has resulted in the loss of 80% of devils. The cancerous cells are transmitted when the devils fight. This foreign material would be normally be recognised and destroyed by the immune system. In Tasmanian devils, immune diversity is so low that cancerous cells can be transferred without causing an immune response in a recipient. In some more positive news, recent evidence shows that some populations are developing immunity to DFTD. This may originate in individuals with MHC alleles distinctly different from the susceptible individuals.



**There have been recent reintroductions of devils to the mainland. See the Resource Hub video.*

1. Endangered species are often subjected to genetic bottlenecks. Explain how genetic bottlenecks affect the ability of a population of an endangered species to recover from its plight:

_____

_____

2. (a) What has been the genetic consequence of bottleneck events in the Tasmanian devil population?_____

_____

_____

(b) How has this led to increased susceptibility to disease, specifically infectious cancer? _____

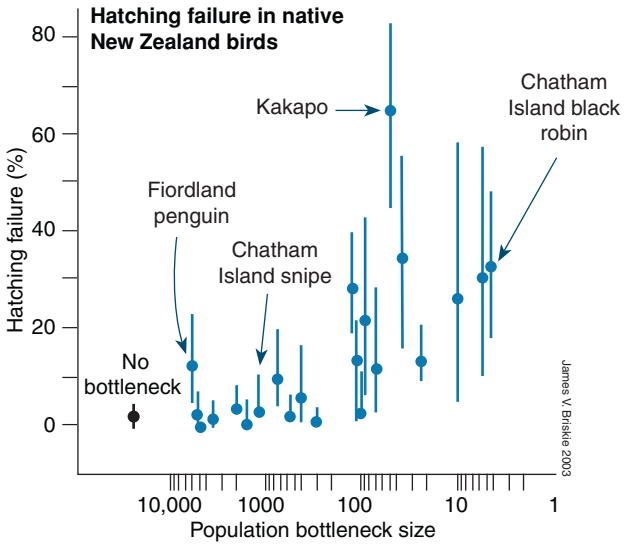
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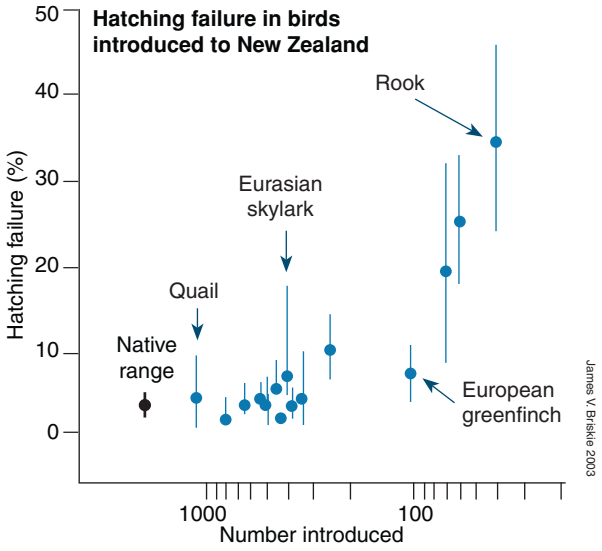
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Genetic bottlenecks affect hatching rates in birds

- Severe bottlenecks reduce genetic diversity. It can sometimes be difficult to identify the many effects of this reduction. New Zealand has many native birds that have gone through recent bottlenecks, and many are still critically endangered. Many birds were introduced into New Zealand during the 1800s by acclimatisation societies. Some of these introductions were small and so these founder populations also represented genetic bottlenecks. They are useful illustrations of genetic bottlenecks.
- The effect of these bottlenecks on egg hatching was investigated in 22 native birds. As a comparison, the hatching rates of introduced birds were also investigated.







Kakapo were once common throughout New Zealand, but by 1995 there were just 51 left. The population is now around 150.



The Chatham Island black robin population was just 5 in 1980, but has risen to 200. It has very little genetic diversity.



Small numbers of rook were introduced in the 1860s. Its population reached over 60,000 by 1975 before culling reduced it.

3. What events might cause a population (genetic) bottleneck? _____

_____

4. (a) What has been the genetic consequence of bottleneck events in the Tasmanian devil population? _____

_____

_____

(b) How has this led to increased susceptibility to disease, specifically infectious cancer? _____

_____

_____

_____

5. (a) What was the effect of genetic bottlenecks on hatching failure in native and introduced birds in New Zealand birds?

_____

(b) Why might this be? _____

_____

_____

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108 Selection for Skin Colour in Humans

Key Idea: Skin colour is the result of a dynamic balance between two different selection pressures linked to fitness.

Pigmented skin of varying tones is a feature of humans that evolved after early humans lost the majority of their body hair. However, the distribution of skin colour globally is not random; people native to equatorial regions have darker skin tones than people from higher latitudes. For many years, biologists postulated that this was because darker skins had evolved to protect against skin cancer. The problem with this explanation was that skin cancer is not tied to evolutionary fitness because it affects post-reproductive individuals and cannot therefore provide a mechanism for selection. Physiological and epidemiological evidence has now shown that selection pressures on skin colour are finely balanced to produce a skin tone that regulates the effects of the sun's UV radiation on the nutrients vitamin D and folate, both of which are crucial to successful reproduction and therefore evolutionary fitness. The selection is stabilising within each latitudinal region.



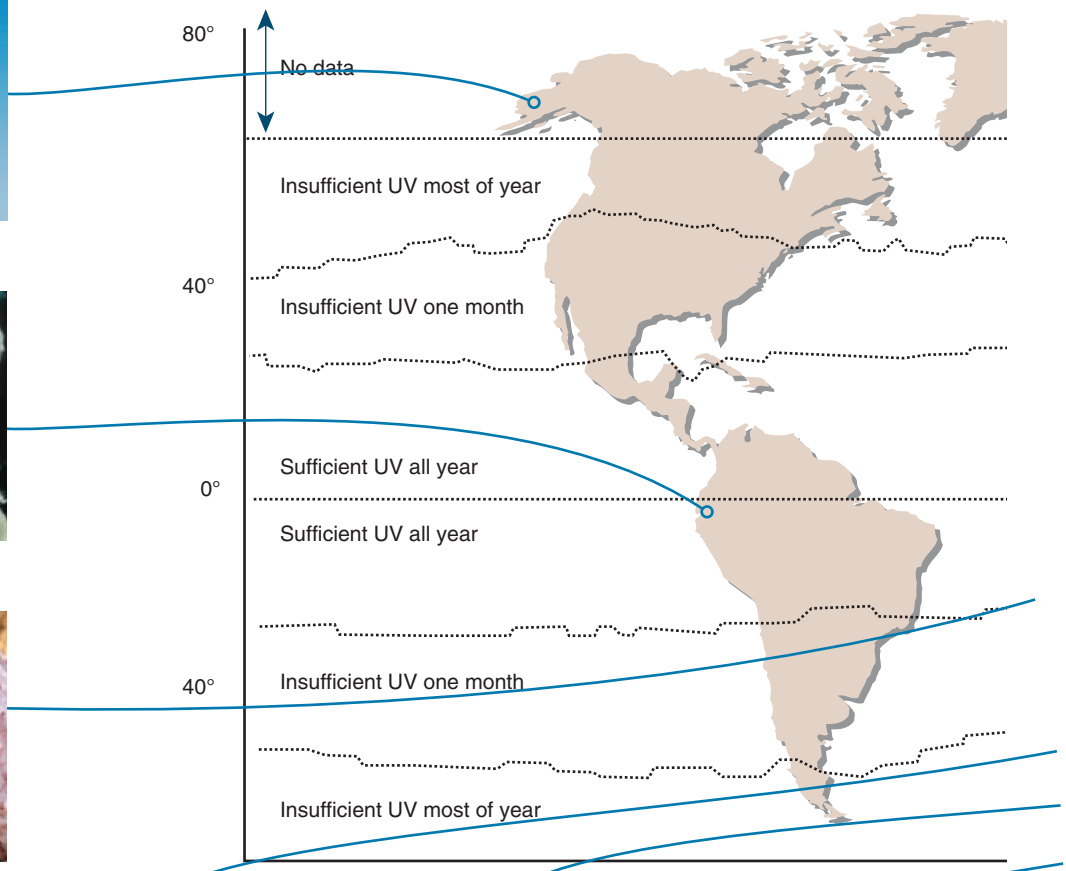
Alaska



Peru



Liberia





Burundi



Botswana

Photo: Lisa Grey



Iraq

1. (a) Describe the role of folate in human physiology: _____

_____

(b) Describe the role of vitamin D in human physiology: _____

_____

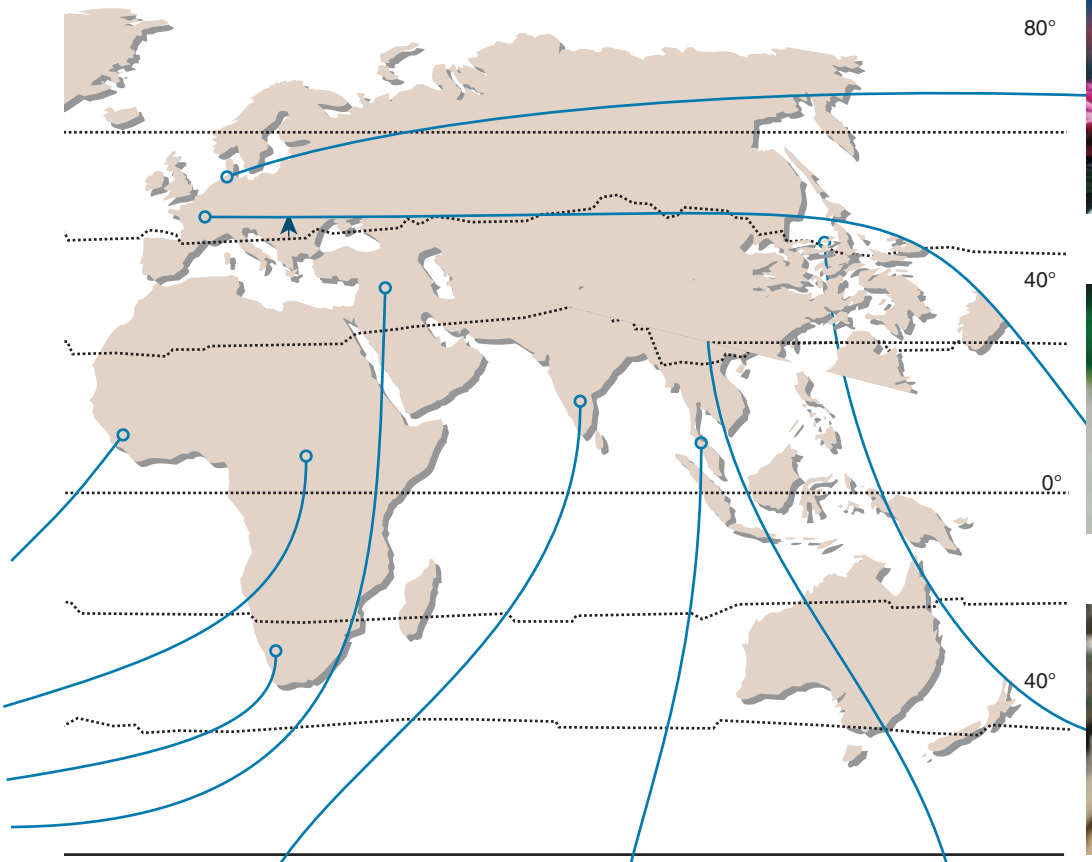
2. How does UV light intensity change from north to south across the globe? _____

_____

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Skin colour in humans: a product of natural selection

- Human skin colour is the result of two opposing selection pressures. Skin pigmentation has evolved to protect against destruction of folate from ultraviolet light, but the skin must also be light enough to receive the light required to synthesise vitamin D.
- Vitamin D synthesis is a process that begins in the skin and is inhibited by dark pigment. Folate is needed for healthy neural development in humans and a deficiency is associated with fatal neural tube defects. Vitamin D is required for the absorption of calcium from the diet and therefore normal skeletal development.
- Women also have a high requirement for calcium during pregnancy and lactation. Populations that live in the tropics receive enough ultraviolet (UV) radiation to synthesise vitamin D all year long. Those that live in northern or southern latitudes do not. In temperate zones, people lack sufficient UV light to make vitamin D for one month of the year. Those nearer the poles lack enough UV light for vitamin D synthesis most of the year (above). Their lighter skins reflect their need to maximise UV absorption (the photos show skin colour in people from different latitudes).



Adapted from Jablonski & Chaplin, Sci. Am. Oct. 2002



The Netherlands



France



Japan



Southern India



Malaysia



China

3. Explain why, in any given geographical region, women tend to have lighter skins (by 3-4% on average) than men:

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Long-term resident **Recent immigrant**

1 Southern Africa: ~ 20-30˚S



Khoisan-Namibia



Zulu: 1000 years ago

2 Australia: ~ 10-35˚S



Australian Aboriginal



European: 300 years ago

3 Banks of the Red Sea: ~ 15-30˚N



Rita Willaert-Flickr

Nuba-Sudan



Arab: 2000 years ago

4 Northern India: ~ 10-30˚S



West Bengal



Aptychus-Flickr

Tamil: ~100 years ago

The skin of people who have inhabited particular regions for millennia has adapted to allow sufficient vitamin D production while still protecting folate stores. In the photos above, some of these original inhabitants are illustrated to the left of each pair and compared with the skin tones of more recent immigrants (to the right of each pair, with the number of years since immigration). The numbered locations are on the map.

4. (a) Early hypotheses to explain skin colour linked pigmentation level only to the degree of protection it gave from UV-induced skin cancer. Explain why this hypothesis was inadequate in accounting for how skin colour evolved:

(b) Explain how the new hypothesis for the evolution of skin colour overcomes these deficiencies: _____

5. The Inuit people of Alaska and northern Canada have a diet rich in vitamin D and their skin colour is darker than predicted on the basis of UV intensity at their latitude. Explain this observation:

6. (a) What health problems might be expected for people of African origin (right) now living in northern UK?

(b) How could these people avoid these problems in their new higher latitude environment?



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109 Why Don't Mutations Disappear?

Assessment Task, Outcome 2: *Analysis and evaluation of a selected biological case study*

- Natural selection operates on phenotypes (and therefore their genotypes) in the prevailing environment. For some phenotypic conditions controlled by a single gene with two alleles, a heterozygote (an individual with two different alleles for a gene) may have a higher fitness than either of the homozygous conditions. This situation is called **heterozygous advantage**.
- In the case of the sickle cell allele outlined below, susceptibility to malaria is high in the homozygous dominant condition, but lower in the heterozygous condition. Consequently, the heterozygote has a higher fitness in malaria-prone regions. Heterozygous advantage can result in the stable coexistence of different phenotypes in a population (a state called balanced polymorphism) and can account for the persistence of detrimental alleles. The maintenance of the sickle cell mutation in malaria-prone regions is one of the few well documented examples in which the evidence for heterozygous advantage is conclusive.

The sickle cell allele (Hb^S)

Sickle cell disease is caused by a mutation in a gene encoding haemoglobin. Genetic analyses show that the mutation arose spontaneously in different regions. The mutant allele (**Hb^S**) produces a form of haemoglobin that differs from the functional form by just one amino acid in the β-chain. This small change causes 'sickling' of the red blood cells. The sickling causes the red blood cells to clump together, blocking blood vessels, and causing numerous circulatory and organ problems. Destruction of the red blood cells also leads to anaemia.

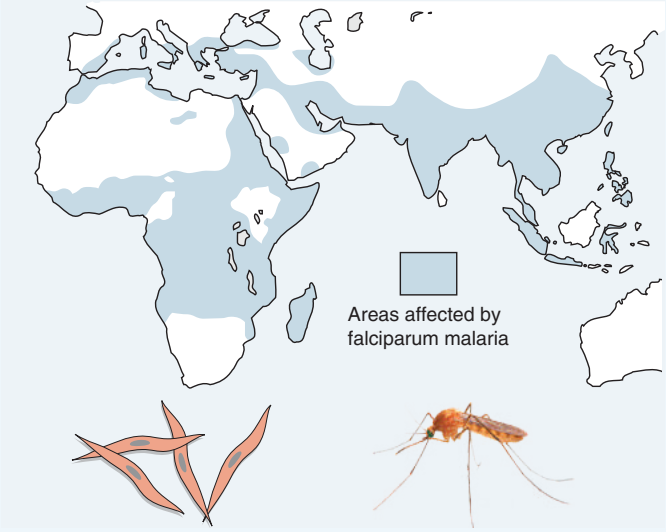
- In heterozygotes (Hb^SHb), there is a mixture of both normal and sickle cells and they are said to carry the sickle cell trait. They are generally unaffected by the disease except in low oxygen environments.
- People with two Hb^S genes (Hb^SHb^S) suffer severe illness and often die prematurely. Hb^S is therefore considered to be a lethal allele.

Heterozygous advantage in malarial regions

Falciparum malaria is widely distributed throughout central Africa, the Mediterranean, Middle East, and tropical and semi-tropical Asia (Figure 1). It is transmitted by the *Anopheles* mosquito, which spreads the protozoan *Plasmodium falciparum* from person to person as it feeds on blood. Symptoms appear 1-2 weeks after being bitten, and include headache, shaking, chills, and fever. Falciparum malaria is more severe than other forms of malaria, with high fever, convulsions, and coma. Death can occur within days of the first symptoms appearing.

- **The paradox**: The Hb^S allele offers considerable protection against malaria. Sickle cells have low potassium levels, which causes *Plasmodium* parasites inside these cells to die. Those with a normal phenotype are very susceptible to malaria, but heterozygotes (Hb^SHb) are much less so. This situation, called **heterozygous advantage**, has resulted in the Hb^S allele being present in moderately high frequencies in parts of Africa and Asia despite its harmful effects (Figure 2).
- This is a special case of balanced polymorphism, called a **balanced lethal system** because neither of the homozygotes produces a phenotype that survives, but the heterozygote is viable.

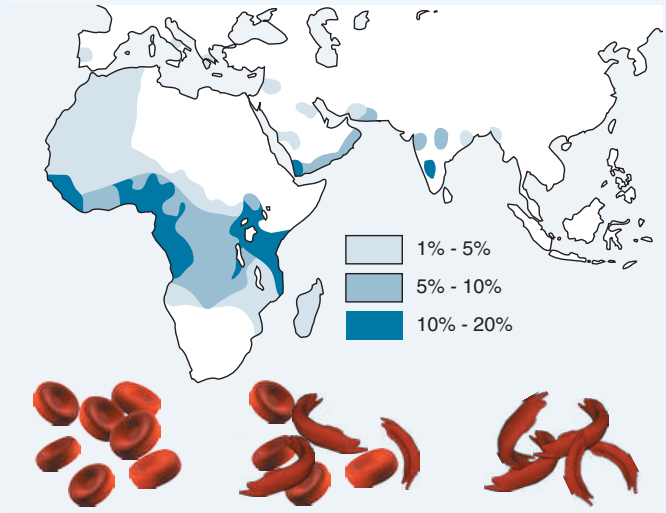
Figure 1: Incidence of falciparum malaria



Four species of *Plasmodium* cause malaria but the variety caused by *P. falciparum* is the most severe.

Anopheles mosquito, the insect vector responsible for spreading *Plasmodium*.

Figure 2: Frequency of the sickle cell allele



HbHb
All red blood cells normal
Susceptible to malaria

Hb^SHb
Normal and sickle cells
Malaria resistance

Hb^SHb^S
All cells are sickled
Sickle cell disease

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- Figure 3 shows the general distributions of various haemoglobin disorders that all produce abnormal red blood cells to some degree.
- Hb^E is a mutation that appears to have arisen about 5000 years ago and is caused by a change in the 26th amino acid in the β-chain from glutamic acid to lysine.
- The Hb^S mutation changes the 6th amino acid from glutamic acid to valine.
- Hb^C is a mutation that occurs in the same position as Hb^S, but the mutation produces the amino acid lysine instead of valine.
- Both Hb^E and Hb^C heterozygotes show virtually no (and certainly much less than Hb^S) symptoms of anaemia (reduced haemoglobin levels).
- Thalassaemia is a disease in which gene mutations result in the lowered production of haemoglobin and red blood cells. The effects can be very severe.

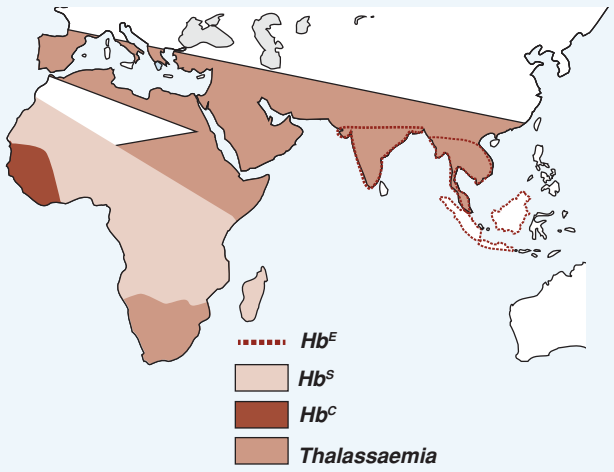


Fig. 3: Distribution of abnormal blood conditions

1. Study the three diagrams in this activity. Analyse the distribution of blood abnormalities caused by abnormal haemoglobin and explain why these abnormalities continue to exist in the population despite severe cases being lethal.

   Your analysis and answer should include:

   A description of the distribution of malaria throughout the world
   A comparison of the distribution of malaria and frequency of the Hb^s allele
   An explanation of why carriers of the Hb^s allele have an advantage in malaria-prone regions
   An explanation how heterozygous advantage can lead to balanced polymorphism

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110 Selective Breeding in Animals

Key Idea: Selective breeding is the process of breeding together organisms with desirable qualities (e.g. high milk yield) so the trait is reliably passed on to the next generation.

Selective breeding (or artificial selection) is the process by which humans select organisms with desirable traits and breed them together so the trait appears in the next generation. The process is repeated over many generations until the characteristic becomes common. Selective breeding often uses reproductive technologies, such as artificial insemination, so that the desirable characteristics of one male can be passed onto many offspring. This increases the rate at which the desirable trait is passed to progeny. There are problems associated with selective breeding. The gene pool becomes more constrained and some alleles may be lost. A reduction in genetic diversity may also decrease the ability of a species to adapt to changes in the environment.

The origin of domestic animals



PIG

Wild ancestor: Boar (left)

Origin: Anatolia, 9000 years BP

Now: More than 12 distinct modern breeds, including the Berkshire (meat) and Tamworth (hardiness).

DOMESTIC FOWL

Wild ancestor: Red jungle fowl (right)

Origin: Indus Valley, 4000 BP

Now: More than 60 breeds including Rhode Island red (meat) and leghorn (egg production).



Each domesticated breed has been bred from the wild ancestor. The date indicates the earliest record of the domesticated form (years before present or BP). Different countries have different criteria for selection, based on their local environments and consumer preferences.



Bezoar ibex goat



Mouflon



Zebu: derived from Indian aurochs

GOAT

Wild ancestor: Bezoar goat

Origin: Iraq, 10,000 years BP

Now: approx. 35 breeds including Spanish (meat), Angora (fibre) and Nubian (dairy).

SHEEP

Wild ancestor: Asiatic mouflon

Orign: Iran, Iraq, Levant, 10,000 years BP

Now: More than 200 breeds including Merino (wool), Suffolk (meat), Friesian (milk), and dual purpose (Romney).

CATTLE

Wild ancestor: Auroch (extinct)

Origin: SW Asia, 10,000 years BP

Now: 800 modern breeds including the Aberdeen Angus (meat), Friesian and Jersey (milk), and zebu (draught).

1. (a) What is selective breeding? _____

_____

(b) What are the advantages of selective breeding? _____

_____

_____

2. What effect would selective breeding have on the genetic diversity of a population? _____

_____

_____

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The origins of domestic dogs

- All breeds of dog are members of the same species, *Canis familiaris* and provide an excellent example of selective breeding. The dog was the first domesticated species and, over centuries, humans have selected for desirable traits, so extensively that there are now more than 400 breeds of dogs.
- Until very recently, the grey wolf was considered to the ancestor of the domestic dog. However, recent (2015) genetic studies provide strong evidence that domestic dogs and grey wolves are sister groups and shared a now extinct wolf-like common ancestor, which gave rise to the dog before the agricultural revolution 12,000 years ago. Based on genetic analysis, four major clusters of ancient dog breeds are recognised. Through selective breeding, all other breeds are thought to have descended from these clusters.



1: Older lineages
The oldest lineages, including Chinese breeds, basenji, huskies, and malamutes.



2: Mastiff-type
An older lineage that includes the mastiffs, bull terriers, boxers, and rottweilers.



3: Herding
Includes German shepherd, St Bernard, borzoi, collie, corgi, pug, and greyhound



4: Hunting
Most arose in Europe. Includes terriers, spaniels, poodles, and modern hounds.

Problems with selective breeding

- Selection for a desirable phenotype can result in undesirable traits being emphasised, often because genes for particular characteristics are linked and selection for one inadvertently selects for the other. For example, the German shepherd is a working dog, originally bred for its athleticism and ability to track targets.
- In German shepherds bred to meet the specific appearance criteria of show dogs, some traits have been exaggerated so much that it causes health issues. The body shape of the show German shepherd has been selected for a flowing trot and it has a pronounced slope in the back. This has resulted in leg, hip, and spinal problems. In addition, selective breeding has increased the incidence of some genetic diseases such as epilepsy and blood disorders.





3. List the physical and behavioural traits that would be desirable (selected for) in the following uses of a dog:

   (a) Hunting large game (e.g. boar and deer): _____

   (b) Stock control (sheep/cattle dog): _____

   (c) Family pet (house dog): _____

   (d) Guard dog: _____

4. As a group, discuss the ethical considerations of using selective breeding to "improve" dog breeds. What would it take to change breed standards to avoid health issues?

_____

_____

_____

_____

_____

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111 Selection in Livestock

Key Idea: The performance of livestock can be improved by selective breeding based on measurable physical traits.

Most of the economically important traits in dairy cattle (below right) are expressed only in females, but the main opportunity for selection is in males. Selection of the best bulls, combined with their worldwide use through artificial insemination and frozen semen has seen a rapid **genetic gain** (i.e. the increase in performance as a result of genetic changes) in dairy cattle since the 1970s. Bulls are assigned breeding values based on the performance of their daughters and granddaughters. In this way, the bulls and cows with the best genetics can be selected to produce the next generation.



Hereford

Beef breeds: Selection is for large breeds with a high proportion of lean muscle. **Desirable traits**: high muscle to bone ratio, rapid growth and weight gain, hardy, easy calving, docile temperament.

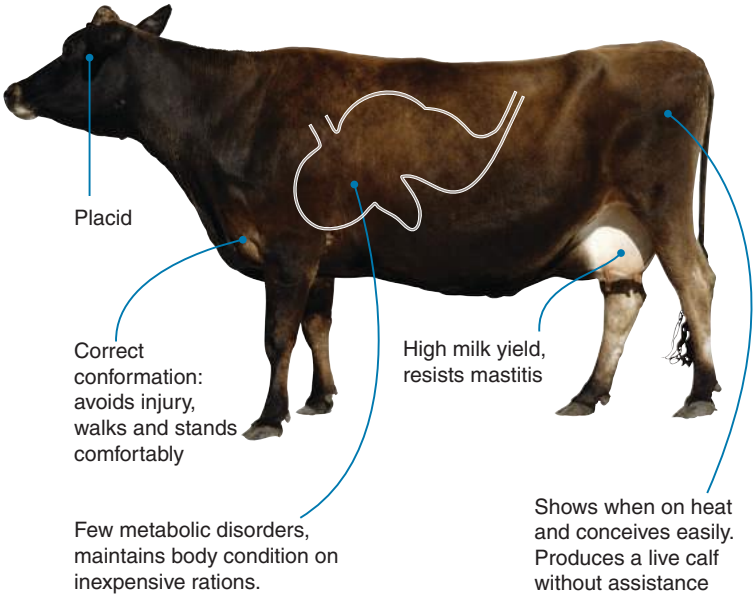


Dairy breeds: Selection is based primarily on high milk production, but good health and fertility are also selected for. **Desirable traits**: high milk yield with good protein and fat content, fast milking speed, docile temperament, good udder characteristics (e.g. good teat placement).



Special breeds: Some cattle are bred for their suitability to climate or terrain. Scottish highland cattle (above) are a hardy, long coated breed and produce well where other breeds cannot thrive.

The perfect dairy cow



Breeding programs select not only for milk production, but also for fertility, udder characteristics, and good health. In addition, artificial selection can be based on milk composition, e.g. high butterfat content (a feature of the Jersey breed, above).

A2 milk, which contains the A2 form of the beta casein protein, has recently received worldwide attention for claims that its consumption lowers the risk of childhood diabetes and coronary heart disease. Selection for the A2 variant in **Holstein cattle** has increased the proportion of A2 milk produced in some regions. A2 milk commands a higher price than A1 milk, so there is a commercial incentive to farmers to produce it.



1. Why can artificial selection produce changes in phenotype much more rapidly than natural selection? _____

2. Suggest why selective breeding has proceeded particularly rapidly in dairy cattle: _____

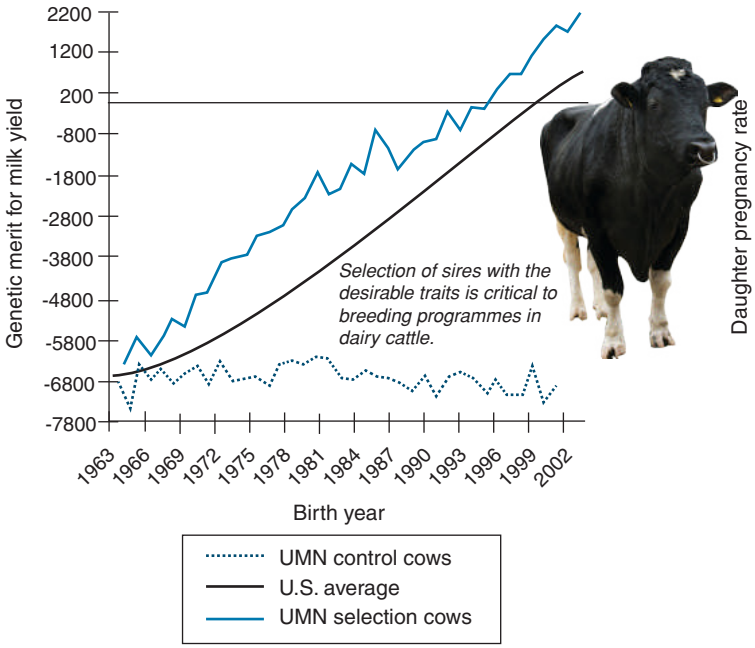
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112 Selective Breeding for Milk Production

Key Idea: Selective breeding is able to produce rapid change in the phenotypic characteristics of a population.

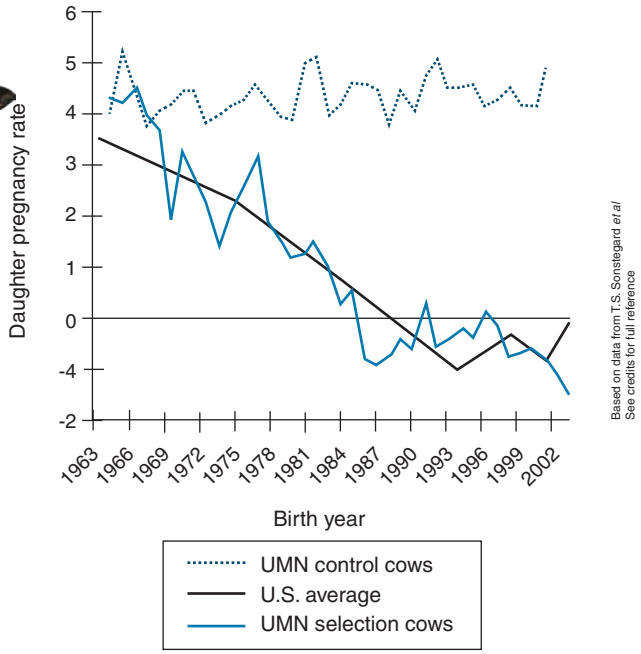
Humans may create the selection pressure for evolutionary change by choosing and breeding together individuals with particular traits. The example of milk yield in Holstein cows (below) illustrates how humans have directly influenced the genetic makeup of Holstein cattle with respect to milk production and fertility. Since the 1960s, the University of Minnesota has maintained a Holstein cattle herd that has not been subjected to any selection. They also maintain a herd that was subjected to selective breeding for increased milk production between 1965 and 1985. They compared the genetic merit (this is essentially the breeding value) for milk yield in these groups to that of the USA Holstein average.

Gain in genetic merit for milk yield



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

Fertility in Holstein cows



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

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

   _____

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

   _____

   _____

2. What limits might this place on maximum milk yield? _____

   _____

3. Why is sire selection important in selective breeding, even if the characters involved are expressed only in the female?

   _____

4. Natural selection is the mechanism by which organisms with favourable traits become proportionally more common in the population. How does selective breeding mimic natural selection? How does the example of the Holstein cattle show that reproductive success is a compromise between many competing traits?

   _____

   _____

   _____

   _____

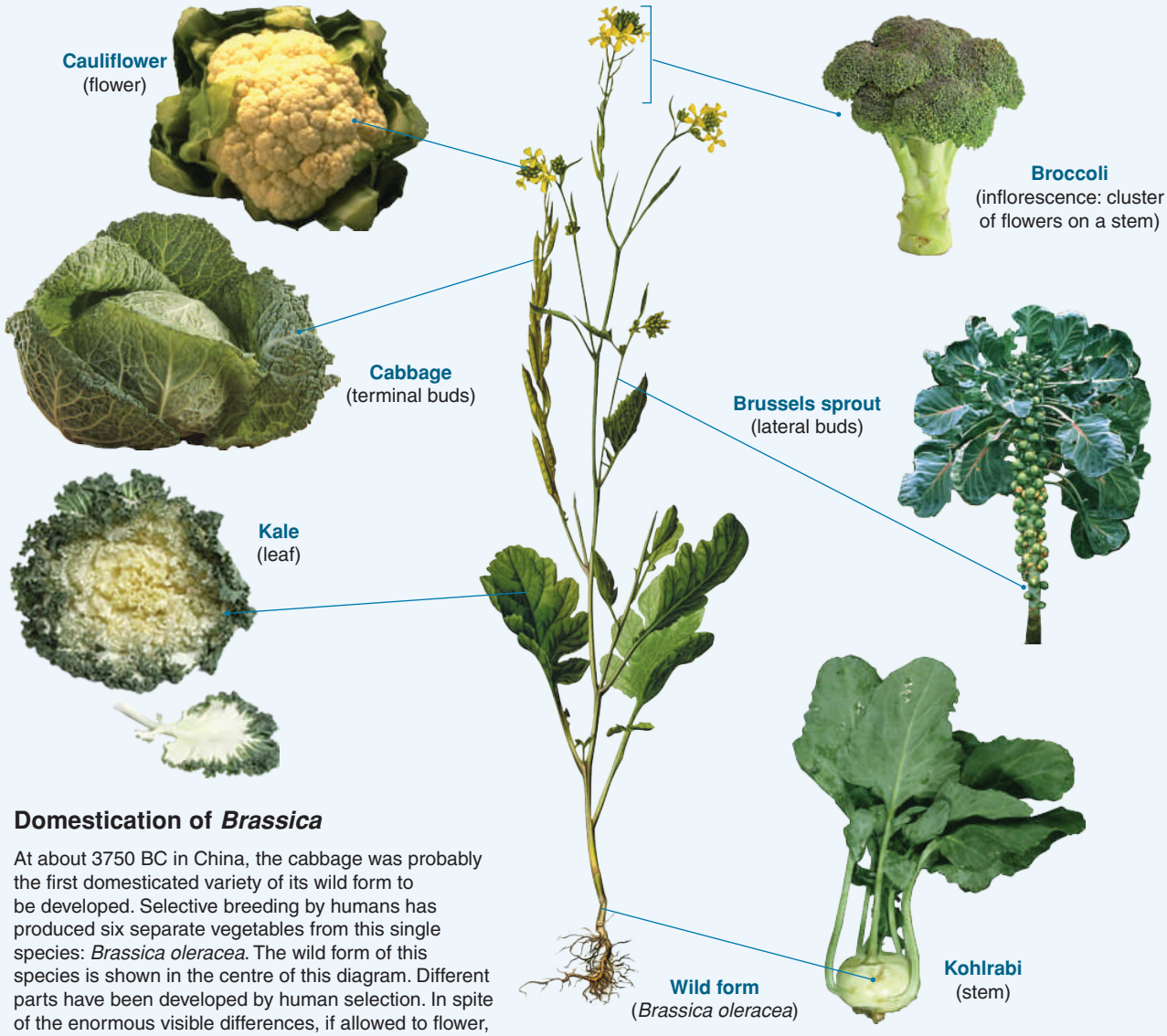
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113 Selective Breeding in Crop Plants

Key Idea: The genetic diversity within crop varieties provides options to develop new crop plants through selective breeding.

For thousands of years, farmers have used the variation in wild and cultivated plants to develop crops. *Brassica oleracea* is a good example of the variety that can be produced by selectively growing plants with desirable traits. Not only are there six varieties of *Brassica oleracea*, but each of those has a number of sub-varieties as well. Although brassicas have been cultivated for several thousand years, cauliflower, broccoli, and brussels sprouts appeared only in the last 500 years.



Domestication of *Brassica*

At about 3750 BC in China, the cabbage was probably the first domesticated variety of its wild form to be developed. Selective breeding by humans has produced six separate vegetables from this single species: *Brassica oleracea*. The wild form of this species is shown in the centre of this diagram. Different parts have been developed by human selection. In spite of the enormous visible differences, if allowed to flower, all six can cross-pollinate. Kale is closer to the wild type than the other related varieties.

1. Study the diagram above and identify which part of the plant has been selected for to produce each of the vegetables:

   (a) Cauliflower: _____ (d) Brussels sprout: _____

   (b) Kale: _____ (e) Cabbage: _____

   (c) Broccoli: _____ (f) Kohlrabi: _____

2. Describe the feature of these vegetables that suggests they are members of the same species: _____

   _____

3. What features of *Brassica oleracea* would humans have selected to produce broccoli? _____

   _____

   _____

   _____

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USDA

The number of apple varieties available now is a fraction of the many hundreds grown a century ago. Apples are native to Kazakhstan and breeders are now looking back to this centre of diversity to develop apples resistant to the bacterial disease that causes fire blight.



In 18th-century Ireland, potatoes were the main source of food for about 30% of the population, and farmers relied almost entirely on one very fertile and productive variety. That variety proved susceptible to the potato blight fungus which resulted in a widespread famine.



Hybrid corn varieties have been bred to minimise damage by insect pests such as corn rootworm (above). Hybrids are important because they recombine the genetic characteristics of parental lines and show increased heterozygosity and hybrid vigour.

4. (a) Describe a phenotypic characteristic that might be desirable in an apple tree: _____

   (b) Outline how selective breeding could be used to establish this trait in the next generation: _____

5. (a) Explain why genetic diversity might decline during selective breeding for particular characteristics: _____

   (b) With reference to an example, discuss why retaining genetic diversity in crop plants is important for food security:

6. Cultivated American cotton plants have a total of 52 chromosomes (2N = 52). In each cell there are 26 large chromosomes and 26 small chromosomes. Old World cotton plants have 26 chromosomes (2N = 26), all large. Wild American cotton plants have 26 chromosomes, all small. How might cultivated American cotton have originated from Old World cotton and wild American cotton:

7. The Cavendish is the variety of banana most commonly sold in world supermarkets. It is seedless, sterile, and under threat of extinction by Panama disease Race 4. Explain why Cavendish banana crops are so endangered by this fungus:

8. Why is it important to maintain the biodiversity of wild plants and ancient farm breeds? _____

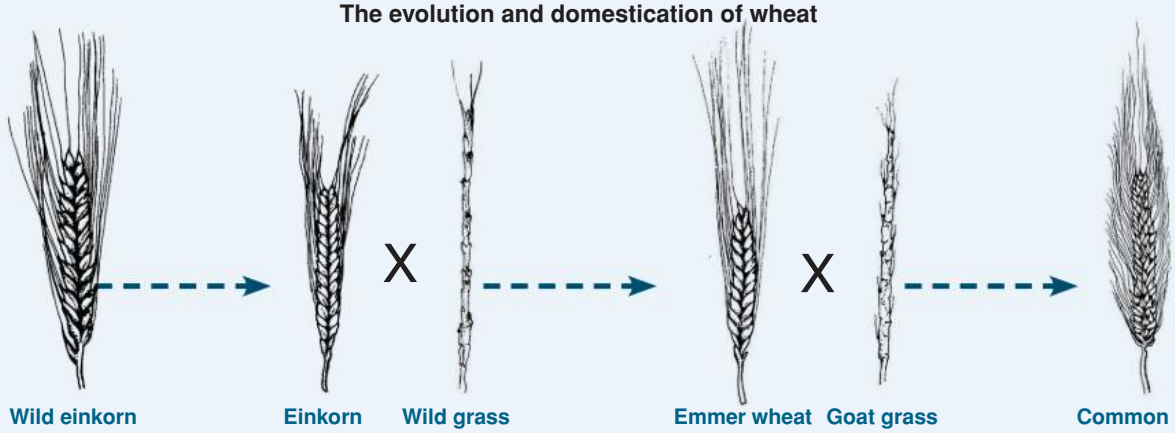
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114 Breeding Modern Wheat

Key Idea: Modern wheat evolved as a result of two natural hybridisation events and the doubling of its chromosomes.

Wheat has been cultivated for more than 9000 years and has undergone many genetic changes during its domestication. The evolution of modern bread wheat from its wild ancestors (below) involved two natural hybridisation events, accompanied by polyploidy (increasing number of complete sets of chromosomes). Once wheat became domesticated, selective breeding emphasised characteristics such as high protein (gluten) content, high yield, and pest and disease resistance. Hybrid vigour (improved characteristics) in wheat cultivars is produced by crossing inbred lines and selecting for desirable traits in the progeny (offspring). Increasingly, research is focused on enhancing the genetic diversity of wheat to provide for future crop development. With this in mind, there is renewed interest in some of the lower yielding, ancient wheat varieties, which possess alleles no longer present in modern inbred varieties.

The evolution and domestication of wheat

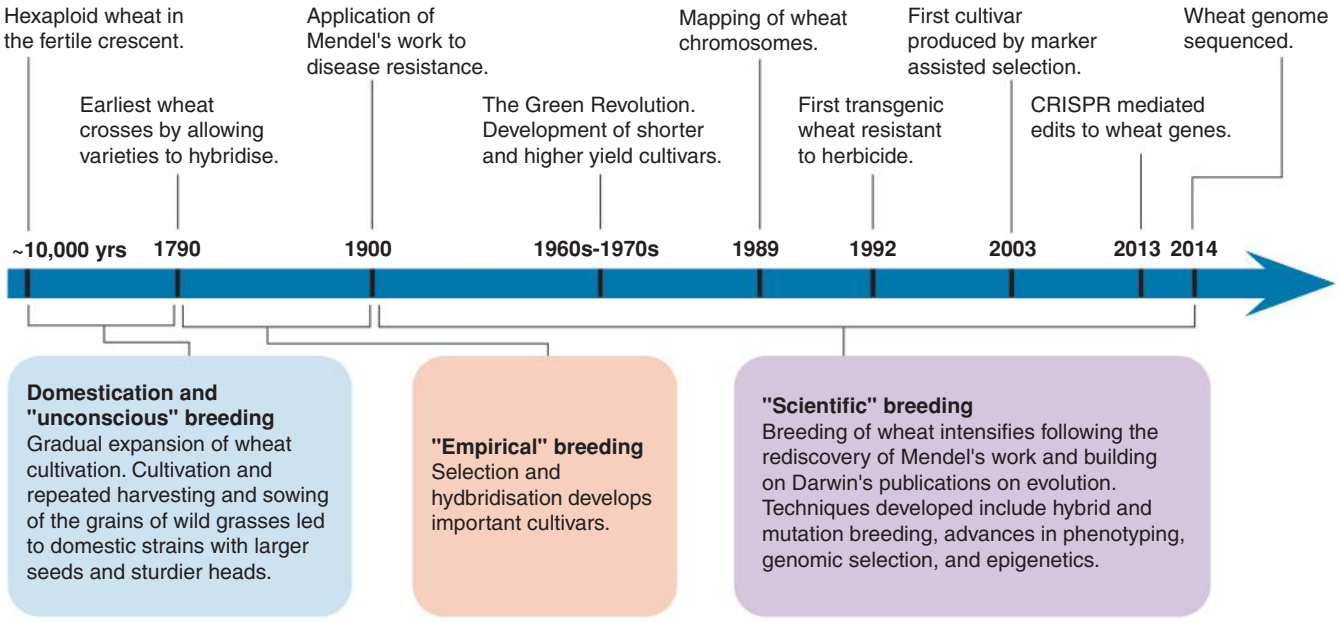


Wild einkorn becomes domesticated in the Middle East. There are slight changes to phenotype but not chromosome number.

A sterile hybrid between einkhorn and wild grass undergoes a chromosome doubling to create fertile emmer wheat.

A sterile hybrid between emmer wheat and goat grass undergoes a chromosome doubling to create fertile common wheat.

Time line of wheat development



1. List three phenotypic characteristics that would be desirable in a wheat plant: _____

2. How have both natural events and selective breeding contributed to the high yielding modern wheat varieties?

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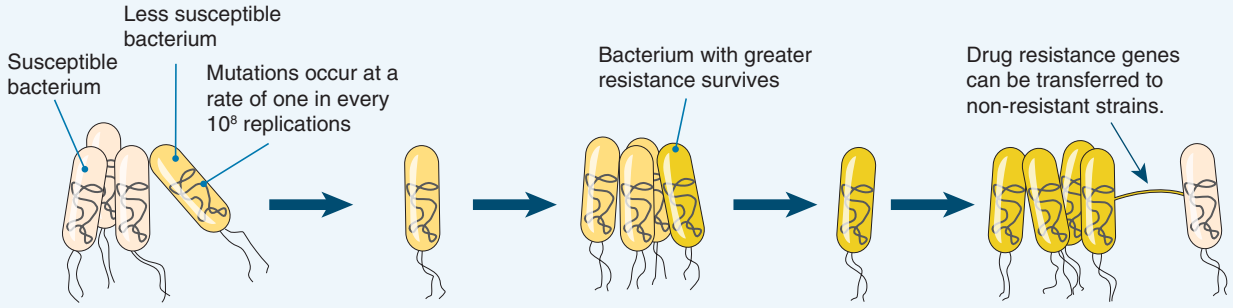
115 The Evolution of Antibiotic Resistance

Key Idea: Widespread use of antibiotics and pesticides has created a selective environment for the proliferation of chemical resistance in microbial populations.

Resistance to antibiotics is becoming a more common and concerning occurrence in the modern world. It arises and spreads when chemical control agents do not remove all the targeted organisms. Those that survive because of their suite of specific inherited characteristics are able to pass on these genes and so resistance becomes more common in subsequent generations (i.e. natural selection). Antibiotic resistance in bacteria, particularly to multiple antibiotics, poses serious threats to human health.

The evolution of antibiotic resistance in bacteria

Antibiotic resistance arises when genetic changes allow bacteria to tolerate levels of antibiotic that would normally inhibit growth. Resistance may arise spontaneously through mutation or by transfer of DNA between microbes (**horizontal gene transfer**). Genomic analyses from 30,000 year old permafrost sediments show that the genes for antibiotic resistance predate modern antibiotic use. In the current selective environment of widespread antibiotic use, these genes have proliferated and antibiotic resistance has spread. For example, methicillin resistant strains of *Staphylococcus aureus* (MRSA) have acquired genes for resistance to all penicillins. Such strains are called superbugs.



Any population, including bacterial populations, includes variants with unusual traits, in this case reduced sensitivity to an antibiotic. These variants arise as a result of mutations in the bacterial chromosome.

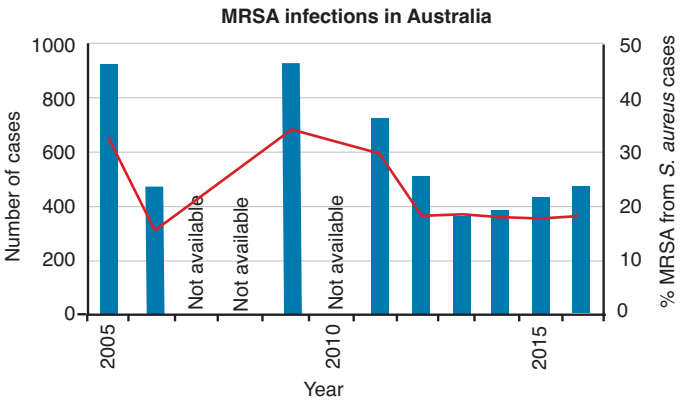
When a person takes an antibiotic, only the most susceptible bacteria will die. The more resistant cells remain alive and continue dividing. Note that the antibiotic does not create the resistance; it provides the environment in which selection for resistance can take place.

If the amount of antibiotic delivered is too low, or the course of antibiotics is not completed, a population of resistant bacteria develops. Within this population too, there will be variation in susceptibility. Some will survive higher antibiotic levels than others.

A highly resistant population has evolved. The resistant cells can exchange genetic material with other bacteria (via horizontal gene transmission), passing on the genes for resistance. The antibiotic initially used against this bacterial strain will now be ineffective.



Staphylococcus aureus is a common bacterium responsible for several minor skin infections in humans. MRSA is a strain that has evolved resistance to penicillin and related antibiotics. MRSA is troublesome in hospital-associated infections because patients with open wounds, invasive devices (e.g. catheters), or poor immunity are at greater risk for infection than the general public.



In Australia MRSA cases have remained relatively steady since 2012. Greater reporting measures and strict hygiene standards have reduced the number of cases since the early 2000s.

1. Describe how resistance develops in a population: _____

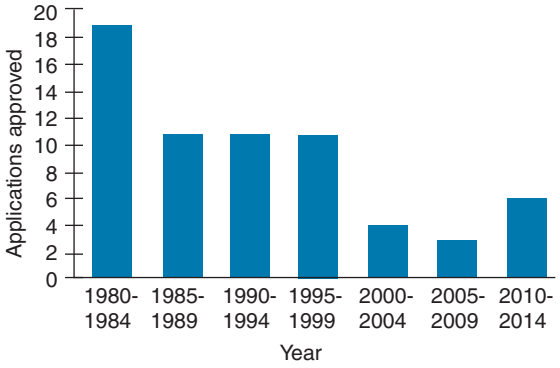
2. How can antibiotic resistance be transferred between strains of bacteria? _____

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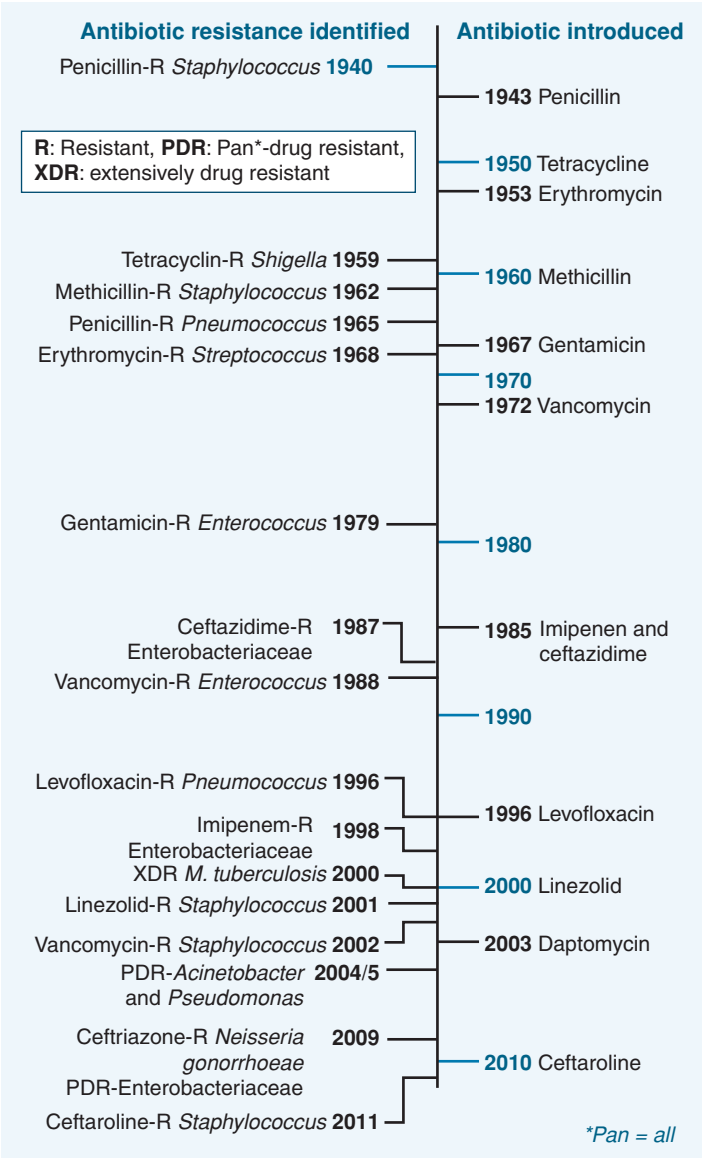
The development of antibiotics and the evolution of antibiotic resistance

- The modern era of antibiotics began in 1928 with Alexander Fleming's discovery of penicillin. By the mid 1940s penicillin was being produced in vast quantities, mainly to treat World War II soldiers.
- However, even then, Alexander Fleming warned about overuse causing antibiotic resistance. Indeed, by the mid 1950s, penicillin resistance in bacteria was already becoming a problem. There are now various levels of resistance in bacteria to all commonly used antibiotics.
- Incorrectly prescribed antibiotics contribute to resistance. Studies have shown incorrect antibiotic therapies in up to 50% of cases.
- Extensive use of antibiotics in agriculture have also contributed to resistance. Farmers use antibiotics to prevent infection in large, high density, livestock and poultry operations, where crowded conditions are potentially "disease-inducing".
- Very few new types of antibiotic have been produced since 2000 compared to previous decades. In fact, many of the world's major pharmaceutical companies no longer research or develop new antibiotics because they cost so much to develop and have a very short useful life span.

New antibiotic drug applications approved since 1980



Historical timeline of antibiotic use



3. With reference to MRSA, describe the implications to humans of widespread antibiotic resistance:

4. Comment on any relationship between the year of an antibiotic's production and the year resistance first appears:

5. Suggest why so few new antibiotics are being produced:

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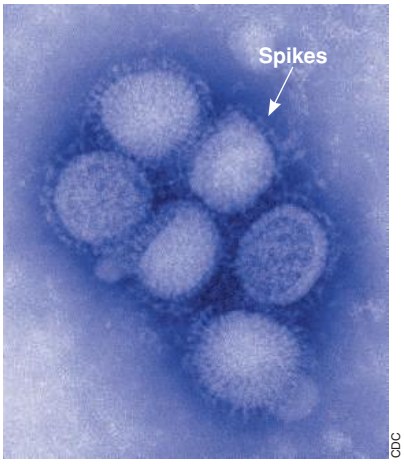
116 Antigenic Variability in Pathogens

Key Idea: Rapid evolution in pathogens, especially viruses, help them evade the human immune system.

The immune system has evolved to "remember" previous infections and so prevent reinfection by the same pathogen. Pathogens, especially viruses, have evolved mechanisms to evade this response by changing their surface features so that a reinfection by the same pathogen is treated as a new infection by the immune system. This gives the pathogen time to replicate before the immune system can respond. Viruses, especially *Influenzavirus*, make these changes by having very high mutation rates and being able to recombine genetic material with other strains of the virus to produce new strains, often never encountered by humans. For example, seasonal flu mutates so rapidly that vaccinations only last a year.

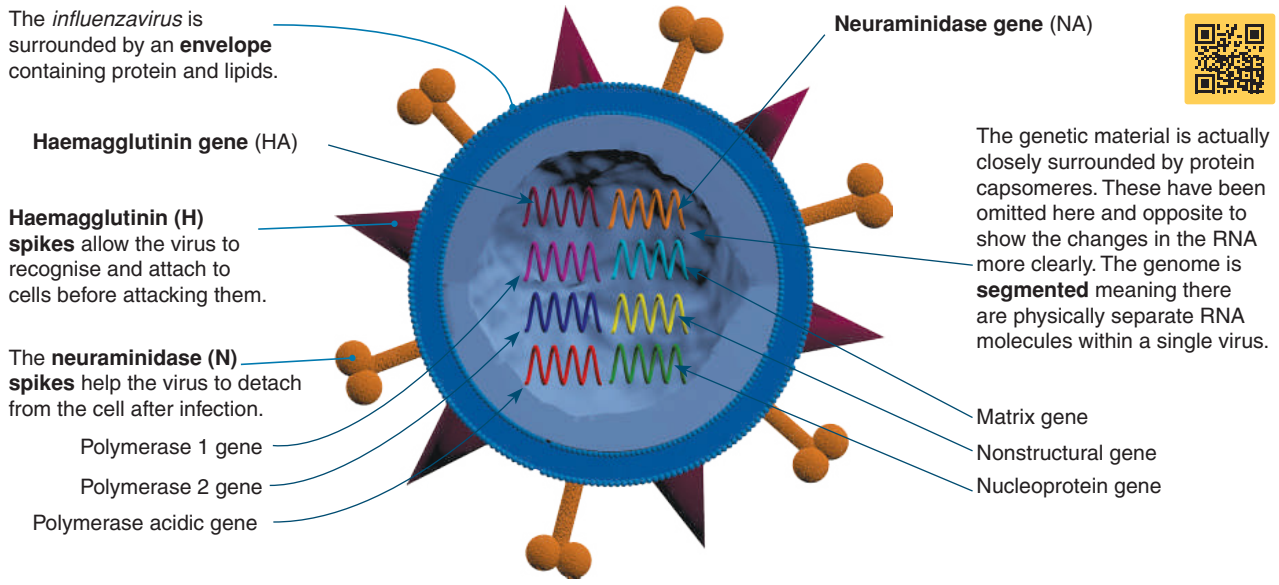
Influenzavirus

- Influenza (flu) is a disease of the upper respiratory tract caused by the viral genus *Influenzavirus*. Three types of *Influenzavirus* (A, B, and C) affect humans.
- The most common and most virulent of these is *Influenzavirus* A, (below). Influenza viruses undergo genetic changes continually, either by **antigenic drift** (small continual changes) or by **antigenic shift** (two strains recombining to create a new subtype).
- These genetic changes result in changes to the proteins presented on the viral surface. The changes to the proteins prevent the human immune system from detecting the virus easily, and allow the virus to reinfect people who may have previously had the flu.



Structure of *Influenzavirus*

- Viral strains are identified by the variation in their haemagglutinin (H) and neuraminidase (N) surface antigens. If two different virus subtypes infect a cell they are able to recombine and readily rearrange (reassort) their RNA segments, which alters the protein composition of their H and N glycoprotein spikes.
- Eight RNA segments (genes) code for the viral proteins (below).



1. The *Influenzavirus* is able to mutate readily and alter the composition of H and N spikes on its surface.

   (a) Why can the virus mutate so rapidly? _____

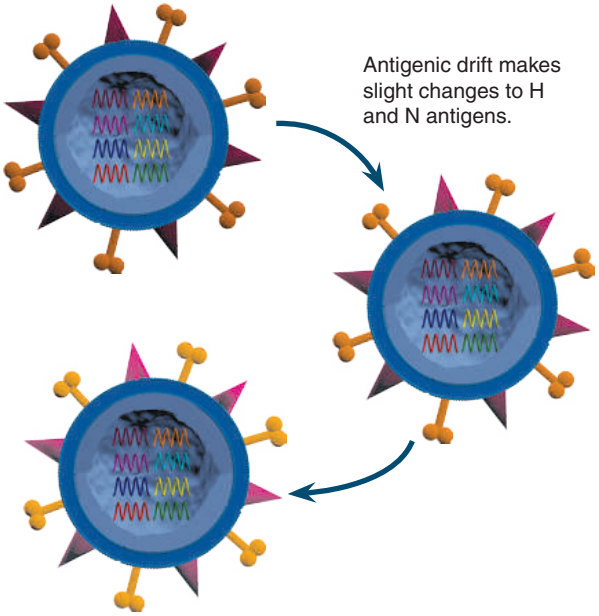
   (b) How does this affect the ability of the immune system to recognise and respond to the virus?

2. What is the difference between antigenic drift and antigenic shift: _____

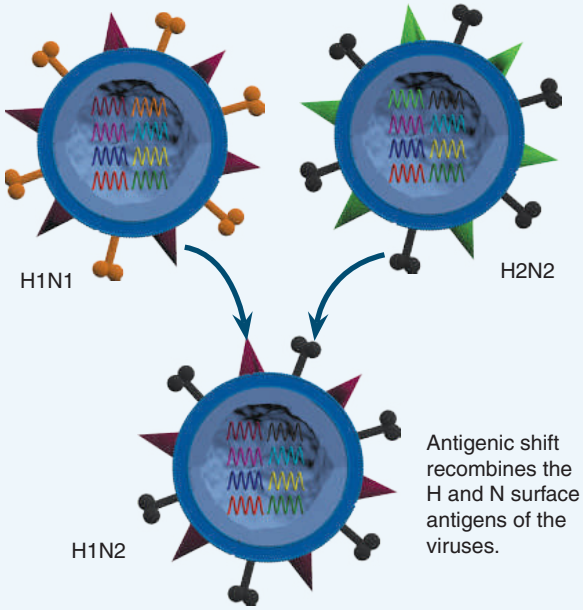
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Change in *Influenzavirus*

Antigenic drifts are small changes (caused by mutations) in the virus which happen continually over time. The changes affect the H and N surface antigens. Accumulated changes result in the immune system not recognising the virus. As a result, the influenza vaccine, which prepares the immune system for infection, must be adjusted each year to include the most recently circulating influenza viruses.

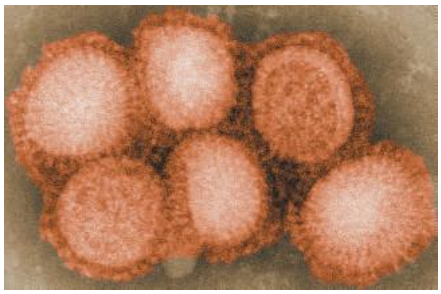


Antigenic shift occurs when two or more different viral strains (or different viruses) recombine to form a new subtype. The changes are large and sudden, and most people lack immunity to the new subtype. New influenza viruses arising from antigenic shift have caused influenza pandemics that have killed millions people over the last century. *Influenzavirus* A is dangerous to human health because it is capable of antigenic shift.

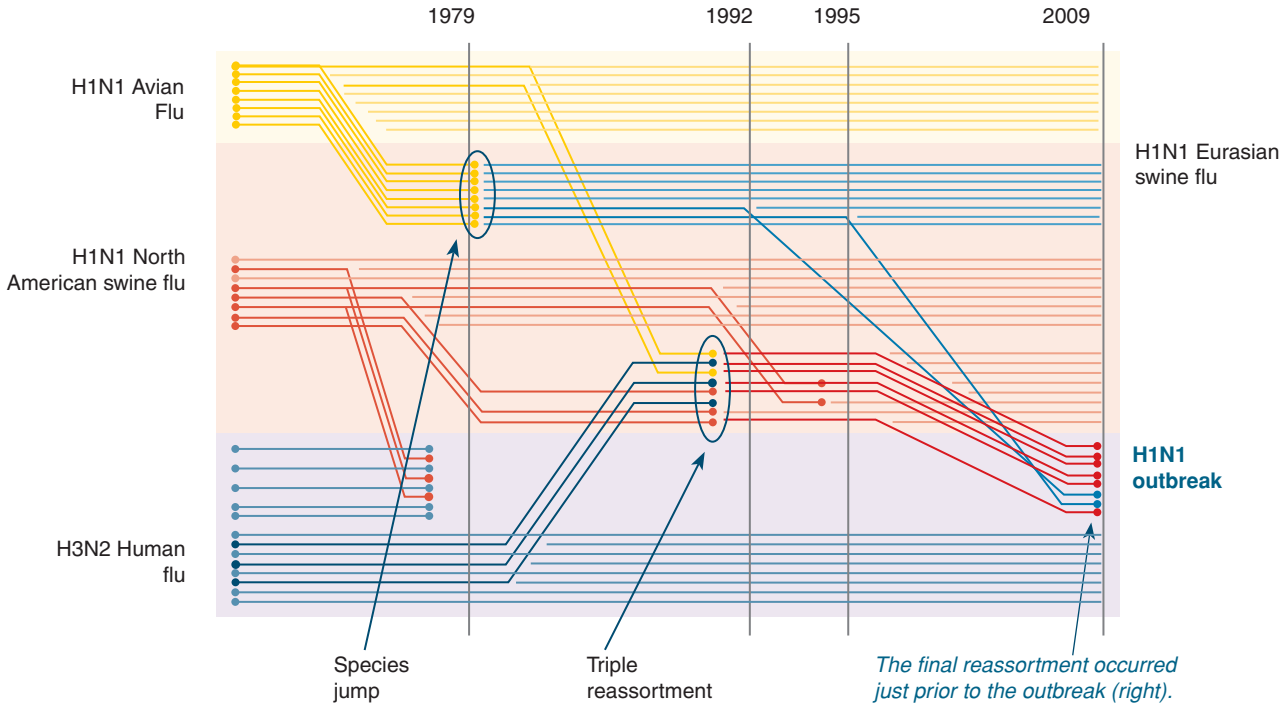


Antigenic shift and outbreaks

- *Influenzavirus* subtypes are classified by the N and H antigens (e.g. H1N1) which are used to illustrate antigenic drift (above). However, all eight RNA segments are able to be reassorted.
- Reassortment is a genetic recombination exclusive to segmented RNA viruses in which infection of a host cell with multiple viruses results in complete gene segments being reshuffled to generate novel viruses.
- For example, the novel H1N1 *Influenzavirus* (right), which caused the 2009 swine flu pandemic, evolved from several reassortments of genes from human, pig, and avian viruses over several decades.



Cybercobra CC 3.0

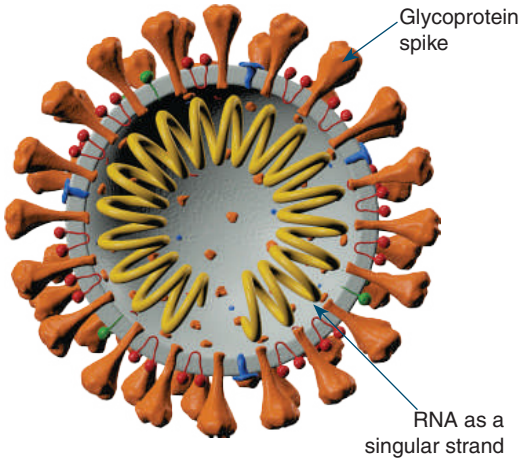


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Coronavirus

- Like *Influenzavirus*, SARS-CoV-2 stores its genetic material as strands of RNA. However, observation has shown that SARS-CoV-2 mutates about four times more slowly than *Influenzavirus*. Currently, it is believed that this is because SARS-CoV-2 has a better "proof-reading" system than *Influenzavirus*, so mutations are less common.
- Although various strains of SARS-CoV-2 have been identified, the virus does not appear to be drifting antigenically. Also its genome (as with all CoVs) is non-segmented, so it is not capable of antigenic shift through reassortment. This means that vaccines may last longer and work against a wider range of strains of SARS-CoV-2 than do vaccines for *Influenzavirus*.
- In the case of SARS-CoV-2, producing a vaccine is not necessarily more difficult than for other viruses. The main challenges were developing the vaccine in the shortest time possible, testing it, ensuring safety, and then distributing it.

SARS-CoV-2: the coronavirus that causes Covid-19



But where did SARS-CoV-2 come from?

The exact pathway of SARS-CoV-2 evolution may not be known definitively for some time. Coronaviruses are common in bats and the best evidence to date suggests that the reservoir is bats. Genetic sequences obtained from viruses in bats show a 96.2% similarity with SARS-CoV-2.

CoV sequences similar to SARS-CoV-2 have also been obtained from pangolins, but not from pangolins in China. At some stage, the proto-SARS-CoV-2 virus must have jumped from its animal host to humans where it likely then evolved into the current SARS-CoV-2 virus.

3. Explain why the influenza vaccine needs to be updated every year: _____

4. Influenza B and C are mainly confined to humans and are not generally found in other animals. Influenza A, however, is found in many animals including humans, birds, horses, and pigs. Explain why this makes influenza A much more likely to develop new strains never before encountered:

5. (a) The triple reassortment event in the evolution of the 2009 swine flu involved flu viruses from which animals?

   (b) The final 2009 H1N1 swine flu contained genes from flu viruses from which animals?

6. Why can't SARS-CoV-2 undergo antigenic shift? _____

7. Which animals are thought to be involved in the evolution of SARS-CoV-2? _____

8. What are the main challenges around a Covid-19 vaccine and how were they overcome?

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117 Chapter Review: Did You Get It?

1. Outline the features of the four factors involved in evolutionary change in a population:

   (i) Species population: _____

   _____

   (ii) Genetic variation: _____

   _____

   (iii) Competition: _____

   _____

   (iv) Increase in favourable variants: _____

   _____

2. Test your vocabulary by matching each term to its definition, as identified by its preceding letter code.

adaptation .....

fitness .....

gene pool .....

genetic drift .....

natural selection .....

variation .....

A A measure of an individual's relative genetic contribution to the next generation as a result of its combination of traits.

B Random changes in allele frequency between generations as a result of the different contributions of individuals to the alleles in the gene pool of the next generation

C A heritable characteristic of a species that equips it for survival and reproductive success in its environment.

D The collective group of genes in a population.

E The differences between individuals in a population as a result of genes and environment.

F The process by which favourable heritable traits become more common in successive generations.

3. Explain the characteristics of each of the following types of natural selection and state when each might operate:

   (a) Directional selection: _____

   _____

   (b) Stabilising selection: _____

   _____

   (c) Disruptive selection: _____

   _____

4. (a) Explain why a person vaccinated for the flu one year can still get the flu the next year: _____

   _____

   _____

   (b) Explain why every few years or decades a flu pandemic occurs: _____

   _____

   _____

   _____

5. Explain why genetic drift is an important evolutionary mechanism in a population after a bottleneck or founder event:

   _____

   _____

   _____

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CHAPTER
11

Changes in Species Over Time

Key terms

adaptation
adaptive radiation
allopatric speciation
biogeographical evidence
chronometric dating (= absolute dating)
common ancestor
divergent evolution
fossil
fossil record
geologic time scale
macroevolution
microevolution
palaeontological evidence
postzygotic isolating mechanism
prezygotic isolating mechanism
radiometric dating
relative dating
reproductive isolation
rock strata
speciation
sympatric speciation
transitional fossil

Fossils and the record of species change over time

Key skills and knowledge

Activity number

☐ 1 Recall your understanding of the term evolution from the previous chapter. Distinguish between microevolutionary changes (changes in allele frequencies within a population) and macroevolutionary changes and give examples of each. This chapter will focus on evolutionary divergence as an example of macroevolution. **118**

☐ 2 Outline the significant events in the history of life on Earth including the rise of multicellular organisms, the establishment of terrestrial life, and the rise of the mammals. What were the possible triggers for these major shifts in biodiversity? **119**

☐ 3 Using examples, describe the palaeontological evidence for the change in species over time. Describe types of fossils and explain how fossils are formed. Explain what is meant by the fossil record and how it records the faunal succession over time. Explain the importance of index fossils in interpreting the fossil record. **120 122**

☐ 4 Distinguish between relative and absolute dating of fossils, including recognising some of the methods used and the date ranges for which they are appropriate. Explain the importance of the low of superposition when applying relative dating techniques. **121 - 123**

☐ 5 Explain the significance of transitional fossils. Using examples, describe the trends that transitional fossils record in the evolution of related taxa. **124**







J. Podos

Evidence of speciation

Key skills and knowledge

☐ 6 Using real or hypothetical examples, describe the role of geographic isolation as a precursor to the divergence of species. **125 127**

☐ 7 Describe and explain mechanisms of reproductive isolation, distinguishing between prezygotic and postzygotic reproductive isolating mechanisms. Describe the role of reproductive isolation in the divergence of species. Suggest why postzygotic reproduction isolation occurs only after prezygotic mechanisms are in place. **126**

☐ 8 Describe speciation as a consequence of different selection pressures acting on isolated populations and leading to reproductive isolation. Recognise that in allopatric speciation, isolation occurs as a result of geographic separation. In sympatric speciation isolation occurs by means other than geographical separation. **128**

☐ 9 Describe the evidence of allopatric speciation leading to divergence in Galápagos finches. Describe how repeated speciation events, divergence, and secondary contact lead to the radiation of finch species from a common ancestor. **129**

☐ 10 Describe the evidence of sympatric speciation leading to divergence in Howea palms on Lord Howe Island. Explain how the species managed to diverge while occupying the same restricted range. Whatmechanisms keep the palm species distinct?

118 What is Evolution?

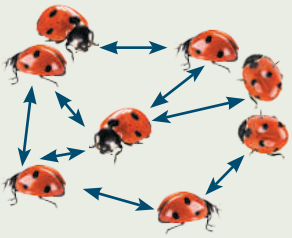
Key Idea: Evolution of taxonomic groups (macroevolution) results from evolution within species (microevolution).

Evolution refers to the heritable genetic changes occurring in a population over time. Importantly, evolution refers to populations, not to individuals and the changes must be passed on to the next generation. Evolutionary processes can be considered at two scales. **Microevolution** refers to changes in the gene or allele frequencies within a species. Microevolution is responsible for changes in a species' genetic make-up in response to environmental changes (e.g. spread of antibiotic resistance). **Macroevolution** refers to evolution above the species level, e.g. the formation of new species or genera. It encompasses increases in taxonomic diversity or morphological differences (evolutionary radiations).

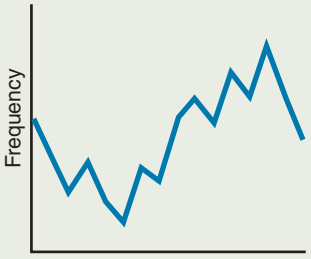


Mutation
Mutations produce new alleles (gene variants) and DNA sequences. Many small mutations can result in large changes over many generations.

Natural selection
Individuals with beneficial variations will have an advantage. They will leave more offspring, increasing the frequency of that variation in later generations.



Gene flow
Genetic information flowing into or out of the population affects the population over time. As populations diverge, gene flow is reduced.



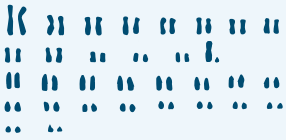
Genetic drift
Random changes in gene frequencies between generations affect the genes available for recombining in later generations.

MICROEVOLUTION

MACROEVOLUTION



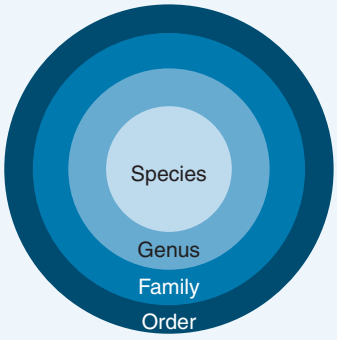
Zebra karyogram

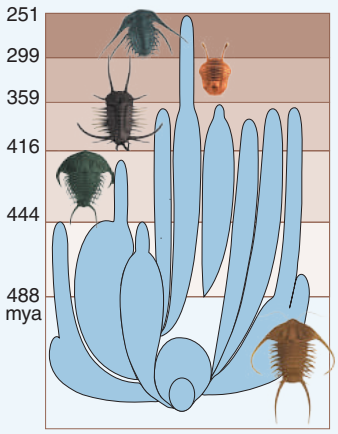


Horse karyogram

Lions and leopards are more closely related than either are to tigers.







Genomic divergence
Large scale patterns in the number of chromosomes, genes, and genomic organisation reflect evolutionary relationships.

Morphological divergence
Evolutionary radiations are often accompanied by differences in the morphology of taxa. The amount of difference indicates the relatedness of organisms.

Taxonomic diversity
Taxonomy is the science of classifying the diversity that has resulted from macroevolution. Organisms form taxa based on shared characteristics.

Trends and patterns
Macroevolution encompasses trends or patterns in evolution above the species level, such as evolutionary radiation (above) and convergence.

1. Define evolution: _____

_____

_____

2. Using examples, distinguish between macroevolution and microevolution: _____

_____

_____

_____

_____

_____

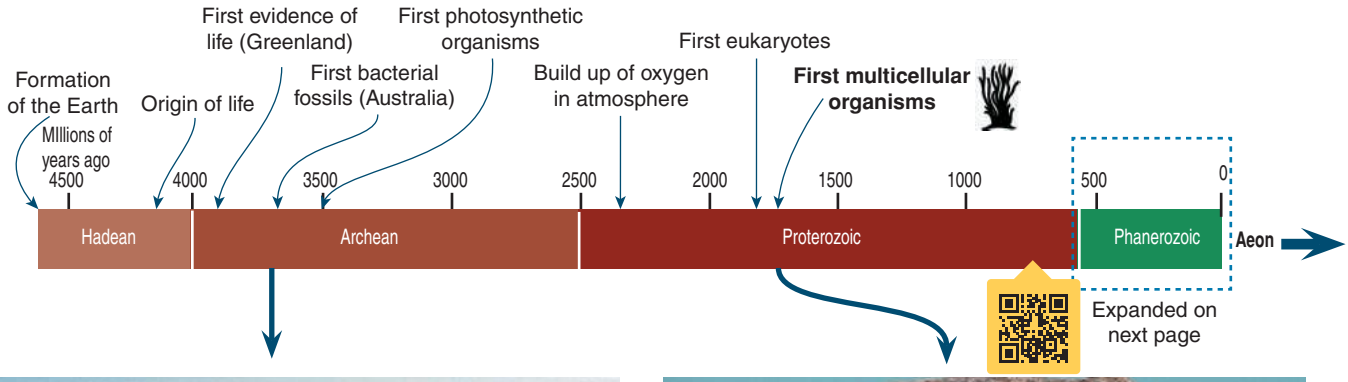
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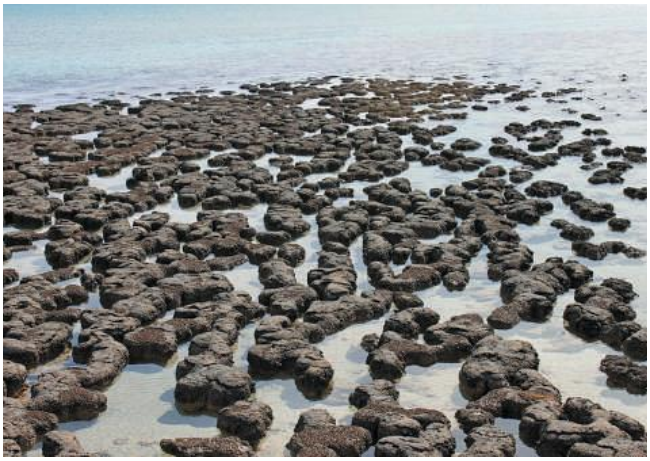
119 Earth's Evolutionary History

Key Idea: Life on Earth originated about 4100 million years ago, but complex life evolved much more recently than this.

Life forms on Earth originally arose from primitive cells living some 4100 million years ago in conditions quite different to those on Earth today. The earliest fossil records of living things show only simple cell types. It is thought that the first cells arose as a result of evolution at the chemical level in a 'primordial soup' (a rich broth of chemicals in a warm pool of water, perhaps near a volcanic vent). Life appears very early in Earth's history, but did not evolve beyond the single cell stage until much later (about 600 mya). This would suggest that the evolution of complex life forms required greater hurdles to be overcome. The build up of free atmospheric oxygen, released as a by-product of oxygenic photosynthesis, was important for the evolution of eukaryotes and paved the way for the evolution of multicellular life.

Time line of Earth's biodiversity





RA



Fossil jellyfish

Stromatolites (such as the ones shown above from Shark Bay, Western Australia), represent some of the most ancient living things on Earth. Few examples exist today, but fossil remains can be dated back to 3.7 billion years ago. Stromatolites are rock like structures formed from the accretion of sediment by microorganisms, especially cyanobacteria (blue-green photosynthetic bacteria). Ancient representatives of cyanobacteria are thought to have been responsible for the production of oxygen in the atmosphere after they evolved oxygenic photosynthesis (light capture and carbon fixation resulting in oxygen production). It resulted in what is called the Great Oxygenation Event (a rise in atmospheric oxygen), which caused the extinction of many anaerobic bacteria but eventually led to the rise of multicellular life forms.

Multicellular organisms arose soon after the evolution of eukaryotes. Multicellularity was a major evolutionary event as it allowed organisms to diversify the tissues and cells of their bodies to perform specialised tasks. The origin of multicellularity is much debated but one hypothesis is that unicellular organisms began to associate together (e.g. cyanobacteria stick together after binary fission and form long chains called filaments). Different cells in the group produced molecules useful to others and so the group benefited by staying together. As the different cell lines became more dependent on others for certain molecules, a greater need to remain together also developed (in low nitrogen conditions, some of the cells in filamentous cyanobacteria transform into nitrogen-fixing cells, and this benefits the other cells in the filament).

1. (a) What was the significance of the buildup of free oxygen in the atmosphere for the evolution of life?

_____

_____

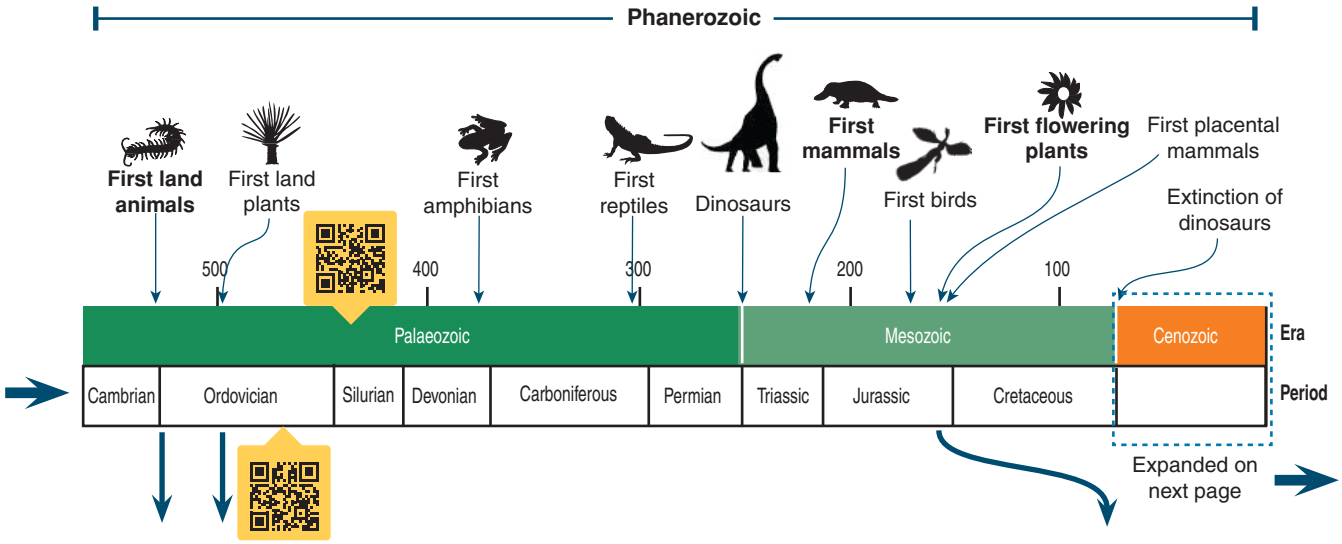
(b) How long did it take for free oxygen to build up in the atmosphere? _____

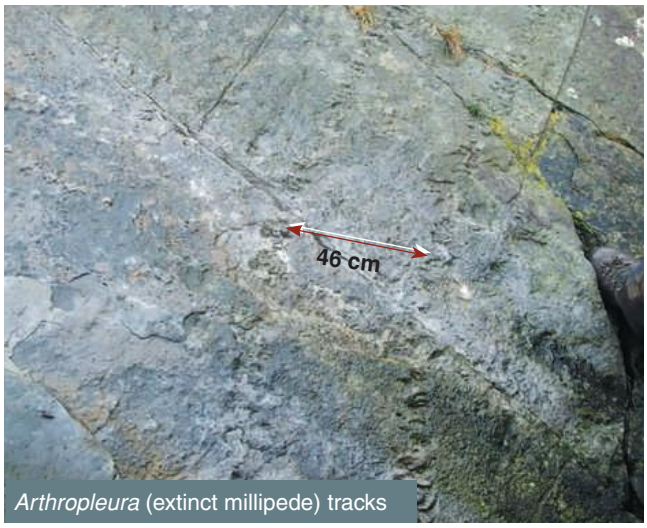
2. Explain how multicellular life evolved: _____

_____

_____

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Arthropleura (extinct millipede) tracks

Ashley Dace



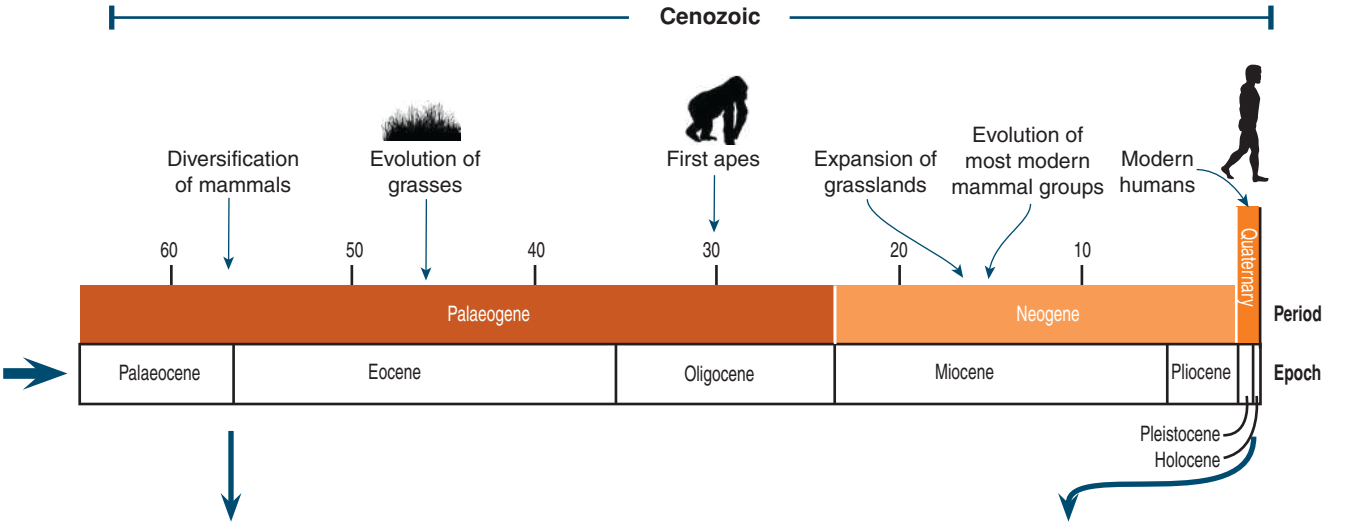
Animals may have ventured onto land before plants, with evidence suggesting they did so about 530 million years ago. The earliest land animals were invertebrates, perhaps similar to horseshoe crabs, which come ashore to lay eggs on the sand. Strategies like this would have been an advantage at a time when there were no land animals to eat the eggs. Similarly, some of the first excursions onto land may have been to take refuge from aquatic predators. **Plants** may have arrived soon after animals, possibly as early as 500 million years ago. The earliest terrestrial plants had no vascular tissue, like mosses and liverworts today. Vascular plants (e.g. ferns) did not appear until about 425 million years ago but (like many animal taxa) underwent a number of **evolutionary radiations** (increases in taxonomic diversity) once they colonised land.

Flowering plants (angiosperms) are the most successful terrestrial plants. With at least 350,000 species, they make up 90% of all living plant species. Flowering plants first appeared about 160 million years ago. They began to diversify rapidly about 120 million years ago. The evolution of flowers helped to make sexual reproduction more efficient. Flowers attracted insects (and later birds) with the use of colours and rewards (such as nectar). The insects and birds then spread pollen from flower to flower. This system has become so successful that many insects and birds now rely on flowers for food and plants rely on their pollinators for reproduction. Genetic evidence suggests the evolution of flowers was linked to at least two rounds of whole genome duplication, which might explain why angiosperms appeared suddenly in the fossil record.

3. What were the earliest land animals and what circumstances may have caused them to come on to the land?

4. Explain why the evolution of flowers was an advantage to plants. What was the result? _____

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Both photos NASA

Mammalian evolution can be traced back to the Carboniferous period with the appearance of the synapsids (e.g. *Dimetrodon*), one of the two major clades of tetrapod vertebrates (the other clade gave rise to the reptiles and birds). However it was not until the Triassic period that the first true mammals appeared. The monotremes (egg laying mammals) appeared about 210 million years ago. Marsupials and placentals probably split about 160 million years ago. Today marsupials are found almost exclusively in Central and South America and Australia (the North American opossum being the exception). While there are 334 species of marsupials, there are nearly 4000 species of placental mammal. The evolutionary radiation of the mammal lineage happened after the extinction of the dinosaurs at the end of the Cretaceous. Mammals diversified rapidly to occupy the vacant niches and give rise to the many taxa we see today.

Human ancestors first appeared about 4 million years ago, with the genus *Homo* appearing about 2 million years ago. Modern humans evolved in Africa about 200,000 years ago. In the short time since then, humans have spread across the globe and now influence every single part of the planet in a way no other living thing ever has since photosynthetic organisms changed the nature of the atmosphere 3 billion years ago. Humans, however, have changed the planet in a much shorter time scale, so much so that it has been suggested that the epoch of recent human existence should be called the Anthropocene. This would have begun the same time that humans began to change the Earth on a large scale, perhaps 12,000 years ago, although some proposals would define it as beginning with the Trinity nuclear test in 1945. In many cases, evidence of human activity can be seen in changes in sediments, especially in lake beds (above).

5. Explain why mammals did not diversify until the Palaeocene epoch, even though they first appeared in the Triassic period.

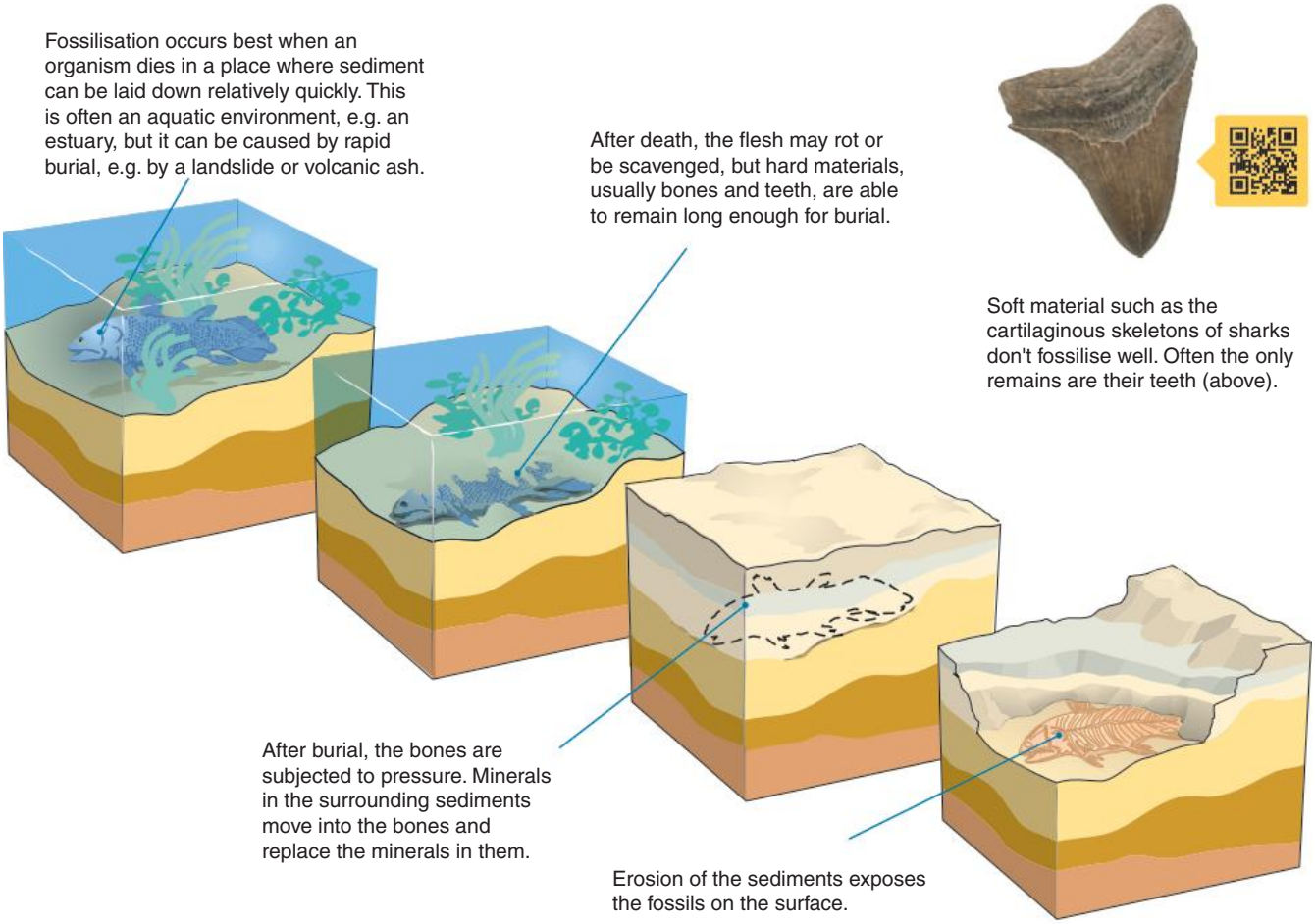
6. The evolution of life on Earth is a history of some lineages diversifying over time and some lineages dying out. Use some examples to explain why diversification takes place:

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120 Fossils

Key Idea: Fossils are the remains of long-dead organisms that have escaped decay and have, after many years, become part of the Earth's crust.

A fossil may be the preserved remains of the organism itself, the impression of it in the sediment (a mould), or marks made by it during its lifetime (trace fossils). For fossilisation to occur, rapid burial of the organism is required (usually in water-borne sediment). This is followed by chemical alteration, whereby minerals are added or removed. Fossilisation requires the normal processes of decay to be permanently arrested. This can occur if the organism's remains are isolated from the air or water and decomposing microbes are prevented from breaking them down. Fossils provide a record of the appearance and extinction of organisms, from species to whole taxonomic groups. Once this record is calibrated against a time scale (by using a broad range of dating techniques), it is possible to build up a picture of the evolutionary changes that have taken place.



Soft material such as the cartilaginous skeletons of sharks don't fossilise well. Often the only remains are their teeth (above).

1. Describe how a fossil forms: _____

2. Explain why the rapid burial of an organism is important in the formation of fossils: _____

3. Explain why the fossil record is biased towards marine organisms with hard parts: _____

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Some fossils act as index fossils

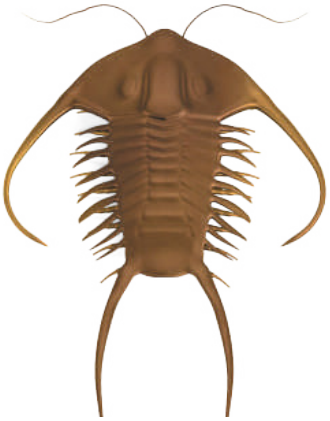
- An **index fossil** is a fossil that is characteristic of a particular span of geologic time or environment. Index fossils help scientists with relative dating (placing rock layers in a relative order to each other), define boundaries in the geologic time scale, and correlate strata from different regions.
- Trilobites make excellent index fossils because they are easily recognisable, abundant in the fossil record, and different families are characteristic of different geographic distributions and different time periods.
- Trilobites (meaning three lobes) are a fossil group of extinct marine arthropods. They first appeared in the fossil record near the beginning of the Cambrian (520 mya) and disappeared in the mass extinction at the end of the Permian (250 mya).
- They were a very diverse group and underwent several radiations during the Cambrian, inhabiting a wide range of marine environments and exhibiting diverse life strategies. The wide diversity in their appearance reflects this.
- The trilobite fossil record provides evidence of several evolutionary trends in the different lineages. These included streamlined shape in swimming forms, broadening of the head in filter feeders, improvement in the ability to curl up into a defensive ball, decreased size, and the evolution of spines as defences (below).



Fossilised trilobite



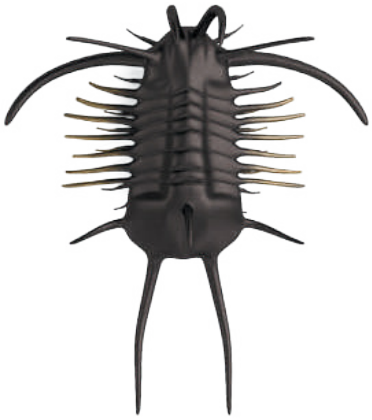
Loss of surface detail could have helped with burrowing. A decrease in size allowed exploitation of new microhabitats.



Spines provided defence from attack and stabilisation on loose surfaces.



An enlargement of the head region was probably an adaptation to filter feeding.



Elongated and streamlined bodies aided swimming in pelagic (open ocean) forms.

4. (a) Explain the importance of index fossils in determining relative time lines: _____

(b) Why do trilobites make good index fossils? _____

5. What fossil evidence do we have that trilobites were a diverse group adapted to many different niches? _____

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121 Methods of Dating Fossils

Key Idea: Accurate dating of fossils is important in being able to piece together evolutionary sequences. Radiometric dating provides absolute dates for constructing a fossil record.

Radiometric dating methods allow an **absolute date** to be assigned to fossils, most commonly by dating the rocks around the fossils. Multiple dating methods for samples provides cross-referencing, which gives confidence in a given date. Absolute, or **chronometric**, dating methods most often involve radiometric dating (e.g. **radiocarbon**, **potassium-argon**, **fission track**), which relies on the known rates of radioisotopic decay of elements to their stable isotopes. Non-radiometric methods can also be used to provide relative dates including the chemical analysis of bones, biostratigraphy, and even using tree rings.

Radiometric dating methods

Uranium-lead

Measures the decay of the two main isotopes of uranium (^{235}U and ^{238}U) into lead in igneous rocks. The ratio of these elements can be used to determine the time since the rocks formed.

Uranium series

Measures the decay of uranium-234 into thorium-230 in marine carbonates (e.g. corals). Uranium-234 is soluble and is incorporated into coral skeletons. As it decays, it produces thorium-230. The age of the coral is determined by the uranium to thorium ratio.

Fission track

When uranium decays, the subatomic particles emitted leave tracks through the mineral. The number of tracks increases over time and this can be used to calculate the time since the rock formed. Fission track dating is useful for dating pottery, glass, and volcanic minerals.

Potassium/argon (K-Ar)

Measures the decay of potassium-40 to argon-40 in volcanic rocks above or below fossil bearing strata. Argon is inert, so it only builds up in minerals by radioisotopic decay of potassium. The amount of argon is therefore related to the age of the rock.

Radiocarbon dating (carbon-14 or ^{14}C)

Measures the amount of the radioisotope carbon-14 (taken up by an organism when it was alive) within its fossilized remains. The older a sample is, the less ^{14}C there is to be detected.

Relative dating methods

Chemical analysis

Bones buried in the ground can absorb elements such as uranium and fluorine. The rate of absorption depends on factors in the immediate environment. Thus bones from the same site can be compared but bones from different sites cannot.

Stratigraphy

Layers of rock laid down oldest at the bottom to youngest at the top. Thus in areas where the layers are not severely deformed the order of deposition can be determined. Displaced deposits can be ordered using knowledge of the order of nearby deposits.

Biostratigraphy

The remains of organisms are incorporated as fossils as deposits are laid down. Older fossils are therefore found below younger ones. Some fossils are characteristic of various layers and times and can be used to date newly found rock layers.

Paleomagnetism

Magnetic minerals in rocks lock-in a record of the Earth's magnetic field when they form. The record of geomagnetic reversals preserved in rock sequences provides a time-scale that is used as a geochronological tool.

Obsidian hydration

Obsidian is a natural volcanic glass used by prehistoric humans to produce blades. The manufacture of these blades zeros the mineral hydration clock. Over time, the mineral becomes hydrated. If the hydration rate is known, an age can be obtained.

1. When the date of a sample has been determined, it is common practice to express it in the following manner: Example: **1.88 ± 0.02** million years old. Explain what the **± 0.02** means in this case:

2. Explain why it is best to test many small samples to find a date when dating a specimen using radiometric dating:

3. Suggest a possible source of error that could account for an incorrect dating measurement using radiocarbon dating:

4. Explain why relative dating methods can not give exact dates of when specimens formed or died: _____

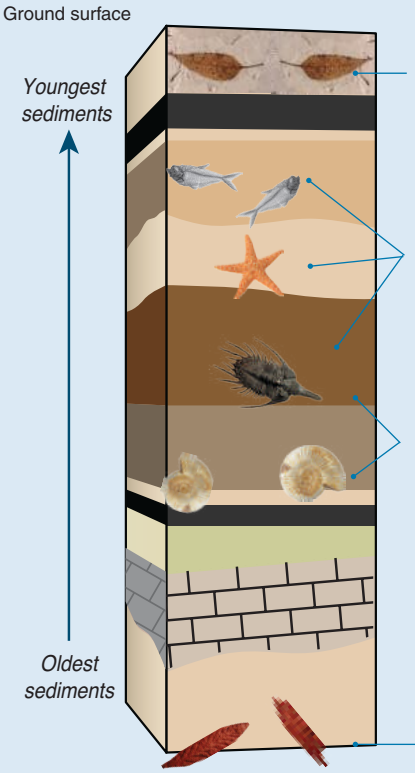
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122 Relative Dating and the Fossil Record

Key Idea: Fossils found in rock at the bottom of a rock profile are older than those found at the top of the rock file.

Relative dating establishes the sequential (relative) order of past events in a rock profile, but it cannot provide an absolute date for an event. Each rock layer (**stratum**) is unique in terms of the type of rock (sedimentary or volcanic) and the type of fossils it contains. Rock layers (**strata**) are arranged in the order that they were deposited, with the oldest layers at the bottom (unless disturbed by geological events). This is called the **law of superposition**. Strata from widespread locations with the same fossils or characteristics can thus be correlated, even when their absolute date is unknown.

Profile with sedimentary rocks containing fossils



Recent fossils are found in more recent sediments
The more recent the layer of rock, the more resemblance there is between the fossils found in it and living forms.

Many extinct species
The number of extinct species is far greater than the number of species living today.

Fossil types differ in each stratum
Fossils in any given layer of sedimentary rock are generally quite different to fossils in other layers.

More primitive fossils are found in older sediments
Phyla are represented by more generalised forms in the older layers, and not by specialised forms (such as those alive today).

What is relative dating?

Relative dating is a way to determine the relative order of past events without necessarily determining absolute (chronometric) age. The same rocks and fossils can then be used to correlate stratigraphic records in different places. Material that can't be dated using absolute methods can therefore be correlated with the same material elsewhere for which an absolute date may be available.

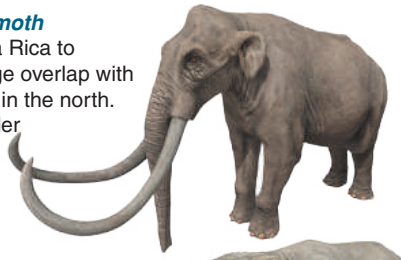
New fossil types mark changes in environment

In the rocks marking the end of one geologic period, it is common to find many new fossils that become dominant in the next. Each geologic period had an environment very different from those before and after. Their boundaries coincided with drastic environmental changes and the appearance of new niches. New selection pressures resulted in new adaptive features as species responded to the changes. An absolute age can be assigned to fossils, usually by dating the rocks around them. Most often, this involves radiometric dating (e.g. radiocarbon, K-Ar).

The fossil record of proboscidea

African and Asian elephants have descended from a diverse group known as **proboscideans** (named for their long trunks). The first pig-sized, trunkless members of this group lived in Africa 40 million years ago. From Africa, their descendants invaded all continents except Antarctica and Australia. As the group evolved, they became larger, an effective evolutionary response to deter predators. Examples of extinct members of this group are illustrated below:

Columbian mammoth
Pleistocene, Costa Rica to northern US. Range overlap with woolly mammoths in the north.
~4 m at the shoulder



Deinotherium
Miocene-Pleistocene, Asia, Africa
~4 m at the shoulder



Gomphotherium
Miocene, Europe, Africa
~ 3 m at the shoulder



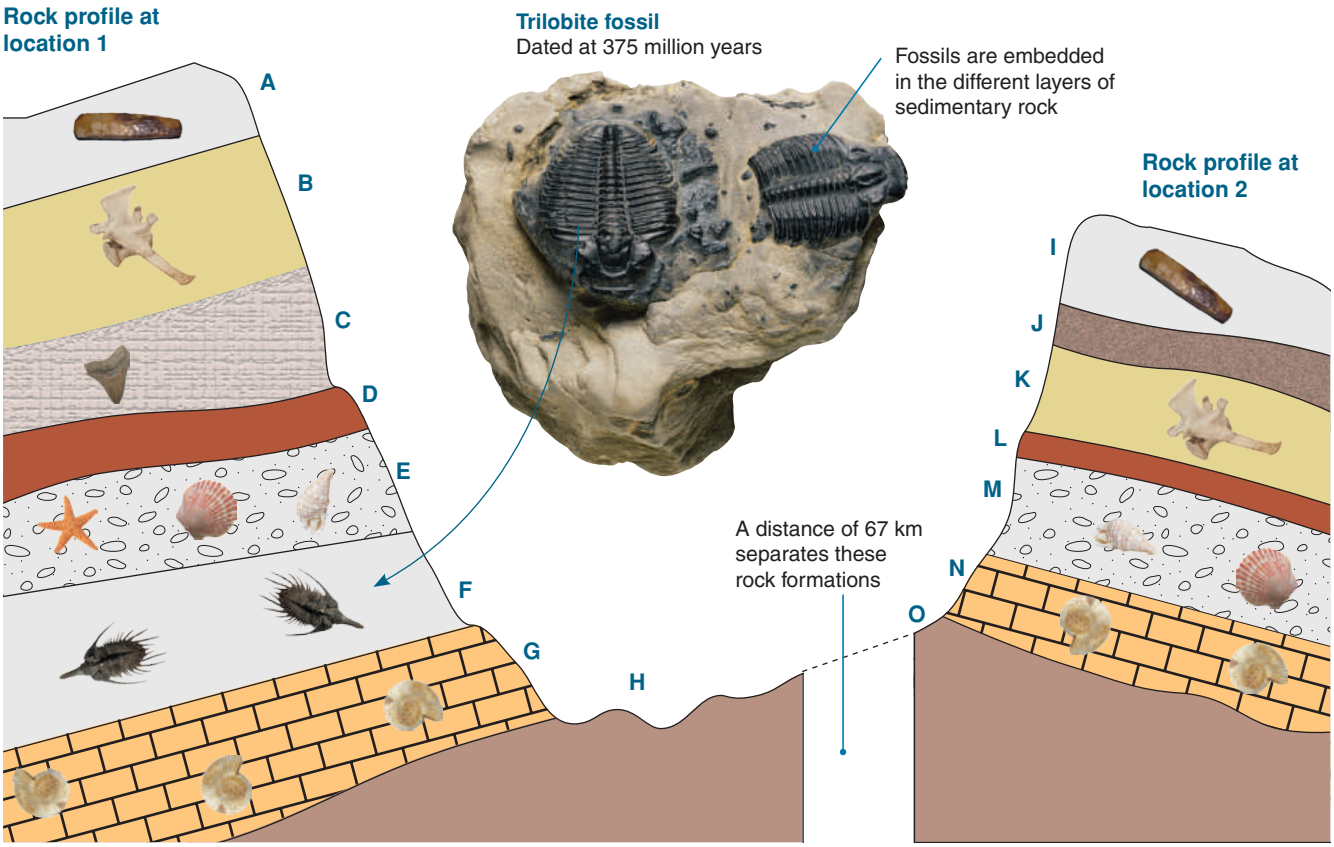
Platybelodon
One of several genera of shovel-tuskers. Middle Miocene, Northern Asia, Europe, Africa
~3 m at the shoulder



- **Modern day species can be traced:** The evolution of many present-day species can be very well reconstructed. For instance, the evolutionary history of the modern elephants is exceedingly well documented for the last 40 million years. The modern horse also has a well understood fossil record spanning the last 50 million years.
- **Fossil species are similar to but differ from today's species**: Most fossil animals and plants belong to the same major taxonomic groups as organisms living today. However, they do differ from the living species in many features.

1. Explain the importance of fossils in relative dating: _____

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The questions below relate to the diagram above, showing a hypothetical rock profile from two locations separated by a distance of 67 km. There are some differences between the rock layers at the two locations. Apart from layers D and L which are volcanic ash deposits, all other layers comprise sedimentary rock.

2. Assuming there has been no geologic activity (e.g. tilting or folding), state in which rock layer (A-O) you would find:

   (a) The youngest rocks at location 1: _____ (c) The youngest rocks at location 2: _____

   (b) The oldest rocks at location 1: _____ (d) The oldest rocks at location 2: _____

3. (a) State which layer at location 1 is of the same age as layer M at location 2: _____

   (b) Explain the reason for your answer above: _____

   _____

4. The rocks in layer H and O are sedimentary rocks. Explain why there are no visible fossils in these layers:

   _____

   _____

5. (a) State which layers present at location 1 are missing at location 2: _____

   (b) State which layers present at location 2 are missing at location 1: _____

6. Using radiometric dating, the trilobite fossil was determined to be approximately 375 million years old. The volcanic rock layer (D) was dated at 270 million years old, while rock layer B was dated at 80 million years old. Give the approximate age range (i.e. greater than, less than, or between given dates) of the rock layers listed below:

   (a) Layer A: _____ (d) Layer G: _____

   (b) Layer C: _____ (e) Layer L: _____

   (c) Layer E: _____ (f) Layer O: _____

7. Suggest why gaps in the fossil record can make it difficult to determine an evolutionary history? _____

   _____

   _____

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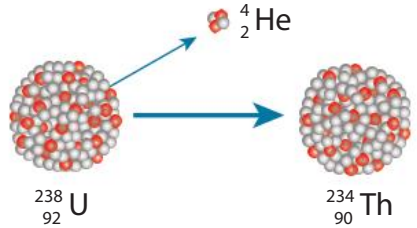
123 Absolute Dating

Key Idea: Chronometric dating uses radioisotopes to produce dates for when a specimen was formed. These dates can be used to calibrate relative dating methods.

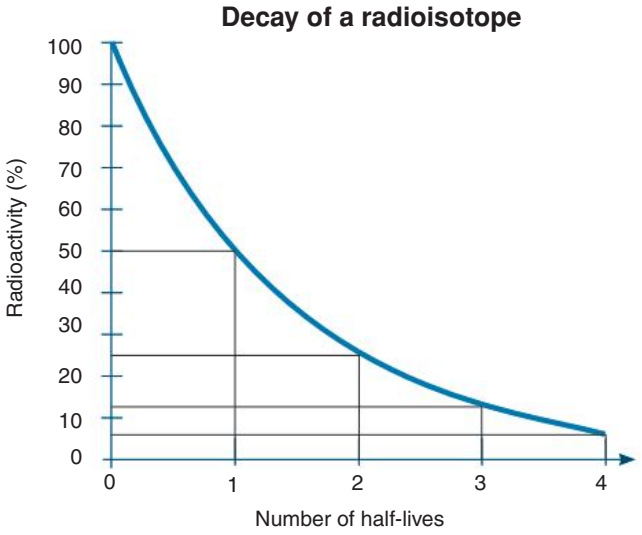
Many chronometric dating methods rely on the decay of radioactive isotopes (radioisotopes) to calculate the age of a specimen. A radioisotope may go through multiple decay events (a decay series) before reaching a stable non-radioactive isotope. The ratio of the original radioisotope to the stable isotope can be used to calculate the age of the specimen. In some cases the ratios of the different elements within the decay series can be used. Radioactivity can be used to date both once living (e.g. bone) or nonliving (e.g. volcanic rocks) materials. Isotopes are taken up by living organisms at relatively constant rates so that when they die the amount of radioisotope left in the body can be compared to that of living organisms to find a date of death.

Radioactive decay

The rate of decay of a radioisotope is measured by its half-life. **A half-life** is the time it takes for half the atoms in a sample of radioactive substance to decay into a new element, or the time in which there is a 50% chance any particular atom will have decayed. For example, uranium-238 has a half life of 4.5 billion years. Starting with a sample of 10 g of uranium-238, after 4.5 billion years there would be 5 g of uranium-238 and 5 g of various other elements (mostly lead) in the sample.



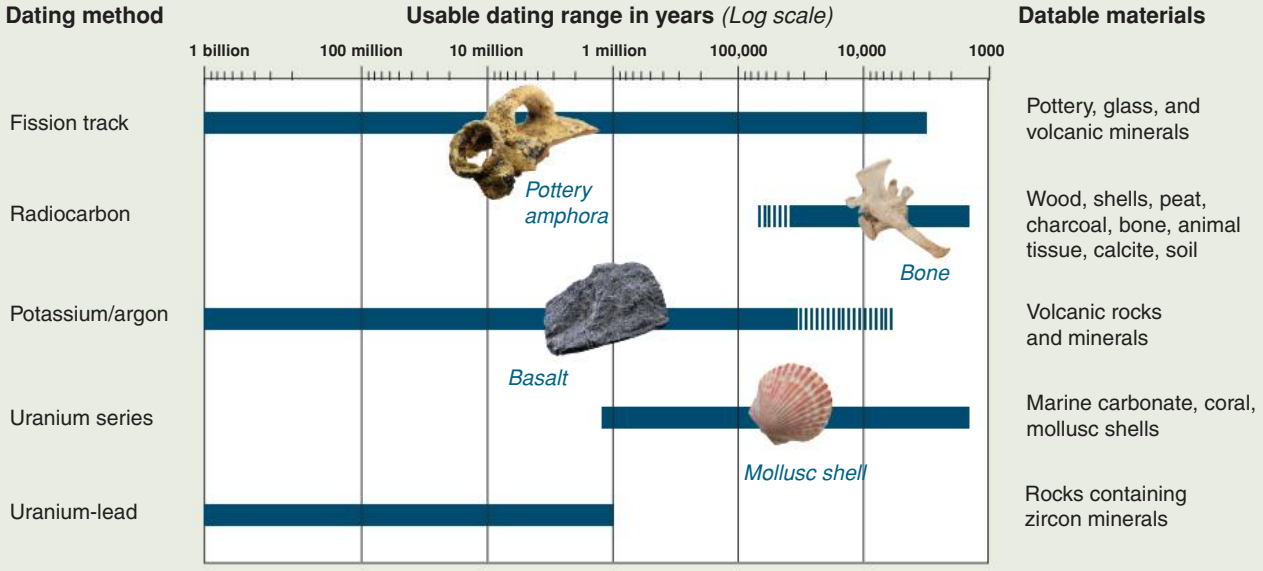
Uranium-238 has a half live of 4.5 billion years and decays to thorium-234. Thorium-234 has a half life of just 24.5 days.



Isotope	Half-life
Uranium-238	4.5 billion years
Uranium-235	700 million years
Thorium-234	24.5 days
Thorium-230	76,000 years
Radium-226	1600 years
Lead-210	22 years
Potassium-40	1.25 billion years
Carbon-14	5730 years

Dating using radioactivity

The length of the half-life and the type of sample being dated are important when considering which dating method to use. Beyond a certain number of half-lives, the amount of radioisotope left may become so small that accurate dating becomes impossible.



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Non-isotopic methods

Some methods for chronometric dating do not rely on measuring half-lives. They instead use other properties of atoms in the sample, including electron spins and emission of light.

Electron spin resonance (ESR)

When minerals are exposed to radiation (e.g. cosmic rays or radioactive decay) their electrons may be knocked from one energy level to a higher energy level. As they return to the lower energy level they may become trapped between energy levels. Under ESR, these electrons can be detected and the number of electrons relates to the length of time since the sample formed. This method is useful for dating objects such as burnt flints, cave sediments, bone, teeth, and loess (wind-blown deposits).

Thermoluminescence

This method is useful for dating objects including ceramics, quartz, feldspar, and carbonates. It works by measuring the accumulated radiation dose (e.g. radioactivity or sunlight) of a crystalline sample. When the sample is heated, it emits light in proportion to the radiation dose. The larger the dose the older the object. Measurements of surrounding radiation can be used to identify the annual dose. The method is relatively cheap but requires the destruction of a significant amount of the sample.

1. Examine the diagram below left (opposite) and determine the approximate dating range and datable materials for each of the methods listed below (Note the logarithmic time scale on the diagram):

	Dating range	Datable materials
(a) Potassium-argon method:	_____	_____
(b) Radiocarbon method:	_____	_____
(c) Uranium series:	_____	_____

2. Radiocarbon dating compares the amount of carbon-14 in dead material to the amount in living material (adjusted to natural variations).

   (a) A piece of tree branch found in sediments of an ancient swamp is radiocarbon dated and found to have about 3% of the expected amount of ^{14}C in a living tree. Calculate the age of the tree:

   _____

   _____

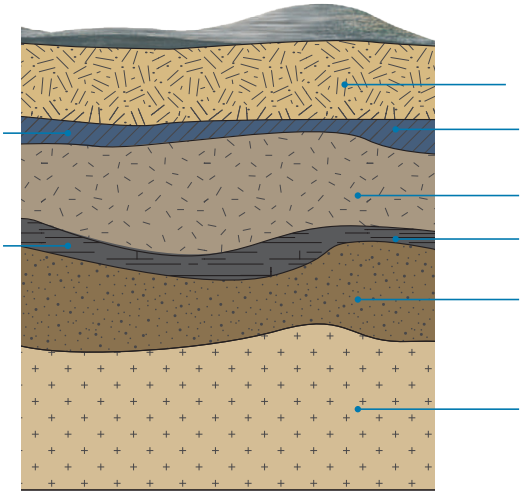
   (b) Another tree branch from the same sediments is radiocarbon dated to 150,000 years. Is this date likely to be accurate? Explain your answer:

   _____

   _____

   _____

3. Use the following data to place the fossils A to E in the correct layers along with the age of the layers:
   Fossil C is never found with fossils A, B, D, and E. Fossil B is found in the same layers as fossil D. Fossil D is found below a layer of volcanic ash dated at 20 million years old. Fossil A is found above a layer of volcanic ash dated at 10 million years old. Fossil E is never found with any other fossils, but it resembles a fossil found elsewhere below a layer of rock dated at 27 million years old. Fossil B shows more derived skeletal features than fossil E.



Layer 1: Fossils: _____ Age: _____

Layer 2: Age: _____

Layer 3: Fossils: _____ Age: _____

Layer 4: Age: _____

Layer 5: Fossils: _____ Age: _____

Layer 6: Fossils: _____ Age: _____

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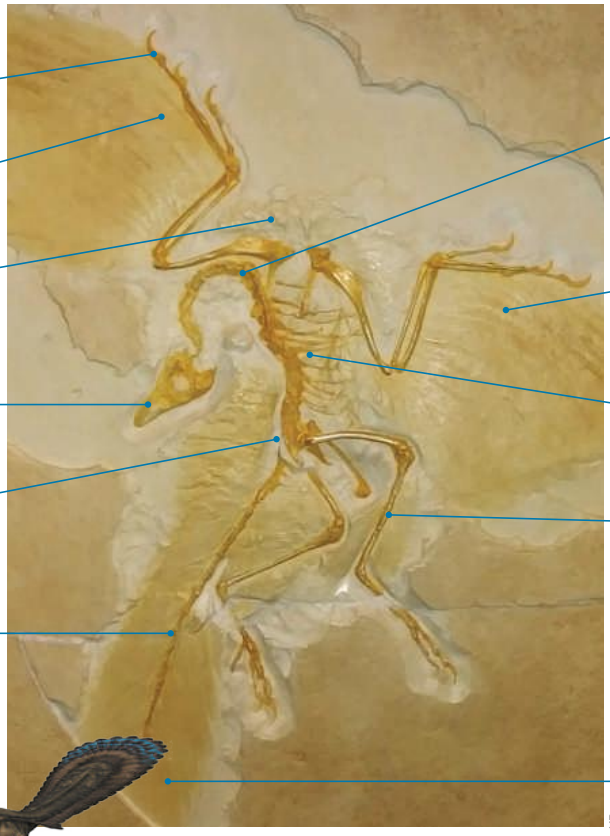
124 Transitional Fossils

Key Idea: Transitional fossils show intermediate states between two different, but related, groups. They provide important links in the fossil record

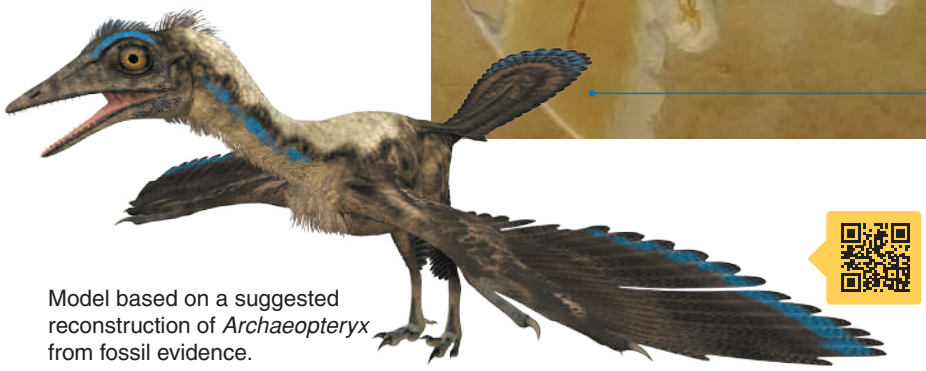
Transitional fossils are fossils with a mixture of features found in two different, but related, groups. Transitional fossils provide important links in the fossil record and provide evidence to support how one group may have given rise to the other by evolutionary processes. Important examples of transitional fossils include horses, whales, and *Archaeopteryx* and other non-avian feathered dinosaurs (below). *Archaeopteryx* was a transitional form between non-avian dinosaurs and birds. *Archaeopteryx* was crow-sized (50 cm length) and lived about 150 million years ago. It is regarded as the first primitive bird and had a number of birdlike (avian) features, including feathers. However, it also had many non-avian features, which it shared with theropod dinosaurs of the time.

Archaeopteryx

Non-avian features	Avian features
Forelimb has three functional fingers with grasping claws	Vertebrae are almost flat-faced
Lacks the reductions and fusions present in other birds	Impressions of feathers attached to the forelimb
Breastbone is small and lacks a keel	Belly ribs
True teeth set in sockets in the jaws	Incomplete fusion of the lower leg bones
The hind-limb girdle is typical of dinosaurs, although modified	Impressions of feathers attached to the tail
Long, bony tail, shared with other dinosaurs of the time	



KP



Model based on a suggested reconstruction of *Archaeopteryx* from fossil evidence.

1. (a) What is a transitional fossil? _____

_____

_____

(b) Why are transitional fossils important in understanding evolution? _____

_____

_____

2. (a) Identify one feature of *Archaeopteryx* that clearly shows it is related to reptiles: _____

_____

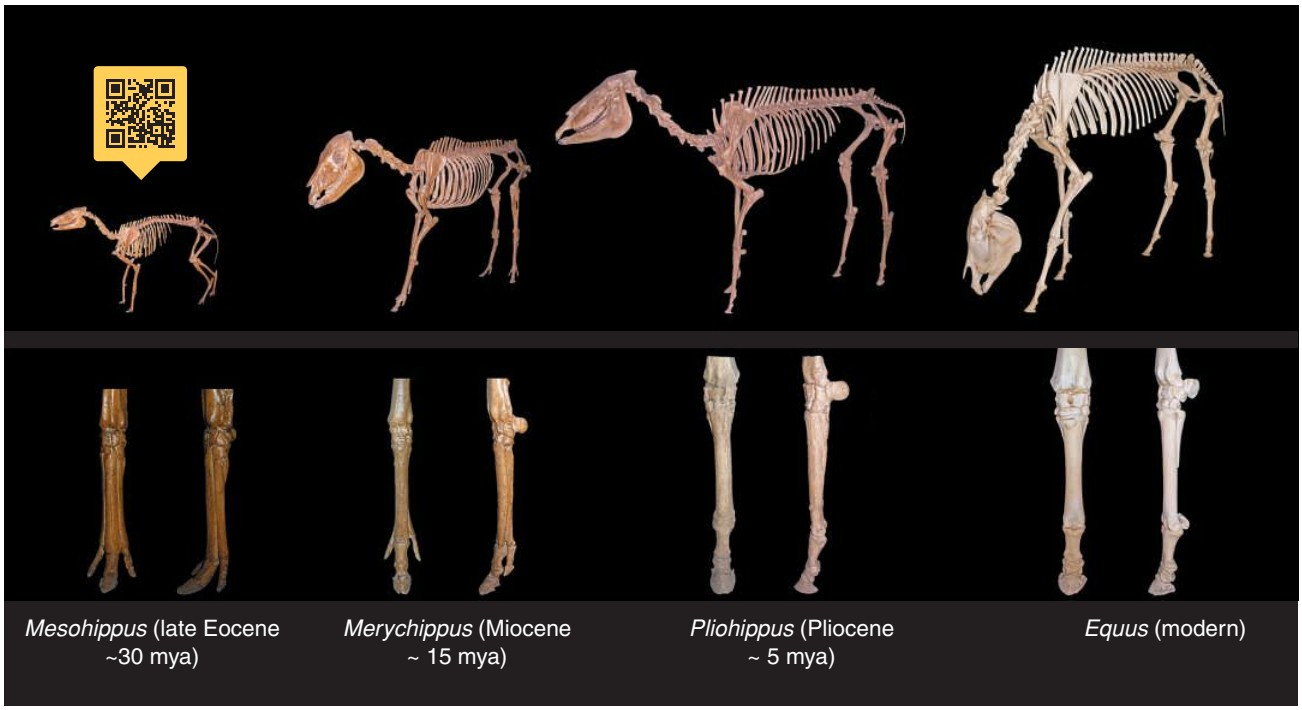
(b) Identify one feature of *Archaeopteryx* that clearly shows it is related to modern birds: _____

_____

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Horse transitional fossils

- The evolution of the horse from the ancestral *Hyracotherium* to modern *Equus* is well documented in the fossil record. The rich fossil record, which includes numerous **transitional fossils**, has enabled scientists to develop a robust model of horse phylogeny. It is a complex tree-like lineage with many divergences, and a diverse array of often coexisting species. The environmental transition from forest to grasslands drove many of the changes observed in the fossil record. These include reduction in toe number, increased size of cheek teeth, and increasing body size.
- Over time, equids became taller and faster, enabling them to detect and escape predators more easily. Their overall size increased and their limbs became more elongated. The reduction in the number of toes from four to one also enabled them to run faster and more efficiently (with less energy expenditure).



H. Zell CC3.0

3. (a) What special condition(s) or pressure(s) do you think acted on the horse's ancestors to produce the equids of today?

(b) Describe the general anatomical changes in horses over the last 30 million years:

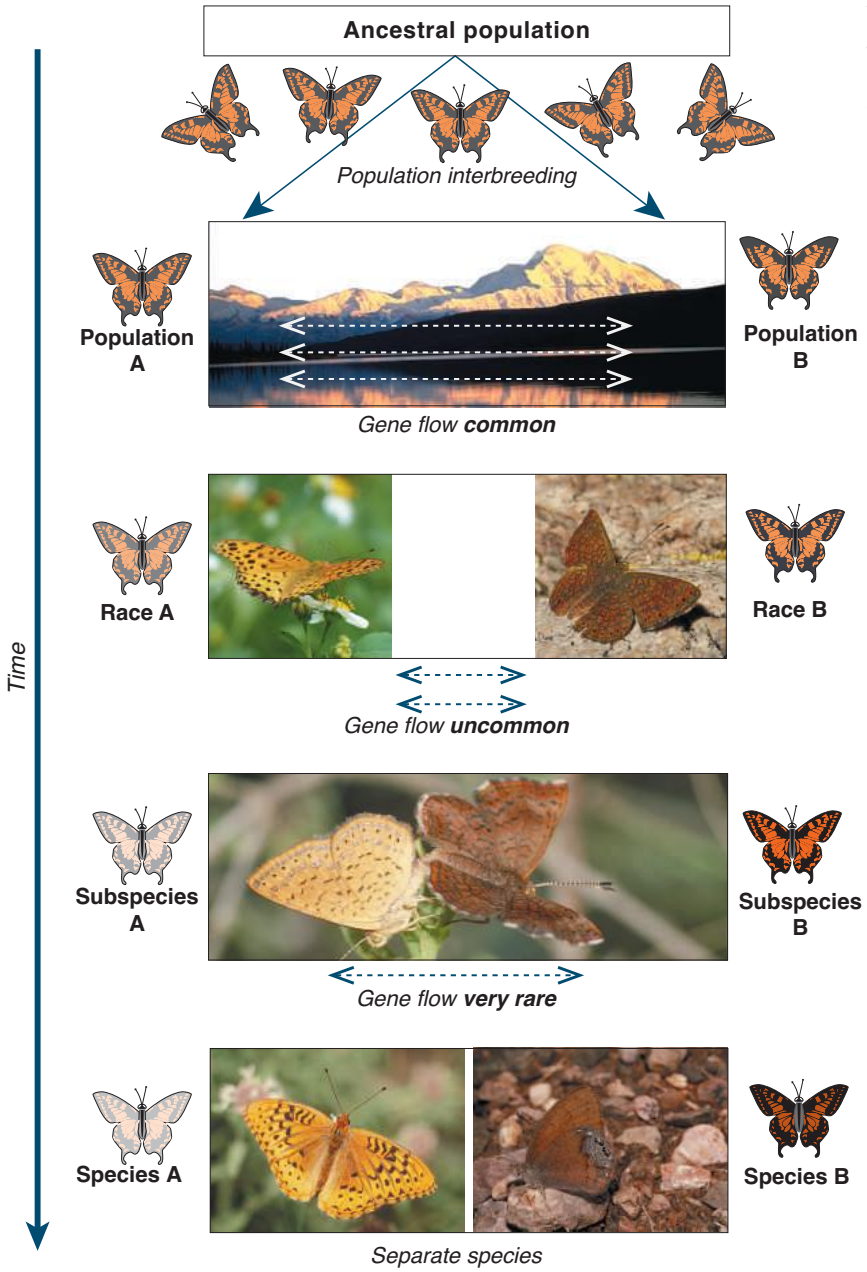
4. In terms of the story they tell, in what way are the transitional fossils of equids and *Archaeopteryx* different and how are these stories important?

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125 Stages in Species Formation

Key Idea: Speciation may occur in stages marked by increasing isolation of diverging gene pools. Physical separation is followed by increasing reproductive isolation.

The diagram below shows a possible sequence of events in the origin of two new species from an ancestral population. Over time, the genetic differences between two populations increase and the populations become increasingly isolated from each other. The isolation of the two gene pools may begin with a geographical barrier. This may be followed by progressively greater reduction in gene flow between the populations until the two gene pools are isolated and they each attain species status.



A species of butterfly lives on a plateau. The plateau is covered with grassland strewn with boulders. During colder weather, some butterflies sit on the sun-heated boulders to absorb the heat, while others retreat to the lower altitude grassland to avoid the cold.

Continued mountain building raises the altitude of the plateau, separating two populations of butterflies, one in the highlands the other in the lowlands.

In the highlands, boulder-sitting butterflies (BSBs) do better than grass-sitting butterflies (GSBs). In the lowlands, the opposite is true. BSBs only mate on boulders with other BSBs. Darker BSBs have greater fitness than light BSBs. (they can absorb more heat from the boulders). In the lowlands, light GSBs blend in with the grass and avoid predators better than darker butterflies.

Over time, only boulder-sitting butterflies are found in the highlands and grass-sitting butterflies in the lowlands. Occasionally wind brings members of the two groups together, but if they mate, the offspring are usually not viable or have a much lowered fitness.

Eventually gene flow between separated populations ceases as variation between the populations increases. They fail to recognise each other as members of the same species.

1. Identify the variation in behaviour in the original butterfly population: _____

_____

2. What were the selection pressures acting on BSBs in the highlands and GSBs in the lowlands respectively?

_____

_____

_____

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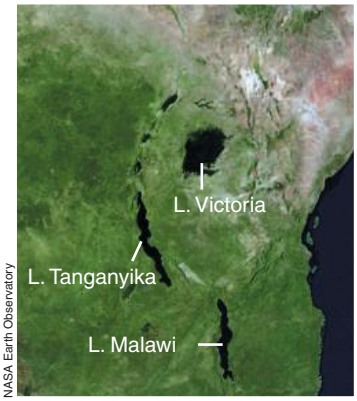
126 Reproductive Isolation

Key Idea: Reproductive isolation maintains separate species by preventing gene flow between populations.

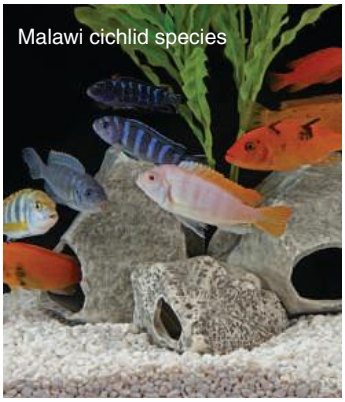
Isolating mechanisms are barriers to successful interbreeding between species. Reproductive isolation is fundamental to the biological species concept, which defines a species by its inability to breed with other species to produce fertile offspring. Prezygotic isolating mechanisms act before fertilisation, preventing species ever mating, whereas postzygotic barriers take effect after fertilisation. Reproductive isolation prevents interbreeding (and therefore gene flow) between species. A hybrid is a cross between two species and any factor that prevents the formation of viable, fertile hybrids contributes to reproductive isolation. Single barriers may not completely stop gene flow, so most species commonly have more than one type of barrier. Single barriers to reproduction (including geographical barriers) often precede the development of a suite of reproductive isolating mechanisms (RIMs). Most operate before fertilisation (prezygotic RIMs) with postzyotic RIMs being important in preventing offspring between closely related species.

Geographical isolation

Geographical isolation describes the isolation of a species population (gene pool) by some kind of physical barrier, e.g. mountain range, water body, isthmus, desert, or ice sheet. Geographical barriers are not regarded as reproductive isolating mechanisms because they are not part of the species' biology, although they are often a necessary precursor to reproductive isolation in sexually reproducing populations. Geographical isolation is a frequent first step in the subsequent reproductive isolation of a species. For example, geologic changes to the lake basins have been instrumental in the subsequent proliferation of cichlid fish species in the rift lakes of East Africa (right). Similarly, many Galápagos Island species (e.g. iguanas, finches) are now quite distinct from the Central and South American species from which they arose after isolation from the mainland.



NASA Earth Observatory



Prezygotic reproductive isolating mechanisms

Temporal isolation

Individuals of different species do not mate because they are active during different times of the day or in different seasons. Plants flower at different times of the year or even at different times of the day to avoid hybridisation (e.g. members of the orchid genus *Dendrobium*, which occupy the same location and flower on different days). Closely related animal species may have different breeding seasons or periods of emergence. Periodical cicadas (right) of the genus *Magicicada* are so named because members of each species in a particular region are developmentally synchronised, despite very long life cycles. Once their period of development underground is over (13 or 17 years depending on the species), the entire population emerges at much the same time to breed.


Bruce Marlin


Lorax

Behavioural isolation

Behavioural isolation operates through differences in species courtship behaviours. Courtship is a necessary prelude to mating in many species and courtship behaviours are species specific. Mates of the same species are attracted with distinctive, usually ritualised, dances, vocalisations, and body language. Because they are not easily misinterpreted, the courtship behaviours of one species will be unrecognised and ignored by individuals of another species. Birds exhibit a remarkable range of courtship displays. The use of song is widespread but ritualised movements, including nest building, are also common. Examples include the courtship bowers of bowerbirds, the elaborate displays of Galápagos frigatebirds, and the strutting and drumming displays of grouse (right).


Albatross courtship


Male tree frog calling


Male frigatebird courtship display


Wing beating in male sage grouse

Mechanical isolation

Structural incompatibility in the reproductive organs prevents sperm transfer between individuals of different species. This is an important isolating mechanism between closely related arthropod species. Many flowering plants have coevolved with their animal pollinators and have flower structures to allow only that insect access. Structural differences in the flowers and pollen of different plant species prevents cross breeding because pollen transfer is restricted to specific pollinators and the pollen itself must be species compatible.


Damselflies mating


Complex flowers in orchids

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Postzygotic isolating mechanisms

Postzygotic isolating mechanisms operate after fertilisation and are important in preventing offspring between closely related species. They involve genetic incompatibilites.

Hybrid sterility

Hybrid sterility may occur due to the failure of meiosis to produce normal gametes in the hybrid. This can occur if the chromosomes of the two parents are different in number or structure (see the "**zebronkey**" karyotype, right).

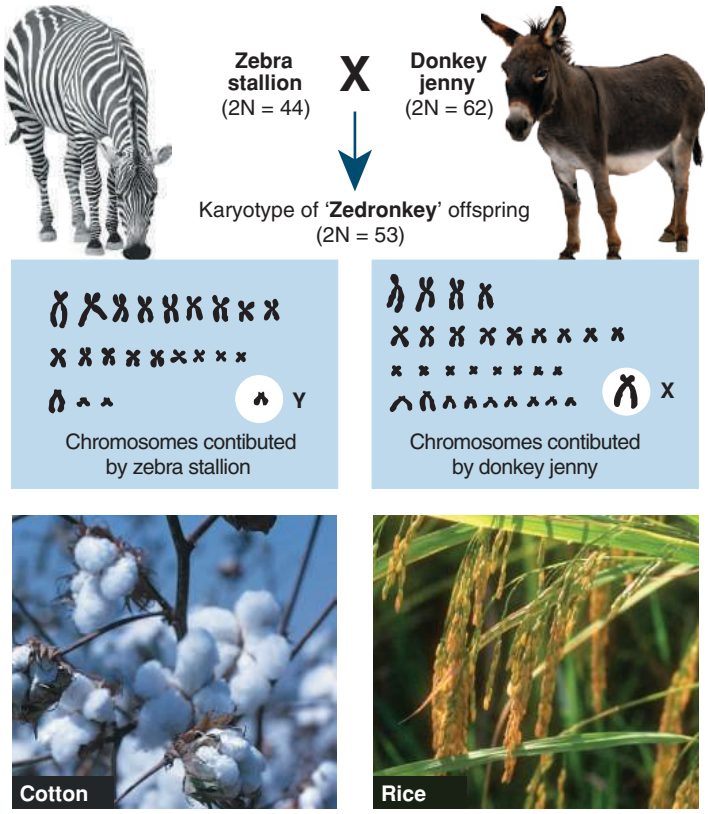
Non-viable hybrids

Mating between individuals of two species may produce a zygote, but genetic incompatibility may stop development of the zygote. Fertilised eggs often fail to divide because of mis-matched chromosome numbers from each gamete.

Hybrid breakdown

Hybrid breakdown is a common feature of some plant hybrids. The first generation (F_1) may be fertile, but the second generation (F_2) are infertile or nonviable. Examples include hybrids between species of cotton (near right), species of *Populus*, and strains of cultivated rice *Oryza*.

In plants, hybridisation can lead to new species formation if there is a doubling of the chromosome number during meiosis. The new plant is immediately genetically (and therefore reproductively) isolated from the parent species due to differences in chromosome number.



1. (a) Why is a geographical barrier not considered a reproductive isolating mechanism? _____

   _____

   _____

   (b) Identify some geographical barriers that could separate populations: _____

   _____

   (c) Why is geographic isolation often an important first step in species formation? _____

   _____

   _____

2. Explain how temporal isolation stops closely related species from interbreeding: _____

   _____

   _____

   _____

3. Explain why many animals have courtship displays and how this prevents breeding between species:

   _____

   _____

   _____

   _____

4. How does the structure of some orchids isolate them from other species of orchid? _____

   _____

   _____

5. What is the name given to reproductive isolating mechanisms that operate before fertilisation? _____

   _____

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127 Speciation and the Role of Habitat

Key Idea: Geographic barriers presented by inhospitable habitat are contributing to speciation in Australian treecreepers.

The geographical barriers isolating populations on continents are often very different to those isolating island populations. In Australia, geographical barriers exist in the form of regions of inhospitable habitat. These create regions of preferred habitat cut off from one another. The species and subspecies of Australian treecreepers, *Climacteris*, are distinguishable by variations in the colour patterns of their plumage. Their distribution is restricted to savannah woodland and a distinctive form is associated with each of the major woodland areas (below). The populations probably evolved from a single common ancestor, isolated by habitat changes and then unable to expand their individual distributions beyond regions of unsuitable dry habitat. Subsequently, the distribution of two of the treecreeper species has undergone a secondary range expansion (arrows), where they have extended their range beyond their region of origin into new habitat.

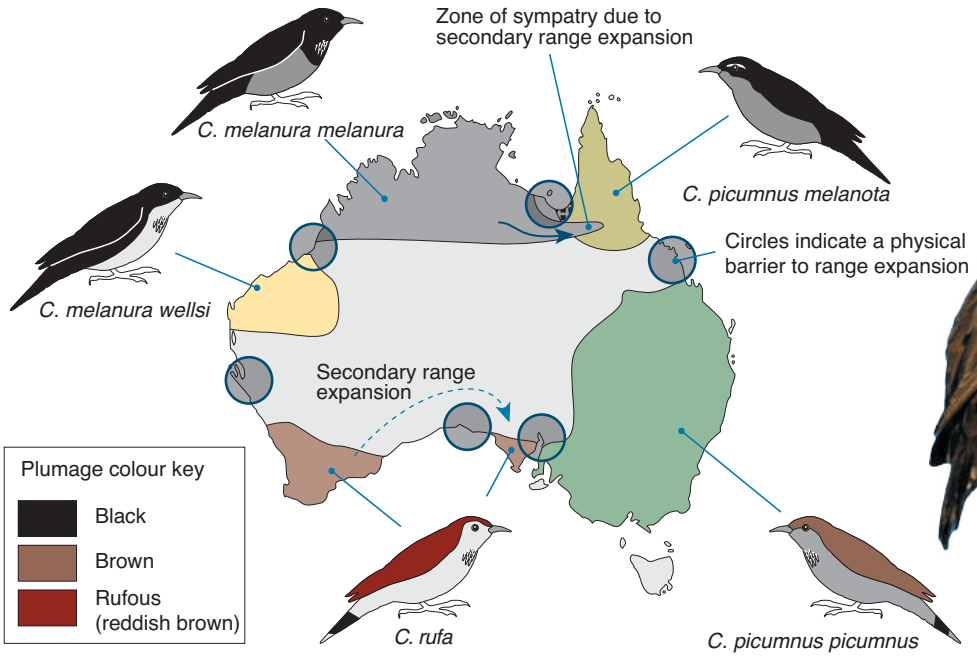




Photo: Aviceda

The Australian treecreeper Genus *Climacteris*

1. (a) How many species are illustrated above? Explain your answer: _____

   _____

   (b) Describe the distribution of these treecreeper populations in Australia: _____

   _____

   _____

2. Explain why there are no treecreeper populations in the central region of Australia: _____

   _____

3. Two species in the NE of Australia, *C. melanura melanura* and *C. picumnus melanota*, exhibit sympatric distribution.

   (a) What is meant by the area of sympatry in this context? _____

   _____

   (b) What mechanisms are most likely to prevent interbreeding between these two species? _____

   _____

4. What is meant by secondary range expansion in the two populations above: _____

   _____

5. Describe the physical barriers that have prevented the neighbouring populations from mixing (in all but one case):

   _____

6. Predict a likely outcome to the distribution of these species, should the climate change to produce more coastal rainfall:

   _____

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128 Allopatric Speciation

Key Idea: Allopatric speciation is the genetic divergence of a population after it becomes subdivided and isolated.

Allopatric speciation refers to the genetic divergence of a species after a population becomes split and then isolated geographically. It is probably the most common mechanism by which new species arise and has certainly been important in regions where there have been cycles of geographical fragmentation, e.g. as a result of ice expansion and retreat (and accompanying sea level changes) during glacial and interglacial periods.

Stage 1: Moving into new environments

There are times when the range of a species expands for a variety of different reasons. A single population in a relatively homogeneous environment will move into new regions of their environment if there is intense competition for resources. Competition between members of the same species is the most intense because they are competing for identical resources in the same habitat. In the diagram on the right there is a 'parent population' of a single species with a common gene pool with regular 'gene flow'. Theoretically any individual can mate with any other individual of the opposite sex.

Stage 2: Geographical isolation

Parts of the population may become isolated by **physical barriers**, such as mountains, deserts, or stretches of water. These barriers may cut off those parts of the population that are at the extremes of the range and gene flow becomes rare or stops altogether. Climate change (e.g. ice ages and a consequent rise and fall in sea level) can leave 'islands' of habitat separated by large inhospitable zones that the species cannot traverse.

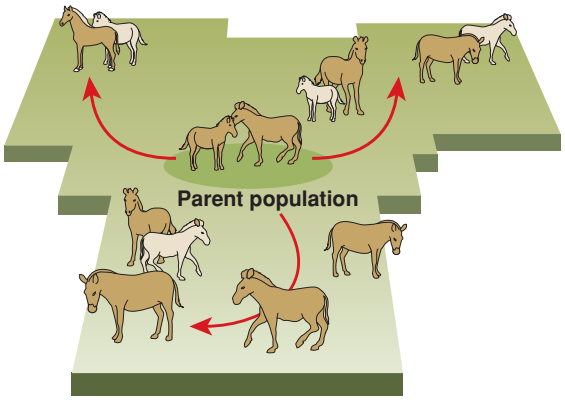
Example: In mountainous regions, alpine species can populate extensive areas of habitat during cool climatic periods. During warmer periods, they may become isolated because their habitat is reduced to 'islands' of high ground surrounded by inhospitable lowland habitat.

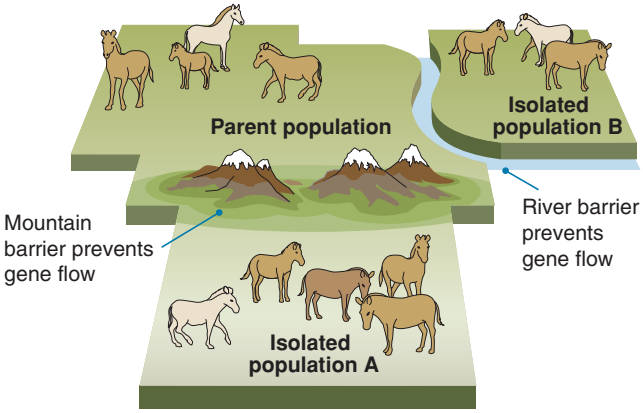
Stage 3: Different selection pressures

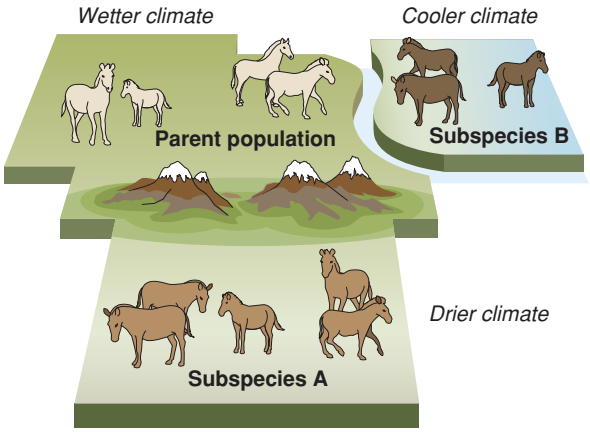
The isolated populations (A and B) may be subjected to quite different selection pressures. These will favour individuals with traits suited to each particular environment. For example, population A will be subjected to selection pressures found in drier conditions, favouring individuals with phenotypes (and genotypes) suited to dry conditions (e.g. better ability to conserve water). This would result in improved survival and reproductive performance. As allele frequencies for certain genes change, the population takes on the status of a subspecies. Reproductive isolation is not yet established but the subspecies are significantly different genetically from related populations.

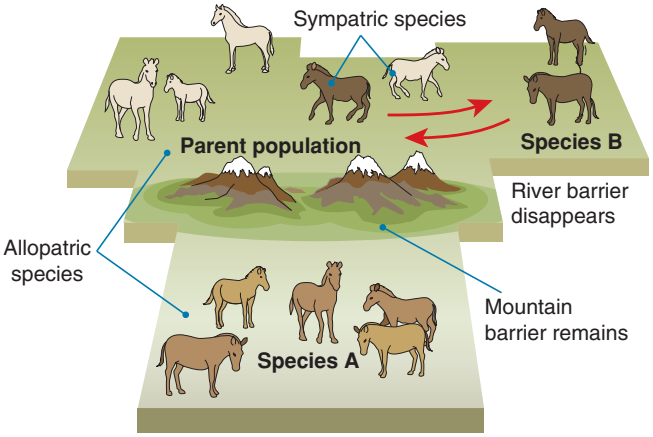
Stage 4: Reproductive isolation

The separated populations (isolated subspecies) undergo genetic and behavioural changes. These ensure that the gene pool of each population remains isolated and 'undiluted' by genes from other populations, even if the two populations should be able to reunite at a later time (if the geographical barrier is removed). Gene flow does not occur but there is a zone of overlap between two species after species B has moved back into the range of the parent population. Closely-related species with an overlapping distribution like this are called **sympatric**. Those that remain geographically isolated are called **allopatric** species.









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1. Why do some animals, given the opportunity, move into new environments? _____

2. Plants are unable to move. How might plants disperse to new environments? _____

3. Describe the amount of gene flow within a parent population prior to and during the expansion of a species' range:

4. Explain how cycles of climate change can cause large changes in sea level (up to 200 m): _____

5. (a) What kinds of physical barriers could isolate different parts of the same population? _____

   (b) How might emigration achieve the same effect as geographical isolation? _____

6. (a) How might selection pressures differ for a population that becomes isolated from the parent population? _____

   (b) Describe the general effect of the change in selection pressures on the allele frequencies of the isolated gene pool:

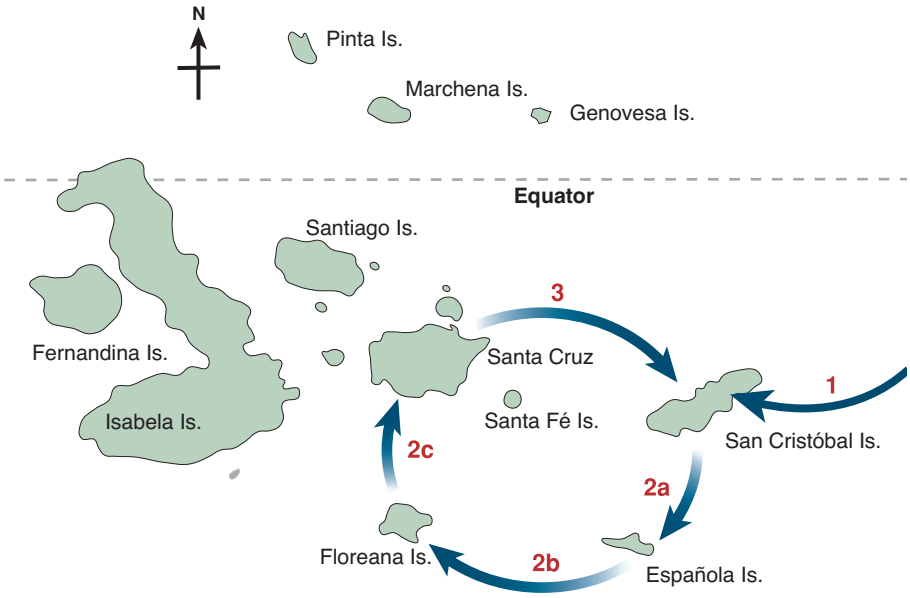
7. Explain how reproductive isolation could develop in geographically separated populations (see previous pages):

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Allopatric speciation in Galápagos finches

- The Galápagos Islands, off the west coast of Ecuador, consist of 16 main islands and six smaller islands. They are home to 14 species of finches, each of which has evolved from a single species of grassquit, which arrived from Ecuador. A fifteenth species inhabits Cocos Island. The evolution of these finches is an example of allopatric speciation.
- Three allopatric speciation events can be seen in the finches. The first is the speciation arising from the colonisation of the Galápagos Islands by the original ancestor (1, right). The second is the speciation arising from the colonising of Cocos Is. from the Galápagos Islands (2, right). The third is the adaptive radiation within the Galápagos Islands archipelago (below).





After the initial speciation following colonisation from the mainland (#1 above), further allopatric speciation events occurred within the Galápagos. Populations spread to other islands and diverged in different environments (#2 stages). Secondary contact then occurred between divergent populations (#3 above). If divergence (speciation) was complete, these species could remain distinct, exploiting different niches. Repeated cycles of speciation events gave rise to all the Galápagos finch species.



J. Podos

One of the most studied Galápagos finches are the **ground finch (**genus *Geospiza*). These have crushing type beaks for seed eating. Three species differ mainly in body size and in the size of their beaks. Three other species have longer beaks and supplement their seed diet with cactus flowers and pulp (the cactus finches) or the eggs and blood of other birds and reptile ticks (the sharp-beaked ground finch).

8. What are the three allopatric speciation events related to the evolution of the Galápagos Island finches as a group?

9. How has allopatric speciation of the finches occurred within the Galápagos Islands? _____

10. How would fitness play a part in speciation when the two divergent species come in contact again (stage 3 above)?

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129 Sympatric Speciation

Key Idea: Sympatric speciation is speciation which occurs even when there is no physical barrier separating gene pools. In sympatric (same place) speciation, a new species evolves from a single ancestral species while inhabiting the same geographic region. Sympatric speciation is rarer than allopatric speciation, although it is not uncommon in plants which form polyploids. There are two situations where sympatric speciation is thought to occur. These are described below.

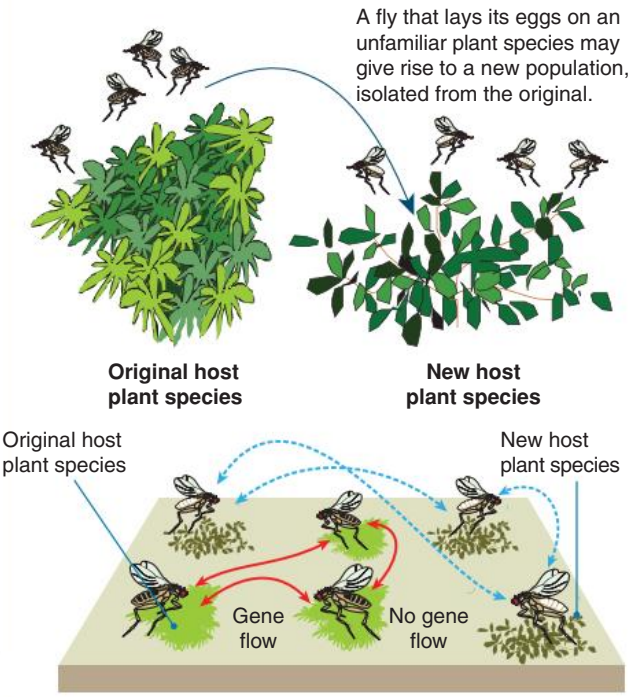
Speciation through niche differentiation

Niche isolation

There are many microhabitats within a heterogeneous environment (one that is not the same everywhere). Some individuals in a population may preferentially occupy to occupy one particular microhabitat, only rarely coming in contact with those that select other microhabitats. Some organisms become so dependent on the resources offered by their particular microhabitat that they never interact with others of their species in different microhabitats.

Reproductive isolation

Sub-populations, which have remained genetically isolated because of their microhabitat preferences, become reproductively isolated. They have become new species with subtle differences in behaviour, structure, and physiology. Gene flow (via sexual reproduction) is limited to organisms that share similar microhabitat preferences (as shown right). **Example**: Some host-specific phytophagous insects (insects that feed on plants) lay eggs on plants identical to the species they themselves hatched on. Host plant preference leads to isolation despite the populations being sympatric.

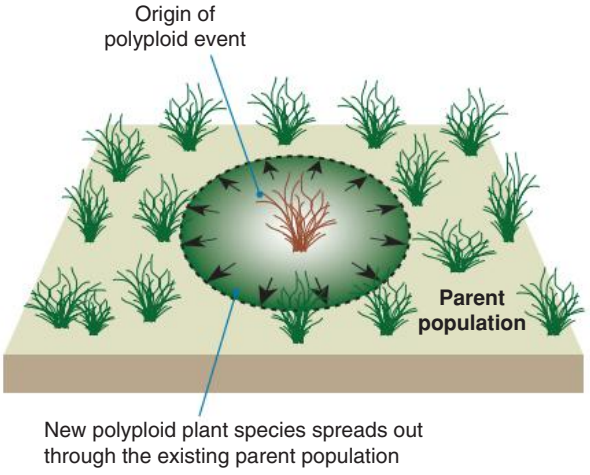


Instant speciation by polyploidy

Polyploidy may result in the formation of a new species without isolation from the parent species. This event, occurring during meiosis, produces sudden reproductive isolation for the new group. Because the sex-determining mechanism is disturbed, animals are rarely able to achieve new species status this way (they are sterile). Many plants, on the other hand, are able to reproduce vegetatively, or self pollinate. This ability to reproduce on their own enables such polyploid plants to produce a breeding population.

Speciation by allopolyploidy

This type of polyploidy usually arises from the doubling of chromosomes in a hybrid between two different species. The doubling often makes the hybrid fertile. **Examples**: Modern wheat. Swedes are a polyploid species formed from a hybrid between a type of cabbage and a type of turnip.



1. Explain what is meant by sympatric speciation: _____

_____

2. What is the mechanism for instant speciation? Explain why it is more common in plants than in animals: _____

_____

_____

_____

3. Explain how niche differentiation could result in the formation of a new species: _____

_____

_____

_____

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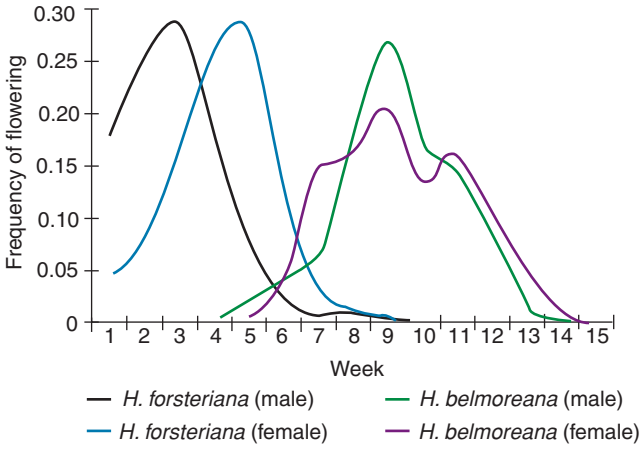
Sympatric speciation in *Howea* palms

- The palms *Howea forsteriana* (below) and *Howea belmoreana* are endemic to Lord Howe Island. Studies show differences between the species in flowering times, tolerance of soil pH, and altitude preference. Hybrids are rare.

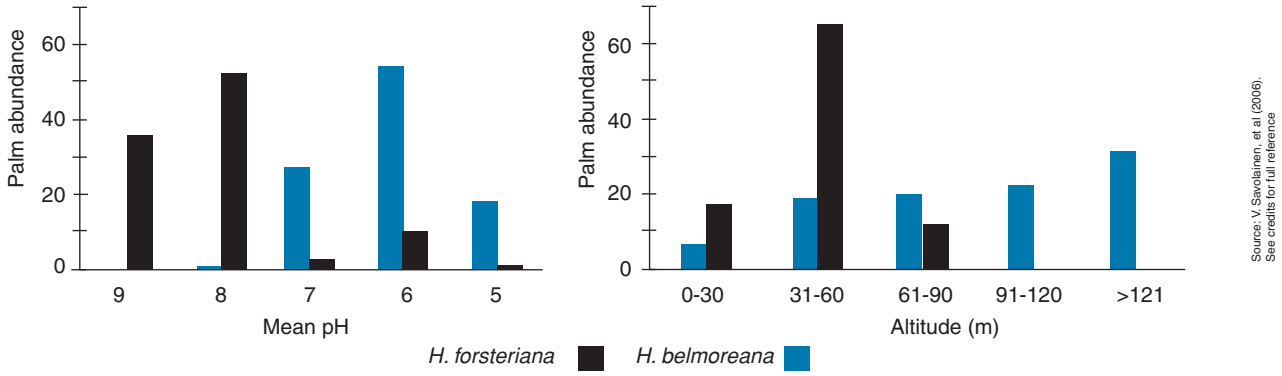


Black Diamond Images CC 2.0

Differences in flowering times



Palm distribution by soil pH and by altitude



Source: V. Savolainen, et al (2006). See credits for full reference



David Stanley CC 3.0

- Lord Howe Island is the eroded remains of a 7 million year old volcano. It is located in the Tasman sea about 700 km from Sydney.
- The island is about 10 km long and 2 km wide at its widest point and covers 14.5 km^2. The highest point is 875 m above sea level.
- About half the island's plants are endemic. *Howea* palms are found throughout the island. They are also cultivated and exported.
- As the island is so small, the palms are believed to have evolved by sympatric speciation.

With respect to the palm species *H. forsteriana* and *H. belmoreana:*

4. Approximately how many weeks difference is there in flowering times between them? _____

5. Describe the difference in preference for soil pH between the two palm species: _____

_____

6. Describe the difference in altitude preference between the two palm species: _____

_____

_____

7. In what way are the observations above consistent with sympatric speciation? _____

_____

_____

_____

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130 Chapter Review: Did You Get It?

1. For each of the following examples, classify the reproductive isolating mechanism (RIM) as prezygotic or postzygotic and describe the mechanisms by which the isolation is achieved (e.g. morphological isolation, hybrid sterility etc.):

   (a) Some different cotton species can produce fertile hybrids, but breakdown of the hybrid occurs in the next generation when the offspring of the hybrid die in their seeds or grow into defective plants:

   (i) RIM: _____ (ii) Mechanism of isolation: _____

   (b) Many plants have unique arrangements of their floral parts that stops transfer of pollen between plants:

   (i) RIM: _____ (ii) Mechanism of isolation: _____

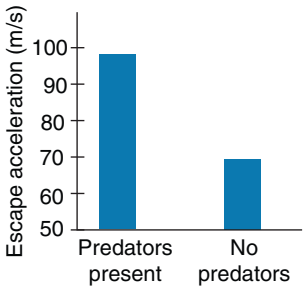
   (c) Two skunk species do not mate despite having habitats that overlap because they mate at different times of the year:

   (i) RIM: _____ (ii) Mechanism of isolation: _____

   (d) Several species of the frog genus *Rana* live in the same regions and habitats, where they may occasionally hybridise. The hybrids generally do not complete development, and those that do are weak and do not survive long:

   (i) RIM: _____ (ii) Mechanism of isolation: _____

2. The graph on the right shows the difference in escape acceleration in two populations of mosquitofish (*Gambusia*) in the Bahamas. One population is subject to heavy predation, the other is not. Suggest how this difference could lead to speciation over time if the populations remain separate:



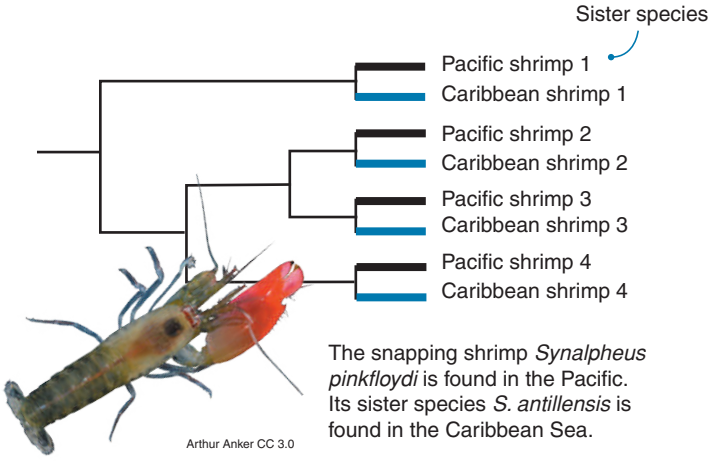
3. The half life of carbon-14 is 5730 years. A bone from a cave is radiocarbon dated and it is found to have about 12% of the carbon 14 it would have if in a living specimen. Approximately how old is the bone?

4. What is the difference between allopatric speciation and sympatric speciation? _____

5. The Isthmus of Panama separates the Pacific Ocean and Caribbean Sea in the region of Central America. The isthmus closed about 3 million years ago. On either side of the isthmus are numerous species of snapping shrimp. Every species on the Pacific side has a sister species on the Caribbean side. The diagram below shows the relationship between the shrimp species.

   (a) What type of evolutionary process has occurred here?

   (b) Why has this occurred?



Arthur Anker CC 3.0

The snapping shrimp *Synalpheus pinkfloydi* is found in the Pacific. Its sister species *S. antillensis* is found in the Caribbean Sea.

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CHAPTER 12

Determining Species Relatedness

Key terms
common ancestor
cladogram
DNA hybridisation
homology
molecular clock
monophyletic
mtDNA
phylogenetic tree
phylogeny
taxon

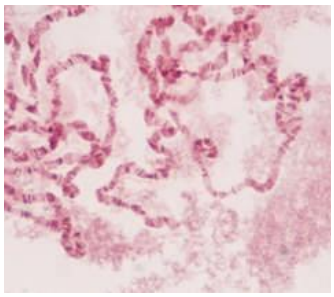
Evidence of relatedness between species

Key skills and knowledge

Activity number

- ☐ 1 Understand that the evidence for evolution and the relatedness of species comes from many scientific disciplines. Identify these lines of evidence and their contribution to our understanding of species relatedness. — 131
- ☐ 2 Understand the biochemical and structural evidence for the common ancestry of all living organisms. Include reference to the universality of DNA, amino acids, and protein structures, and the bacterial origin of eukaryotic mitochondria and chloroplasts. — 132
- ☐ 3 Explain how comparisons of structural morphology and physiology have contributed to our understanding of evolutionary relationships among organisms. Describe examples of homologous structures and recognise them as evidence of common ancestry. — 133
- ☐ 4 Explain what is meant by a vestigial character and provide examples. How does vestigiality arise and why are vestigial characters not completely lost? — 134







Molecular homology

Key skills and knowledge

- ☐ 5 Describe the molecular evidence that reveals similarities between closely related organisms with reference to DNA and protein sequence data, mitochondrial DNA (mtDNA), and genome-wide comparisons (including DNA hybridisation). — 135 136 137 139
- ☐ 6 Describe how biochemical variations can be used as a molecular clock to determine the probable dates of divergence from a common ancestor. How are molecular clocks calibrated and what are their benefits and disadvantages? — 138 139

The use and interpretation of phylogenetic trees

Key skills and knowledge

- ☐ 7 Describe how phylogenetic trees are used to show species relatedness. What are phylogenetic trees based on and how are they constructed? Recognise phylogenetic trees as hypotheses for the possible evolutionary relationships among species. How do we decide which tree is the most likely to be a true representation of the evolutionary history of a group (taxon)? — 140 141
- ☐ 8 Recognise a cladogram as a phylogenetic tree produced using cladistic analysis (analysis of shared derived characteristics). Construct a cladogram to show the evolutionary history of a group of related organisms. — 141 - 143
- ☐ 9 Explain how phylogenetic trees can change in the light of new information or improved technology. Use an example to illustrate your point. — 143

131 Evidence for the Relatedness of Species

Key Idea: Evidence for the fact that populations evolve from a common ancestor comes from many fields of science.

Evolution is simply the heritable genetic changes occurring in a population over time. There are two important points to take from this definition: that evolution refers to populations, not individuals, and that the changes must be inherited.

The evidence for evolution and the relatedness of species comes from many diverse branches of science and includes evidence from both past and present populations. Drawing on evidence from a number of scientific disciplines helps to build a robust explanation for the relatedness and evolutionary history (phylogeny) of taxa.

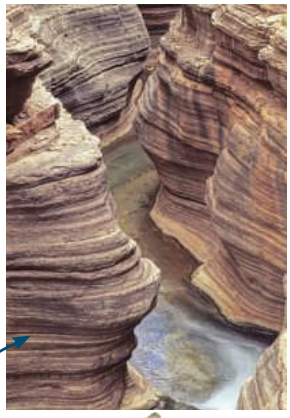


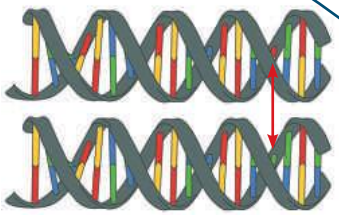
Comparative anatomy

Comparative anatomy examines the similarities and differences in the anatomy of different species. Similarities in anatomy (e.g. the bones forming the arms in humans and the wings in birds and bats) indicate descent from a common ancestor.

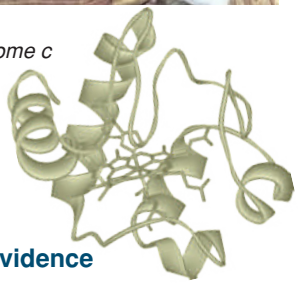
Geology

Geological strata (the layers of rock, soil, and other deposits such as volcanic ash) can be used to determine the relative order of past events and therefore the relative dates of fossils. Fossils in lower strata are older than fossils in higher (newer) strata, unless strata have been disturbed.





Cytochrome c



DNA comparisons

DNA can be used to determine how closely organisms are related to each other. The greater the similarities between the DNA sequences of species, the more closely related the species are.

Protein evidence

Similarities (and differences) between proteins provides evidence for determining shared ancestry. Fewer differences in amino acid sequences reflects closer genetic relatedness.

EVOLUTION



Fossil record

Fossils, like this shark's tooth (left) are the remains of long-dead organisms. They provide a record of the appearance and extinction of organisms.



Developmental evidence

The study of developmental processes and the genes that control them gives insight into evolutionary processes. This field of study is called evolutionary developmental biology (evo-devo).



Biogeography

The geographical distribution of living and extinct organisms provides evidence of common ancestry and can be explained by speciation, extinction, and continental drift. The biogeography of islands, e.g the Galápagos Islands, provides evidence of how species evolve when separated from their ancestral population on the mainland.



Chronometric dating

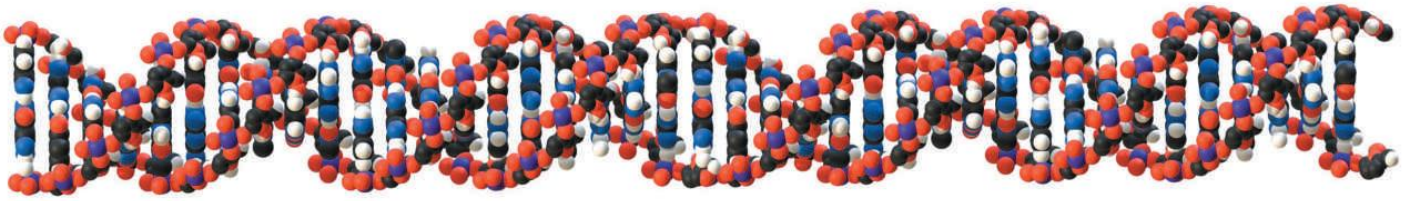
Radiometric dating techniques (such as carbon dating) allow scientists to determine an absolute date for a fossil by dating it or the rocks around it. Absolute dating has been used to assign ages to strata, and construct the geological time scale.

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132 Descent and Common Ancestry

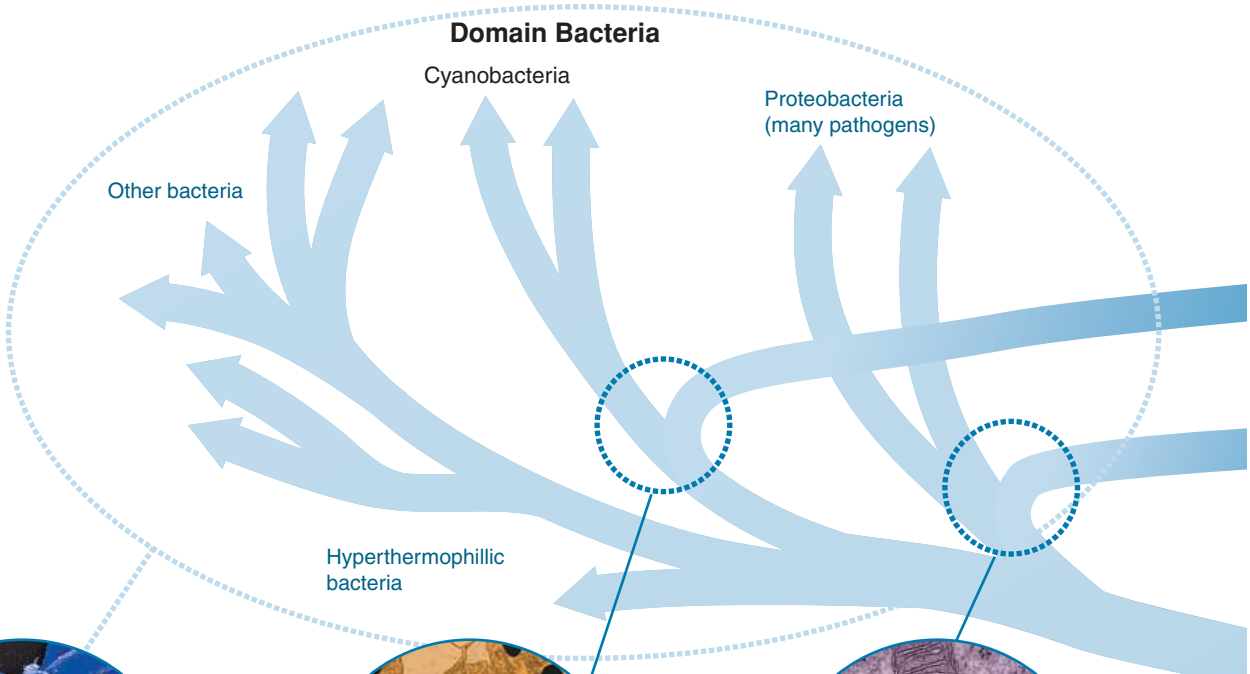
Key Idea: Molecular studies have enabled scientists to clarify the earliest beginnings of the eukaryotes. Such studies provide powerful evidence of the common ancestry of life.

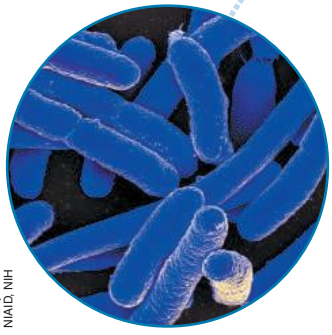
Traditionally, the phylogeny (evolutionary history) of organisms was established using morphological comparisons. In recent decades, molecular techniques involving the analysis of DNA, RNA, and proteins have provided more information about how all life on Earth is related. These newer methods have enabled scientists to clarify the origin of the eukaryotes and to recognize two prokaryote domains. The universality of the genetic code and the similarities in the molecular machinery of all cells provide powerful evidence for a common ancestor to all life on Earth.



There is a universal genetic code

DNA encodes the genetic instructions of all life. The form of these genetic instructions, called the **genetic code**, is effectively universal, i.e. the same combination of three DNA bases code for the same amino acid in almost all organisms. The very few exceptions in which there are coding alternatives are restricted to some bacteria and to mitochondrial DNA.



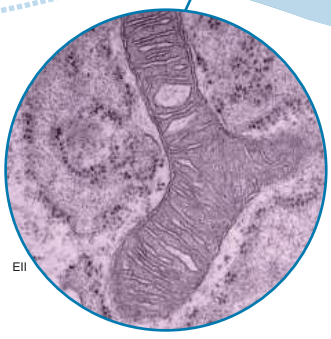


Rocky Mountain Laboratories, NIAID, NIH

Bacteria lack a distinct nucleus and cell organelles. Features of the cell wall are unique to bacteria and are not found among archaea or eukaryotes. Typically found in less extreme environments than archaea.



Chloroplasts have a bacterial origin
Cyanobacteria are considered to be the ancestors of chloroplasts. The evidence for this comes from similarities in the ribosomes and membrane organisation , as well as from genomic studies. Chloroplasts were acquired independently of mitochondria, from a different bacterial lineage, but by a similar process.



EII

Mitochondria have a bacterial origin
Evidence from mitochondrial gene sequences, ribosomes, and protein synthesis indicate that mitochondria have a prokaryotic origin. Mitochondria were probably symbiotic inclusions in an early eukaryotic ancestor.

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1. Identify three features of the metabolic machinery of cells that support a common ancestry of life:

   (a) _____

   (b) _____

   (c) _____

2. Suggest why scientists believe that mitochondria were acquired before chloroplasts: _____

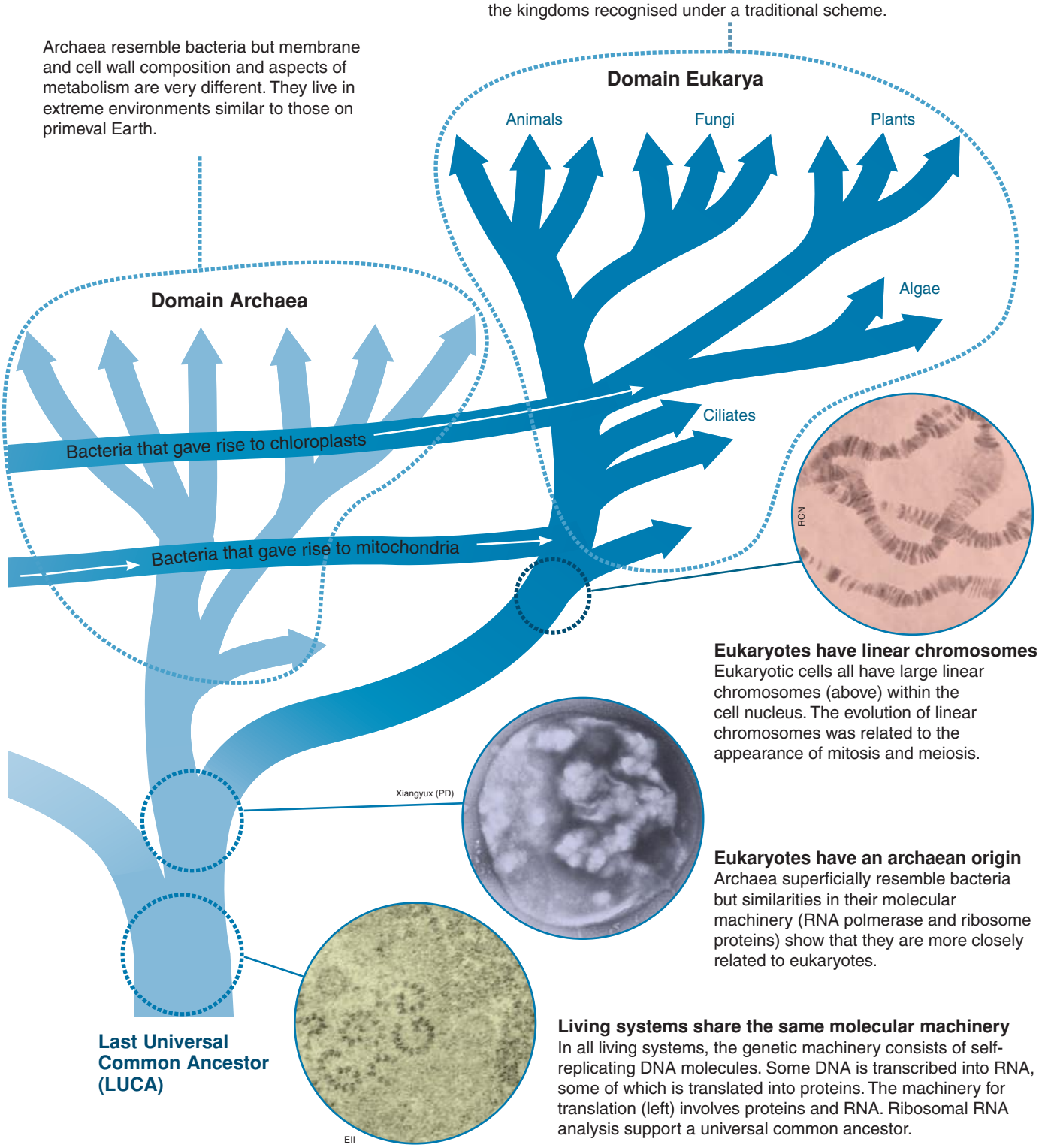
   _____

   _____

   _____

Eukarya (the eukaryotes) are characterised by complex cells with organelles and a membrane-bound nucleus. This domain contains four of the kingdoms recognised under a traditional scheme.

Archaea resemble bacteria but membrane and cell wall composition and aspects of metabolism are very different. They live in extreme environments similar to those on primeval Earth.



Eukaryotes have linear chromosomes
Eukaryotic cells all have large linear chromosomes (above) within the cell nucleus. The evolution of linear chromosomes was related to the appearance of mitosis and meiosis.

Eukaryotes have an archaean origin
Archaea superficially resemble bacteria but similarities in their molecular machinery (RNA polmerase and ribosome proteins) show that they are more closely related to eukaryotes.

Living systems share the same molecular machinery
In all living systems, the genetic machinery consists of self-replicating DNA molecules. Some DNA is transcribed into RNA, some of which is translated into proteins. The machinery for translation (left) involves proteins and RNA. Ribosomal RNA analysis support a universal common ancestor.

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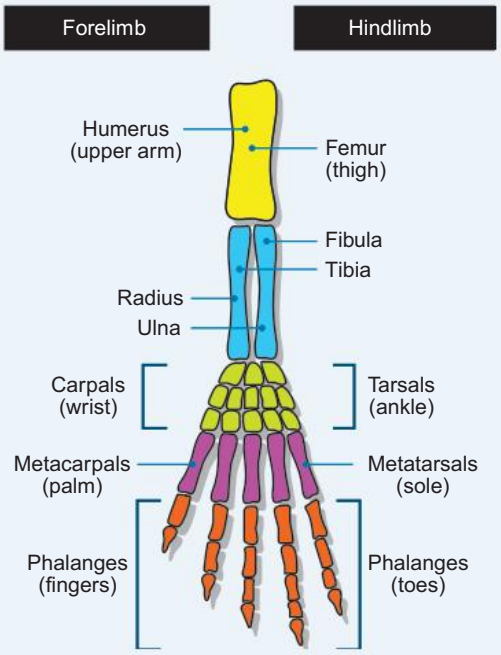
133 Homologous Structures

Key Idea: Homologous structures (homologies) are structural similarities present as a result of common ancestry. The common structural components have been adapted to different purposes in different taxa.

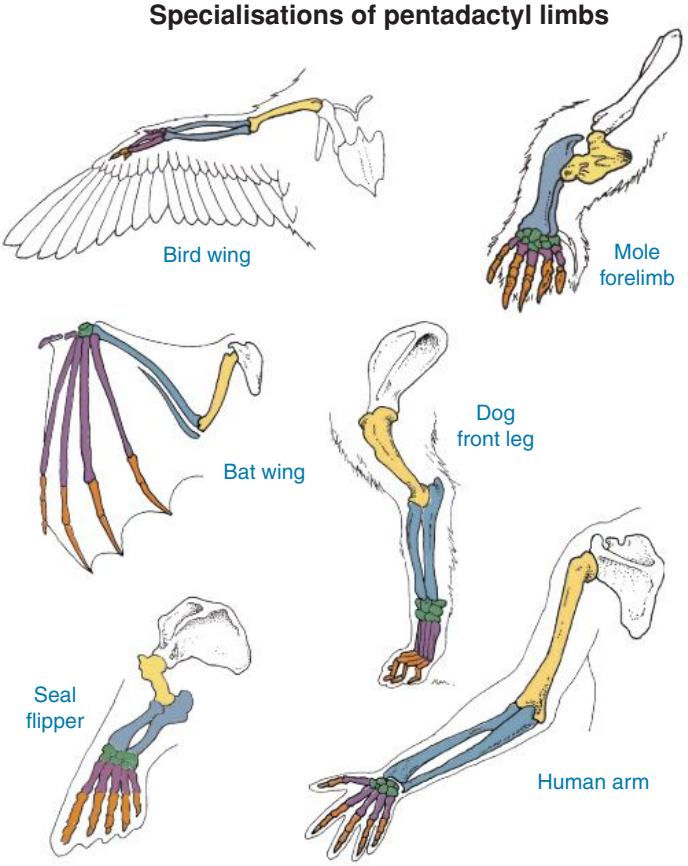
The bones of the forelimb of air-breathing vertebrates are composed of similar bones arranged in a comparable pattern. This is indicative of common ancestry. The early land vertebrates were amphibians with a **pentadactyl limb** structure (a limb with five fingers or toes). All vertebrates that descended from these early amphibians have limbs with this same basic pentadactyl pattern. They also illustrate the phenomenon known as **adaptive radiation**, since the basic limb plan has been adapted to meet the requirements of different niches.

Generalised pentadactyl limb

The forelimbs and hind limbs have the same arrangement of bones but they have different names. In many cases, the basic limb plan has been adapted (e.g. by loss or fusion of bones) to meet the requirements of different niches (e.g. during adaptive radiation of the mammals).



Specialisations of pentadactyl limbs



1. Briefly describe the purpose of the major anatomical change that has taken place in each of the limb examples above:

   (a) Bird wing: Highly modified for flight. Forelimb is shaped for aerodynamic lift and feather attachment.

   (b) Human arm: _____

   (c) Seal flipper: _____

   (d) Dog front leg: _____

   (e) Mole forelimb: _____

   (f) Bat wing: _____

2. Explain how homology in the pentadactyl limb is evidence for adaptive radiation: _____

3. Homology in the behaviour of animals (for example, sharing similar courtship or nesting rituals) is sometimes used to indicate the degree of relatedness between groups. How could behaviour be used in this way:

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134 Vestigial Structures

Key Idea: The persistence of vestigial structures provides evidence for common ancestry.

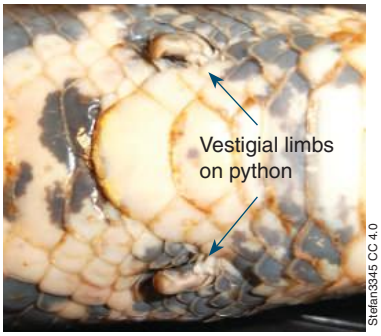
Vestigial structures are anatomical features that have been retained through a species' evolution but have lost most or all of their ancestral function (although they may retain some lesser function). Vestigial structures are often homologous to structures that retain their usual function in other species and so present evidence for biological evolution. For example, the human tail bone (coccyx) has lost its original function (balance and mobility) and is homologous to the fully functioning tail in many other primates. It retains a function in anchoring some pelvic muscles.

Vestigial structures

- Maintaining a structure (or behaviour) that offers no benefit to fitness is costly. This means that there will be selection pressure for regression of characters with a function that is no longer required.
- Vestigial structures can therefore be used as evidence for relatedness by their presence. For example, the vestigial wing bones in emus (and kiwi) show they are related to flighted birds. Flightlessness is an evolved trait in response to an environment where flight was not necessary for survival.
- In another example, along with other evidence, the vestigial limbs in pythons (far right), indicate they are related to reptiles that once walked with all four limbs.



Emus have vestigial wings hidden beneath their feathers. They still have a minor role in balance.



Stefan3345 CC 4.0

The vestigial limbs in pythons and boa constrictors are one indication of their relatedness to lizards.

Vestigial behaviours

- Vestigiality can also be seen in behaviours. Wild dogs turn around several times before lying down, to trample down vegetation and check for safety. Domestic dogs still exhibit this behaviour even through it is no longer needed in human homes.
- The Galápagos flightless cormorant has small stubby wings too small for flying. Yet after coming out of the sea it still hold its wings out to dry the same as its flighted relatives. In flying cormorants, this behaviour dries the wings in preparation for flight. But because flightless cormorants obviously don't fly, this behaviour serves no purpose.





Sharp Photography, sharpphotography CC 3.0

1. In terms of natural selection, explain how structures that were once functional could become vestigial:

2. Explain why some vestigial structures do not disappear altogether:

3. How can vestigial structures show relationships between organisms?

4. How can vestigial behaviours show relationships between organisms?

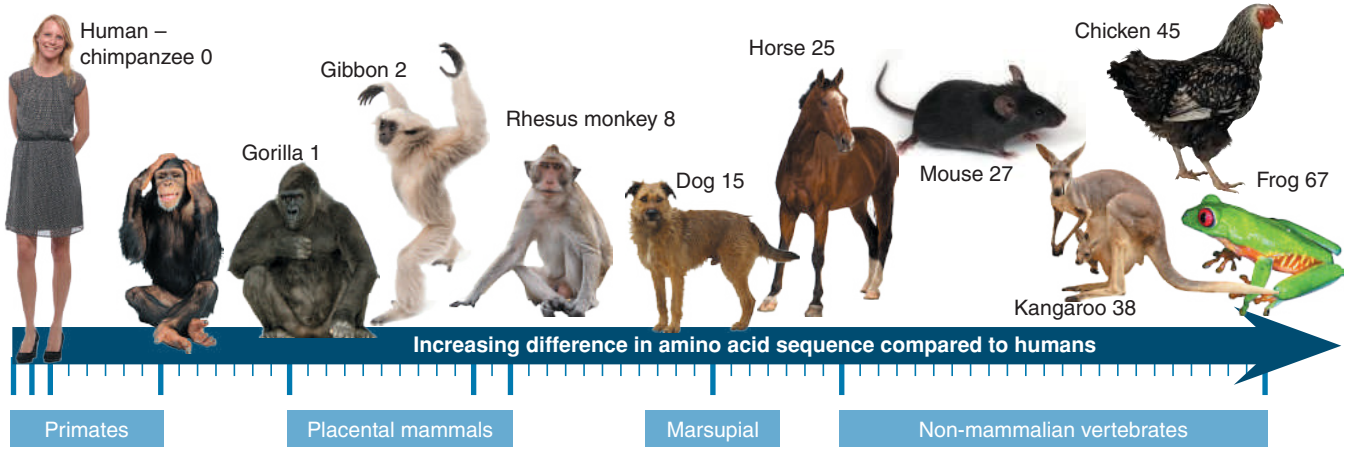
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135 Determining Relatedness Using Proteins

Key Idea: Proteins are the product of gene expression, so an analysis of the differences between the same protein in different taxa gives an indication of species relatedness.

Traditionally, phylogenies were based largely on anatomical traits, and biologists attempted to determine the relationships between taxa based on similarity or by tracing the appearance of key characteristics. With the advent of new molecular techniques, homologies (similarities arising from shared ancestry) could be studied at the molecular level as well and the results compared to phylogenies established using other methods. Protein sequencing provides an excellent tool for establishing homologies. A protein has a specific number of amino acids arranged in a specific order. Any differences in the sequence reflect changes in the DNA sequence. Commonly studied proteins include blood proteins, such as haemoglobin, and the respiratory protein cytochrome c.

Haemoglobin homology



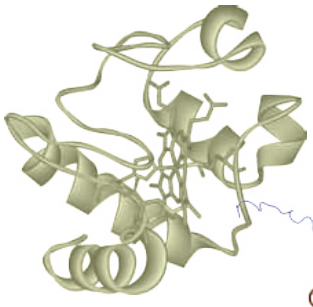
Haemoglobin is the oxygen-transporting blood protein found in most vertebrates. The beta chain haemoglobin sequences from different organisms can be compared to determine evolutionary relationships.

As genetic relatedness decreases, the number of amino acid differences between the haemoglobin beta chains of different vertebrates increases (above). For example, there are no amino acid differences between humans and chimpanzees, indicating they recently shared a common ancestor. Humans and frogs have 67 amino acid differences, indicating they had a common ancestor a very long time ago.

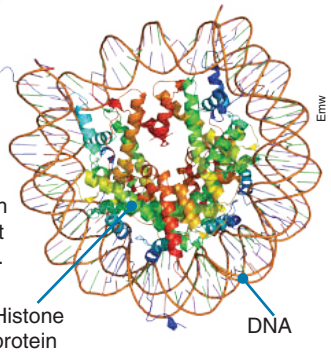
Highly conserved proteins

Some proteins are common in many different species. These proteins are called highly conserved proteins, meaning they change (mutate) very little over time. This is because they have critical roles in the organism (e.g. in cellular respiration) and mutations are likely to be lethal.

Evidence indicates that highly conserved proteins are homologous and have been derived from a common ancestor. Because they are highly conserved, changes in the amino acid sequence are likely to represent major divergences between groups during the course of evolution.



Cytochrome C (left) is a respiratory protein located in the electron transport chain in mitochondria.



Histones (right) are a family of proteins that associate with DNA and organise it so that it can fit inside the cell nucleus.

The Pax-6 protein provides evidence for evolution

- The Pax-6 gene belongs to a family of master genes that regulate the formation of a number of organs, including the eye, during embryonic development.
- The Pax-6 gene produces the Pax-6 protein, which acts as a transcription factor to control the expression of other genes.
- Scientists know the role of Pax-6 in eye development because they created a knockout model in mice where the Pax-6 gene is not expressed. The knockout model is eyeless or has very underdeveloped eyes.
- The Pax-6 gene is so highly conserved that the gene from one species can be inserted into another species, and still produce a normal eye.
- This suggests the Pax-6 proteins are homologous, and the gene has been inherited from a common ancestor.

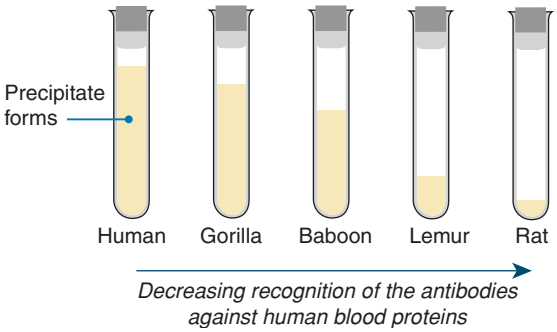
An experiment inserted mouse Pax-6 gene into fly DNA and turned it on in a fly's legs. The fly developed morphologically normal eyes on its legs!

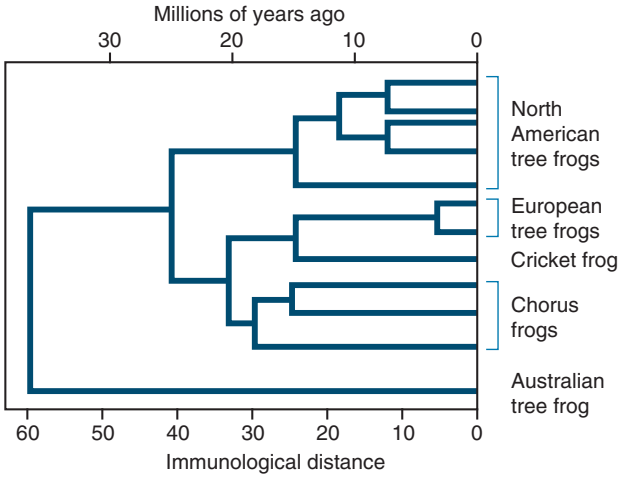


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Using immunology to determine phylogeny

The immune system of one species will recognise the blood proteins of another species as foreign and form antibodies against them. This property can be used to determine the extent of relatedness between species. Blood proteins, such as albumins, are used to prepare **antiserum** in rabbits, a distantly related species. The antiserum contains antibodies against the test blood proteins (e.g. human) and will react to those proteins in any blood sample they are mixed with. The extent of the reaction indicates how similar the proteins are; the greater the reaction, the more similar the proteins. This principle is illustrated (right) for antiserum produced to human blood and its reaction with the blood of other primates and a rat.







The relationships among tree frogs have been established by immunological studies based on blood proteins such as immunoglobulins and albumins. The **immunological distance** is a measure of the number of amino acid substitutions between two groups. This, in turn, has been calibrated to provide a time scale showing when the various related groups diverged.

1. Compare the differences in the haemoglobin sequence of humans, rhesus monkeys, and horses. What do these tell you about the relative relatedness of these organisms?

2. (a) What is a highly conserved protein? _____

   (b) What type of proteins tend to be highly conserved? _____

   (c) Why are the proteins named in (b) highly conserved? _____

   (d) Why are highly conserved proteins good for constructing phylogenies? _____

3. (a) Describe the role of the Pax-6 gene: _____

   (b) What evidence is there that the Pax-6 protein is highly conserved? _____

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136 Determining Relatedness by DNA Hybridisation

Key Idea: DNA hybridisation compares DNA similarity between species and can be used to measure relatedness.

DNA hybridisation is a technique used to quantify the DNA similarity between species. More closely related species have fewer genetic differences than more distantly related species. The method provides information only about how much of the DNA is the same and cannot provide specific information about what the similarities or differences are. Although it has largely been replaced by DNA sequence analysis, DNA hybridisation is still used in microbial studies and has been used to determine the date of human divergence from apes, which has been estimated at 10 and 5 million years ago.

DNA hybridisation technique

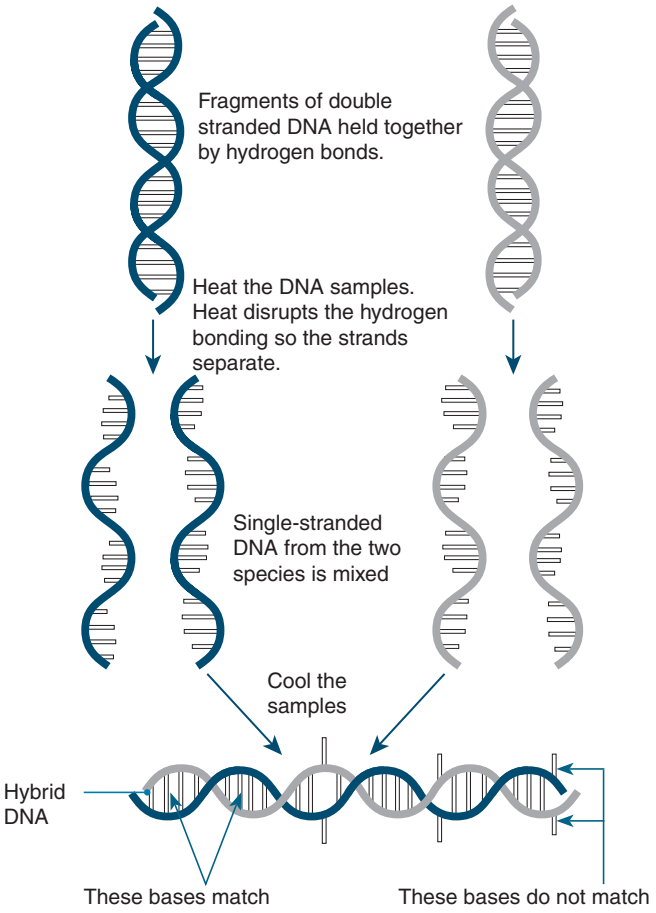
1. DNA from the two species to be compared is extracted, purified and cut into short fragments.
2. The mixture is heated so the DNA separates. The DNA from the two species is mixed together.
3. As it cools, bonds form between compatible nucleotides. Hybrid double-stranded DNA forms.
4. If species share low similarity, the hybrid DNA will have few bonds (and the strands will be weakly held together). The number of bonds (and therefore the strength of the hybrid DNA) increases with increasing similarity.
5. The similarity is measured by heating the hybrid DNA to force it to form single strands. The greater the similarity, the more heat that is required to break the hybrid DNA apart.



Human DNA



Chimpanzee DNA

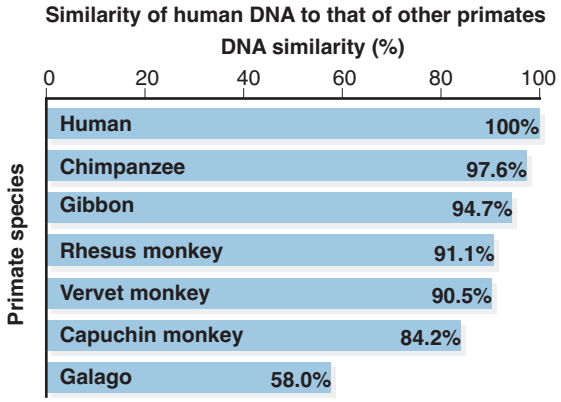


1. How can DNA hybridisation give a measure of genetic relatedness between species?

2. Why do the double strands of DNA break when they are heated?

3. What is responsible for the hybridisation between the DNA strands?

4. The graph below shows the results of a DNA hybridisation between humans and other primates.



(a) Which primate is most closely related to humans?

(b) Which primate is most distantly related to humans?

5. Hybrid DNA from species A and B comes apart at a lower temperature that of species A and C. Which species is A most closely related to?

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137 Genomic Comparisons and Relatedness

Key Idea: Comparing nucleotide sequences in DNA provides detailed information about relatedness between organisms.

DNA sequencing provides the precise order of nucleotides in a DNA molecule. This information, which can now be analysed using sophisticated computing, allows researchers to compare sequences between species in much more detail than is possible with DNA hybridisation. Not only can areas of difference be identified, but the variation between the nucleotides at a certain position can be determined. This information allows researchers to more accurately determine the relatedness between species, even between those with very minor differences.

Comparing DNA sequences

Improved DNA sequencing techniques and powerful computing software have allowed researchers to accurately and quickly sequence and compare entire genomes (all an organism's genetic material) within and between species.

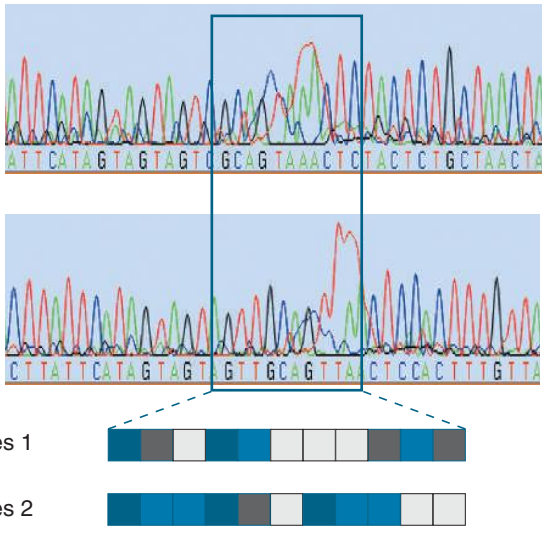
Once DNA sequences have been determined, they are aligned and compared to see where the differences occur (right). DNA sequencing generates large volumes of data and the rise in computing power has been central to modern sequence analyses. The technological advances have been behind the new field of bioinformatics, which uses computer science, statistics, mathematics, and engineering to analyse and interpret biological data.



DNA: Species 1



DNA: Species 2



What type of sequences are compared?

Highly conserved sequences are often used for comparative genomic analysis because they are found in many organisms. The changes (mutations) of the sequences over time can be used to determine evolutionary relationships. As with other forms of molecular analysis, species with fewer nucleotide differences are more closely related than those with many.

Whole genome analysis has been important in classifying the primates. Historical views attributed special status to humans which often confused primate classification schemes. DNA evidence provides impartial quantitative evidence and modern classification schemes have been based on this data.



Based on DNA evidence, chimpanzees are more closely related to humans than they are to gorillas and there is no taxon called "great apes".

1. (a) What advantages does DNA sequence comparison have over DNA hybridisation? _____

_____

_____

(b) How is this an advantage in determining evolutionary relationships? _____

_____

_____

2. Three partial DNA sequences for three different species are presented below.

Species 1	A T G G C C C C C A A C A T T C G A A A A T C G C A C C C C C T G C T C A A A A T T A T C A A C
Species 2	A T G G C A C C T A A C A T C C C C A A C T C C C A C C G T G T A C T C A A A A T C A T C A A G
Species 3	A T G G C A C C C A A T A T C C G C A A A T C A C A C C C C C T G T T A A A A A C A A T C A A C

Based on the number of differences in the DNA sequences:

(a) Identify the two species most closely related: _____

(b) Identify the two species that are least closely related: _____

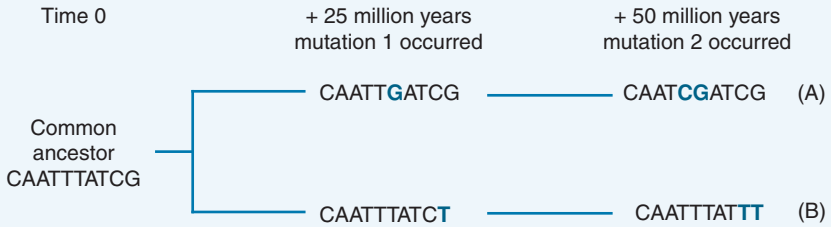
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138 The Molecular Clock Theory

Key Idea: The molecular clock hypothesis proposes that mutations occur at a steady rate and that changes in DNA sequences between species can determine phylogeny.

The molecular clock hypothesis states that mutations occur at a relatively constant rate for any given gene. The genetic difference between any two species can indicate when two species last shared a common ancestor and can be used to construct a phylogenetic tree. The molecular clock for each species, and each protein, may run at different rates, so molecular clock data is calibrated with other evidence (e.g. morphological) to confirm phylogeny. Molecular clock calculations are carried out on DNA or amino acid sequences.

In a theoretical example, the DNA sequence for a gene in two species (A & B, right) alive today differs by four bases. The mutation rate for the gene is approximately one base per 25 million years. Based on this rate, it can be determined that the common ancestor for these two species lived 50 mya.

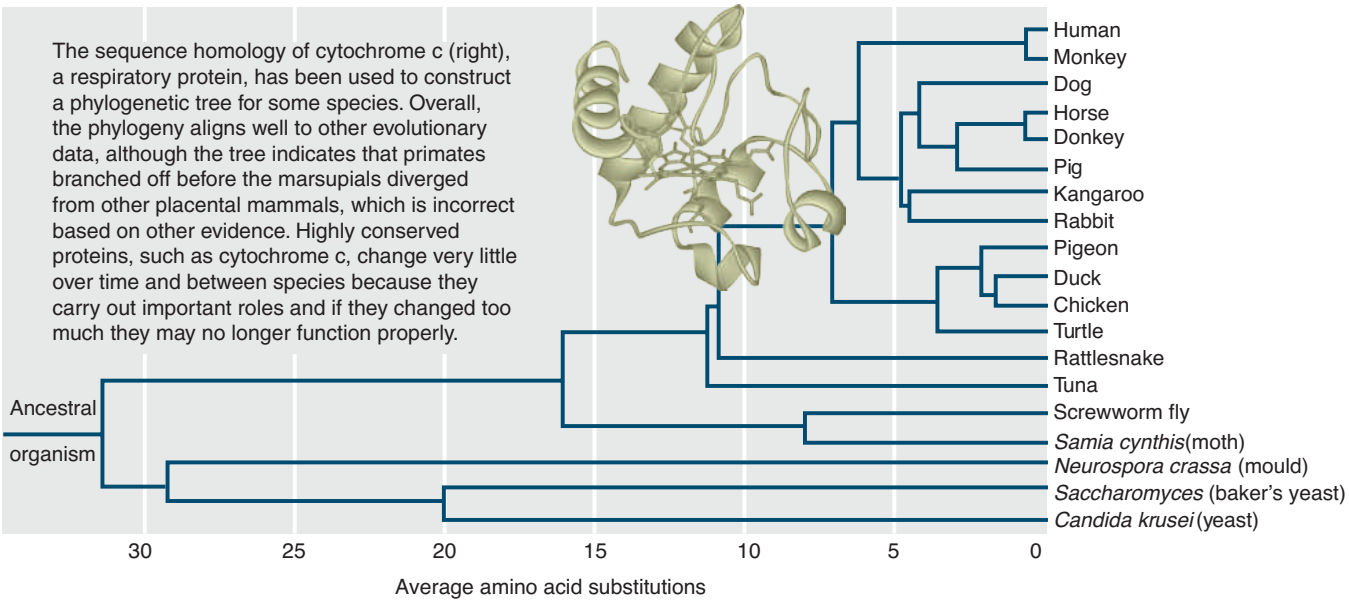


Cytochrome *c* and the molecular clock theory

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Human		Gly	Asp	Val	Glu	Lys	Gly	Lys	Lys	Ile	Phe	Ile	Met	Lys	Cys	Ser	Gln	Cys	His	Thr	Val	Glu	Lys
Pig												Val	Gln			Ala							
Chicken				Ile						Val		Val	Gln			Ala							
Dogfish										Val		Val	Gln			Ala							Asn
Drosophila	<<									Leu		Val	Gln	Arg		Ala							Ala
Wheat	<<		Asn	Pro	Asp	Ala		Ala				Lys	Thr	Arg		Ala						Asp	Ala
Yeast	<<		Ser	Ala	Lys			Ala	Thr	Leu		Lys	Thr	Arg		Glu	Leu						

This table shows the N-terminal 22 amino acid residues of human cytochrome c, with corresponding sequences from other organisms aligned beneath. Sequences are aligned to give the most position matches. A shaded square indicates no change. In every case, the cytochrome's heme group is attached to the Cys-14 and Cys-17. In *Drosophila*, wheat, and yeast, arrows indicate that several amino acids precede the sequence shown.

The sequence homology of cytochrome c (right), a respiratory protein, has been used to construct a phylogenetic tree for some species. Overall, the phylogeny aligns well to other evolutionary data, although the tree indicates that primates branched off before the marsupials diverged from other placental mammals, which is incorrect based on other evidence. Highly conserved proteins, such as cytochrome c, change very little over time and between species because they carry out important roles and if they changed too much they may no longer function properly.



1. How can using molecular clocks help to establish evolutionary relationships (phylogenies) between organisms?

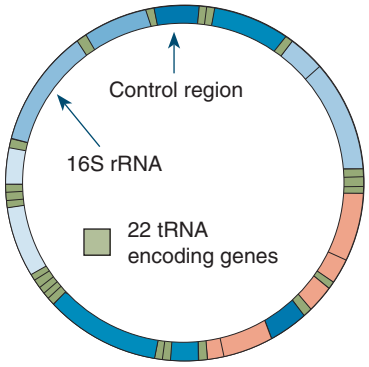
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139 Using Mitochondrial DNA

Key Idea: Mitochondrial DNA (mtDNA) can be used to determine relationships between closely related species.

Mitochondrial DNA (mtDNA) is a single circular piece of DNA found in the mitochondria of eukaryotic organisms. mtDNA mutates at a much higher rate than nuclear DNA and it is inherited, without the usual genetic recombination*, only from the mother (except in extremely rare occurrences). Thus mtDNA mutations are passed on 100% of the time from mother to all her offspring. These two features make mtDNA useful for determining relationships between closely related species or individuals within a species, and for following maternal lineages through time.

The length of the mtDNA can vary between eukaryotes. The human mitochondrial genome contains about 16 kilobases. It encodes 37 genes, 22 of them being for mitochondrial tRNA. Genes that are commonly compared are the 16S rRNA gene and the control region of the mtDNA.



Human mtDNA

Identifying species using mtDNA

Transatlantic mangrove oysters of the genus *Crassostrea* are important commercial oysters on the Atlantic shores of South America and Africa. mtDNA studies have found that the division of this genus into distinct South American and African species (the African *C. gasar* and the South American *C. rhizophorae*) may be incorrect. A study sequenced a 570 base-pair length of mtDNA from the 16S rRNA gene of 18 individuals from nine locations along the African and South American coastline. The study found two distinct DNA sequences. Bases 1 - 60 are shown below:

```
A TTGATTTTTAGTAGTACCTGCCCAGTGCG-TATTATCTTGTTAACGGCCGCCTT
B ..........C..................A.....AG.C..C............
```

In was presumed that the mangrove oyster *C. gasar* was found only on the African side of the Atlantic while *C. rhizophorae* was found only in South America. All samples from the African coastline had the same sequence as A above. However the South American sample was found to have both A and B DNA sequences, showing that *C. gasar* is also present in South America.

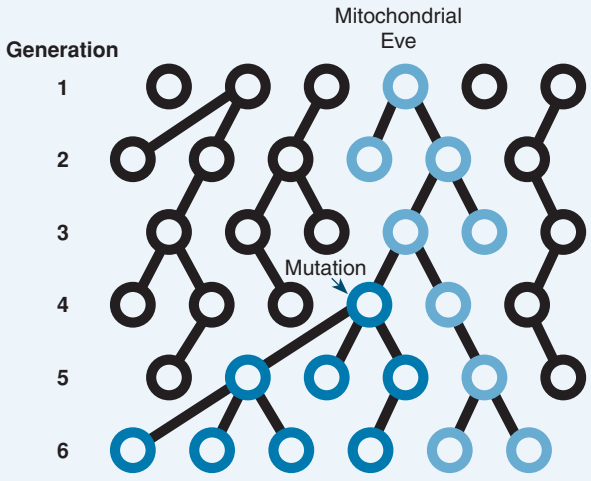


C. gasar on mangrove roots

Using mtDNA to trace human ancestry

Because mtDNA is passed through the maternal line without the usual genetic recombination* it can be used to trace maternal lineage. Barring new mutations, the mtDNA of any one person is the same as their direct maternal ancestor back many generations.

In humans, this concept has been used to trace the most-recent common mitochondrial ancestor of all humans, a single female from Africa (dubbed Mitochondrial Eve or ME). The ME represents that woman whose mitochondrial DNA (with mutations) exists in all humans alive today. Mutations to the mtDNA provide the molecular clock that allows us to determine how much time has elapsed since the ME lived. The existence of a ME does not mean that no other women have left descendents. At some point, these other females must have produced no daughters themselves and so broke the mitochondrial line (right).



**Although mtDNA does recombine, it does so with copies of itself within the same mitochondrion.*

1. (a) How does mtDNA differ from nuclear DNA? _____

   _____

   (b) Why is mtDNA useful for following maternal lineages? _____

   _____

   _____

2. Why is mtDNA useful for determining relationships between closely related species: _____

   _____

   _____

   _____

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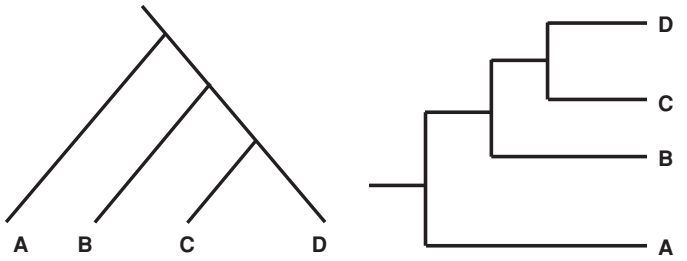
140 What is a Phylogenetic Tree?

Key Idea: Phylogenetic trees represent possible evolutionary histories between organisms. They can be constructed based on appearance or molecular data.

Phylogenetics is the study of the evolutionary history and the relationships among individuals or groups of organisms. These relationships are often shown diagrammatically as a phylogenetic tree. A **phylogenetic tree** represents a likely hypothesis of the evolutionary relationships between biological groups or taxa (*sing*. taxon). A taxon may consist of individual species or be a larger group (e.g. an order). Traditionally phylogenetic trees have been constructed based on similarities or differences in appearance, but in more recent times molecular comparisons have been used. Molecular phylogenetics can reveal differences not seen in morphological comparisons, and have resulted in the revision of some morphological phylogenies where organisms could not be separated on appearance. Phylogenetic trees are often constructed based on cladistics (below).

What do phylogenetic trees look like?

Phylogenetic trees represent possible evolutionary histories, and there are many different ways they can be drawn (right). Depending on how the tree is constructed, some represent evolutionary time through the length of the branches (lines). Phylogenetic trees based on cladistics (cladograms) do not represent evolutionary time.

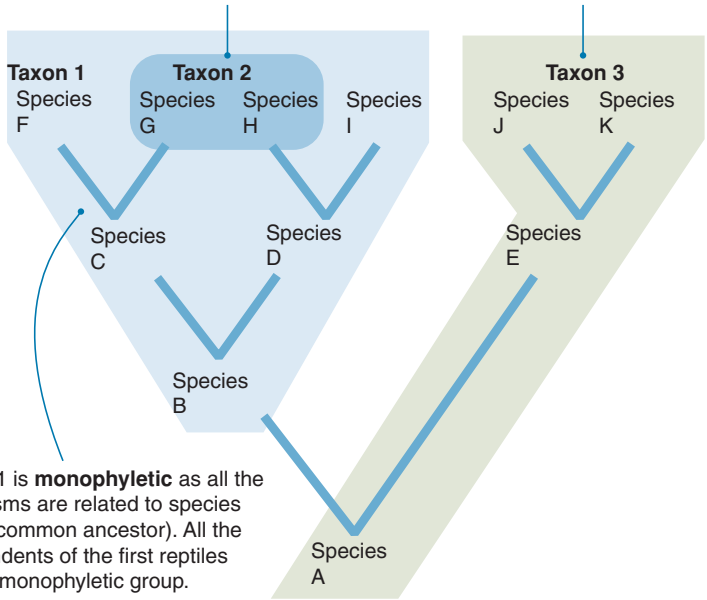


Determining phylogenetic relationships

- Increasingly, analyses to determine evolutionary relationships rely on cladistic analyses of character states. Cladistic analysis groups species according to their most recent common ancestor on the basis of shared derived characteristics or synapomorphies. All other characters are ignored.
- A phylogeny constructed using cladistics thus includes only monophyletic groups, i.e. the common ancestor and all of its descendents. It excludes both paraphyletic and polyphyletic groups (right). It is important to understand these terms when constructing cladograms and to also understand that the terms are relative to whereever you start in the phylogenetic tree (i.e. where the common ancestor is).
- The cladist restriction to using only synapomorphies creates an unambiguous branching tree. One problem with this approach is that a strictly cladistic classification could theoretically have an impractically large number of taxonomic levels and may be incompatible with a Linnaean system.

Taxon 2 is **polyphyletic** as it includes organisms with different ancestors. The group "warm-blooded (endothermic) animals" is polyphyletic as it includes birds and mammals.

Taxon 3 is **paraphyletic**. It includes species A without including all of A's descendents. The traditional grouping of reptiles is paraphyletic because it does not include birds.



Taxon 1 is **monophyletic** as all the organisms are related to species B (the common ancestor). All the descendents of the first reptiles form a monophyletic group.

1. What does a phylogenetic tree show? _____

2. Why might a phylogenetic tree based on molecular differences be preferred over phylogenies based on appearance?

3. Suggest why it is useful to construct phylogenetic trees that are monophyletic: _____

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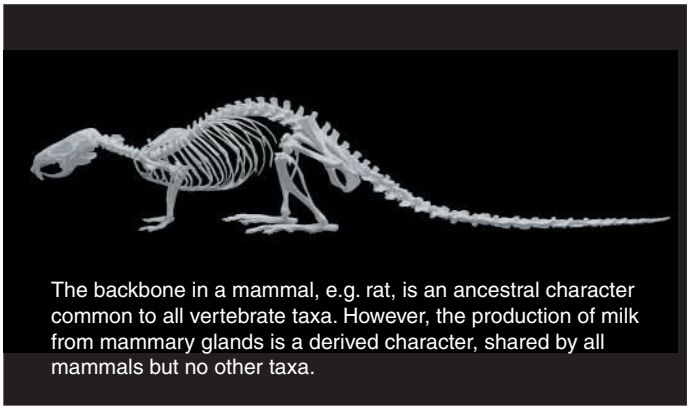
141 Constructing Phylogenies Using Cladistics

Key Idea: Cladograms are phylogenetic trees constructed on the basis of shared derived characteristics.

A **cladogram** is a phylogenetic tree constructed using a taxonomic tool called cladistics. Cladistics groups organisms on the basis of their shared derived characters (features arising in an ancestor and shared by all its descendents) and ignores features that are not the result of shared ancestry. A clade, or branch on the tree, includes a common ancestor and all its descendents (i.e. it is monophyletic). Increasingly, cladistic methods rely on molecular data (e.g. DNA sequences) to determine phylogenies. Highly conserved DNA sequences are used because changes are likely to signal a significant evolutionary divergence. Cladograms may not always agree completely with phylogenies constructed using traditional methods but similarities in the trees indicate that the proposed relationships are likely to be correct.

Derived vs ancestral characters

When constructing cladograms, shared derived characters are used to separate the clades (branches on the tree). Using ancestral characters (those that arise in a species that is ancestral to more than one group) would result in distantly related organisms being grouped together and would not help to determine the evolutionary relationships within a clade. Whether or not a character is derived depends on the taxonomic level being considered. For example, a backbone is an ancestral character for mammals, but a derived character for vertebrates. Production of milk is a derived character shared by all mammals but no other taxa.

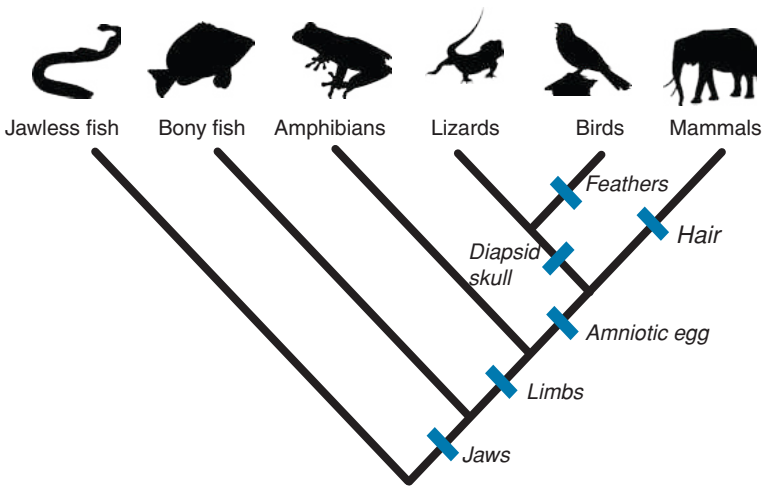


The backbone in a mammal, e.g. rat, is an ancestral character common to all vertebrate taxa. However, the production of milk from mammary glands is a derived character, shared by all mammals but no other taxa.

Constructing a simple cladogram

- A table listing the features for comparison allows us to identify where we should make branches in the cladogram. An outgroup (one which is known to have no or little relationship to the other organisms) is used as a basis for comparison.
- The table (right) lists features shared by selected taxa. The outgroup (jawless fish) shares just one feature (vertebral column), so it gives a reference for comparison and the first branch of the cladogram. As the number of taxa in the table increases, the number of possible trees that could be drawn increases exponentially.
- Several different cladograms can be constructed from the same data. To determine the most likely relationships, the rule of **parsimony** is used. Parsimony assumes that the tree with the simplest explanation (the least number of evolutionary events) is most likely to show the correct evolutionary relationship.
- A possible cladogram for the data in the table is shown on the right. Its construction assumed that six evolutionary events took place (labelled as blue bars on the cladogram). If other cladograms were constructed, but involved more evolutionary events, the one shown would be assumed to be correct because it is the most parsimonious.
- Parsimony can lead to some confusion. Some evolutionary events have occurred multiple times. An example is the evolution of the four chambered heart, which occurred separately in both birds and mammals. The use of fossil evidence and DNA analysis can help to solve problems like this.

	Taxa					
Comparative features	Jawless fish (outgroup)	Bony fish	Amphibians	Lizards	Birds	Mammals
Vertebral column	✔	✔	✔	✔	✔	✔
Jaws	✘	✔	✔	✔	✔	✔
Four supporting limbs	✘	✘	✔	✔	✔	✔
Amniotic egg	✘	✘	✘	✔	✔	✔
Diapsid skull	✘	✘	✘	✔	✔	✘
Feathers	✘	✘	✘	✘	✔	✘
Hair	✘	✘	✘	✘	✘	✔



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1. (a) Distinguish between a shared derived characteristic and a shared ancestral characteristic: _____

(b) Why are ancestral characteristics not useful in constructing evolutionary histories? _____

2. What assumption is made when applying the rule of parsimony in constructing a cladogram? _____

3. Two possible phylogenetic trees constructed from the same character table are shown below. The numbers next to a blue bar represent an evolutionary event.

(a) Which tree is more likely to be correct?

_____

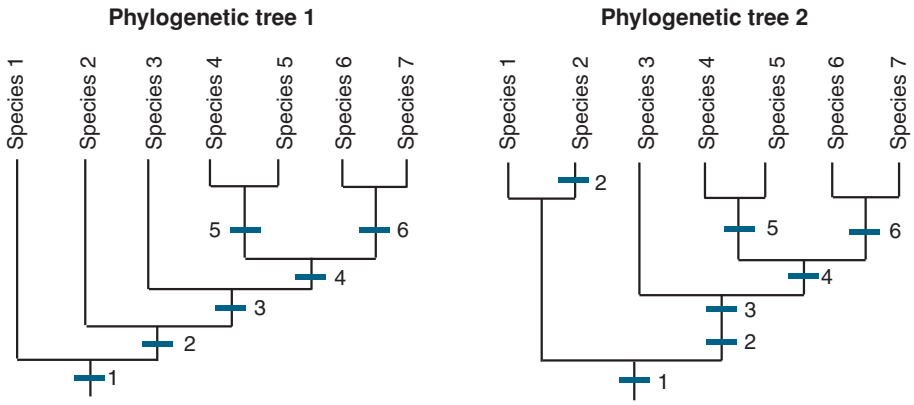
(b) State your reason:

_____

_____

_____

_____



(c) Identify the event which has occurred twice in phylogenetic tree 2: _____

4. A phylogenetic tree is a hypothesis for an evolutionary history. How could you test it? _____

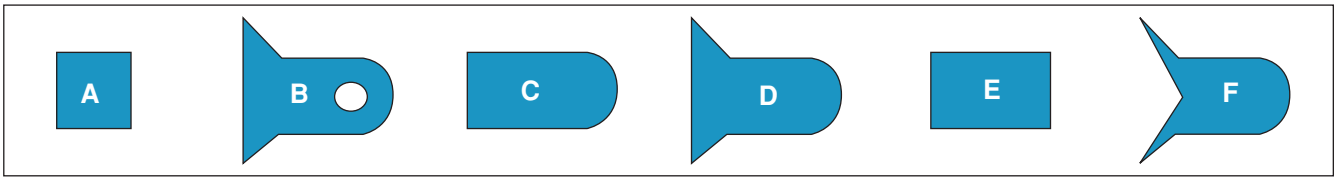
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_____

_____

_____

5. Use the shapes below to construct a cladogram that shows their phylogenetic relationships (hint: A is the outgroup).



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142 Constructing a Cladogram

Key Idea: A table of selected characteristics can be organised systematically as a matrix and used to construct a cladogram.

Taxon	Character 1	2	3	4	5	6	7	8	9	10	11	12	13
Zebra-perch sea chub	0	0	0	0	0	0	0	0	0	0	0	0	0
Barred surfperch	1	0	0	0	0	0	0	0	0	1	1	0	0
Walleye surfperch	1	0	0	0	0	1	0	1	0	1	1	0	0
Black perch	1	1	1	0	0	0	0	0	0	0	0	1	0
Rainbow seaperch	1	1	1	0	0	0	0	0	0	0	0	1	0
Rubberlip surfperch	1	1	1	1	1	0	0	0	0	0	0	0	1
Pile surfperch	1	1	1	1	1	0	0	0	0	0	0	0	1
White seaperch	1	1	1	1	1	0	0	0	0	0	0	0	0
Shiner perch	1	1	1	1	1	1	0	0	0	0	0	0	0
Pink seaperch	1	1	1	1	1	1	1	1	0	0	0	0	0
Kelp perch	1	1	1	1	1	1	1	1	1	0	0	0	0
Reef perch	1	1	1	1	1	1	1	1	1	0	0	0	0



Steve Lonhart (SIMoN / MBNMS) PD NOAA

Surfperches are viviparous (live bearing) and the females give birth to relatively well developed young. Some of the characters (below, left) relate to adaptations of the male for internal fertilisation. Others relate to deterring or detecting predators. In the matrix, characters are assigned a 0 or 1 depending on whether they represent the ancestral (0) or derived (1) state. This coding is common in cladistics because it allows the data to be analysed by computer.

Selected characters for cladogram assembly

1.	Viviparity (live bearing)	0 No	1 Yes
2.	Males with flask organ	0 No	1 Yes
3.	Orbit without bony front wall	0 Yes	1 No
4.	Tail length	0 Short	1 Long
5.	Body depth	0 Deep	1 Narrow
6.	Body size	0 Large	1 Small
7.	Length of dorsal fin base	0 Long	1 Short
8.	Eye diameter	0 Moderate	1 Large
9	Males with anal crescent	0 No	1 Yes
10	Pectoral bone with process	0 No	1 Yes
11.	Length of dorsal sheath	0 Long	1 Short
12.	Body mostly darkish	0 No	1 Yes
13.	Flanks with large black bars	0 No	1 Yes

Notes and working space

1. This activity provides the taxa and character matrix for 11 genera of marine fishes in the family of surfperches. The outgroup given is a representative of a sister family of rudderfishes (zebra-perch sea chub), which are not live-bearing. Your task is to create the most parsimonious cladogram from the matrix of character states provided. To help you, we have organised the matrix with genera having the smallest blocks of derived character states (1) at the top following the outgroup representative. Use a separate sheet of graph paper, working from left to right to assemble your cladogram.

   Identify the origin of derived character states with horizontal bars, as shown in the previous activity. CLUE: You should end up with 15 steps. Two derived character states arise twice independently. Staple your cladogram to this page.

2. (a) Why are the character states organised in a matrix? _____

   _____

   (b) Why is it useful to designate the characters states as 0 (ancestral) or derived (1)?_____

   _____

3. In the cladogram you have constructed for the surfperches, two characters have evolved twice independently:

   (a) Identify these two characters: _____

   (b) What selection pressures do you think might have been important in the evolution of these two derived states?

   _____

   _____

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143 Determining the Relatedness of Whales

Key Idea: The evolutionary relationships of whales can be determined through the fossil record and analysis of DNA.

The evolution of modern whales from an ancestral land mammal is well documented in the fossil record. The fossil record of whales includes many transitional forms, which has enabled scientists to develop an excellent model of whale evolution. The evolution of the whales shows a gradual accumulation of adaptive features that have equipped them for life in the open ocean. The fossil evidence is well supported by the molecular evidence. DNA analysis has played an important role in determining the origin of whales and their transition from a terrestrial (land) form to a fully aquatic form. The evidence shows hippopotamuses to be the closest living ancestor of whales. Both whales and hippos evolved from a family of water-loving extinct, even-toed hoofed mammals (ungulates) more than 50 million years ago. The toothed and baleen whales are thought to have diverged some 28-33 million years ago.

50 mya *Pakicetus*

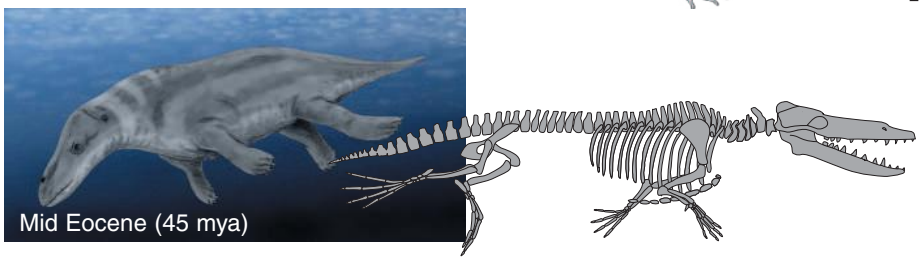
Pakicetus was a transitional species between carnivorous land mammals and the earliest true whales. It was mainly land dwelling, but foraged for food in water. It had four, long limbs. Its eyes were near the top of the head and its nostrils were at the end of the snout. It had external ears, but they showed features of both terrestrial mammals and fully aquatic mammals.



Kevin Guertin cc 2.0

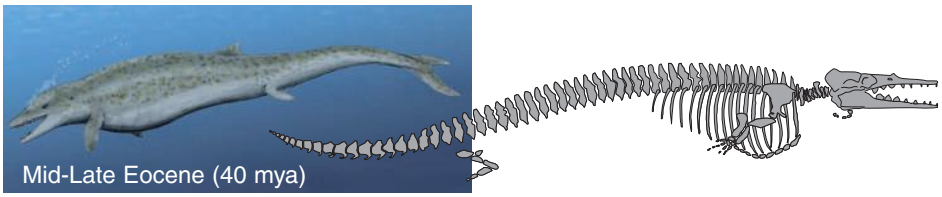
45 mya *Rhodocetus*

Rhodocetus was mainly aquatic (water living). It had adaptations for swimming, including shorter legs and a shorter tail. Its eyes had moved to the side of the skull, and the nostrils were located further up the skull. The ear showed specialisations for hearing in water.



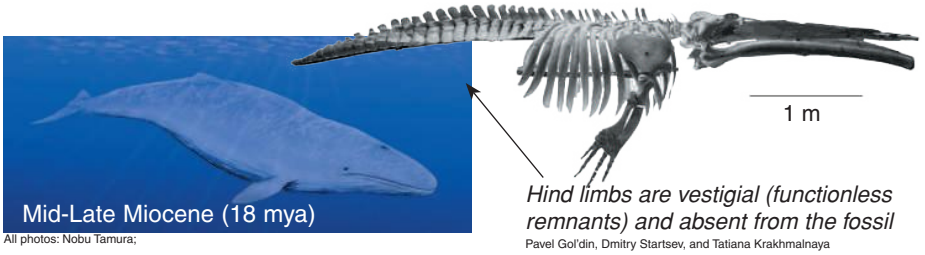
40 mya *Dorudon*

Dorudon was fully aquatic. Its adaptations for swimming included a long, streamlined body, a broad powerful muscular tail, the development of flippers and webbing. It had very small hind limbs (not attached to the spine) which would no longer bear weight on land.



Balaena (recent whale ancestor)

The hind limbs became fully internal and vestigial. Studies of modern whales show that limb development begins, but is arrested at the limb bud stage. The nostrils became modified as blowholes. This recent ancestor to modern whales diverged into two groups (below) about 36 million years ago.



All photos: Nobu Tamura;
Pavel Gol'din, Dmitry Startsev, and Tatiana Krakhmalnaya

Modern whales are categorised into two broad suborders based on the presence or absence of teeth.

- **Toothed whales**: These have full sets of teeth throughout their lives. Examples: sperm whale and orca.
- **Baleen whales**: Toothless whales, which have a comb-like structure (baleen) in the jaw. Baleen is composed of the protein keratin and is used to filter food from the water. Baleen whales have teeth in their early fetal stage, but lose them before birth. Examples: blue whale, humpback whale.



Robert Pittman - NOAA



1. Why does the whale fossil record provide a good example of the evolutionary process? _____

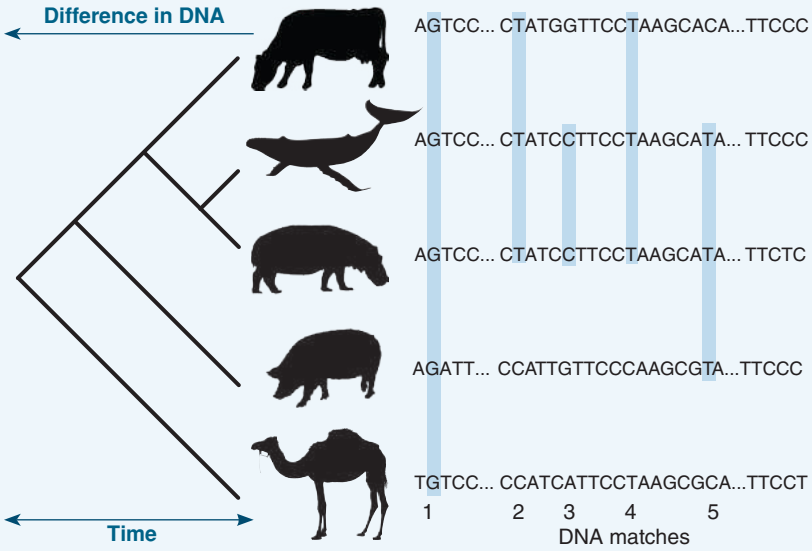
_____

_____

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Using DNA data

- The ancestry of whales has been in debate since Darwin. The radically different morphologies of whales and other mammals makes it difficult work out the correct phylogenetic tree.
- Recently discovered fossil ankle bones, as well as DNA studies, show whales are more closely related to hippopotami than to any other mammal. Coupled with molecular clocks, DNA data can also give the time between each split in the lineage.
- The DNA sequences on the right show part of a the nucleotide subset 141-200 and some of the matching nucleotides used to draw the cladogram. Although whales were once thought most closely related to pigs, based on the DNA analysis the most parsimonious tree disputes this.



2. (a) What adaptations for an aquatic life have evolved in whales over time? _____

(b) What features of the environment do you think would have been involved in the evolution of these adaptations?

3. (a) How does the DNA evidence show hippos are the closest related mammalian group to whales?

(b) How is this confirmed by fossil evidence? _____

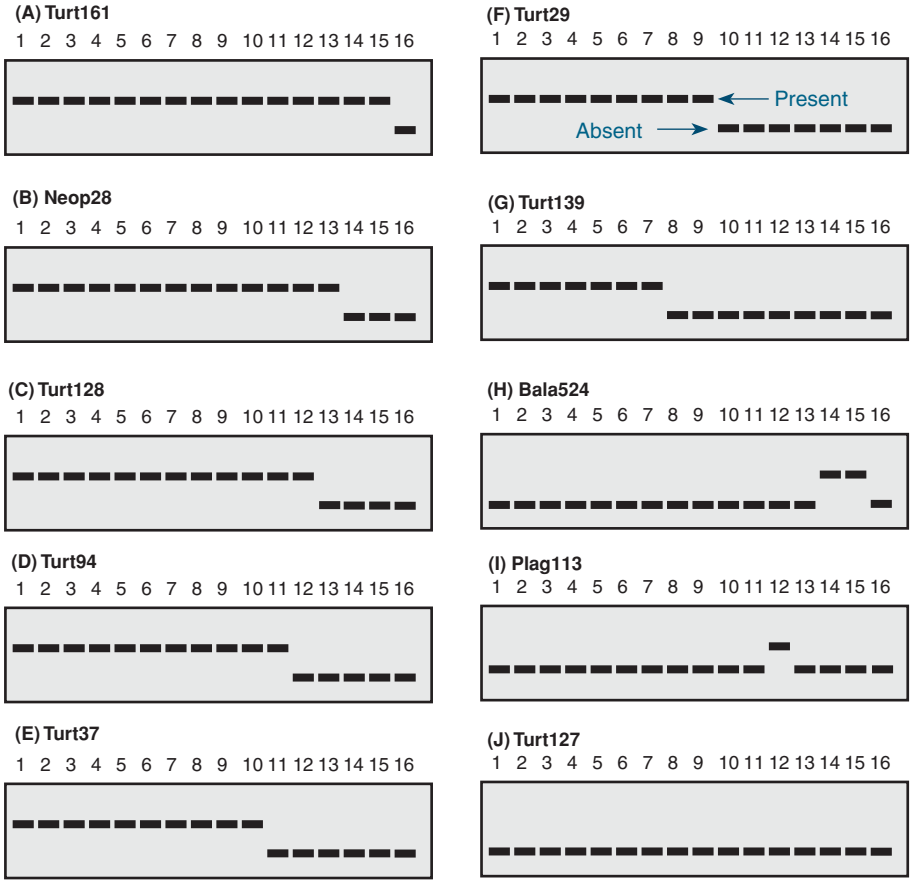
4. Thinking about the size and feeding habits of whales, there are two distinct ways the whales could be placed into related groups. What are these two possible ways of grouping whales and how might you determine which of the grouping is evolutionarily most correct?

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Determining relationships between whales and dolphins

- On the right are DNA profiles for ten Short INterspersed Elements (SINE) in the whale genome (A-J). The profiles simply show if a SINE is present or not. Study of the presence or absence of SINEs can be used to construct a phylogeny. 15 whale (and dolphin) species and the hippopotamus where profiled at each of the ten sites. The whale species are listed below (1-16).
- Note that a present SINE shows as a line towards the top of the profile and an absent SINE shows as a line towards the bottom of the profile.

1 Striped dolphin
2 Risso's dolphin
3 Indo-Pacific bottlenose dolphin
4 Common bottlenose dolphin
5 Long-beaked common dolphin
6 Chinese white dolphin
7 Pantropical spotted dolphin
8 Beluga
9 Finless porpoise
10 Yangtze River dolphin
11 Ginkgo-toothed beaked whale
12 Ganges River dolphin
13 Pygmy sperm whale
14 Omura's whale
15 Common minke whale
16 Hippopotamus



Source: Zhuo Chen *et al* (2011), see credits for the full reference

5. Working in pairs, use the DNA profiles to complete the table below:

6. Next, use the table to produce a cladogram of whale species:

SINE present (1)/absent (0)										
Taxon	A	B	C	D	E	F	G	H	I	J
Striped dolphin										
Risso's dolphin										
Indo-Pacific bottlenose dolphin										
Common bottlenose dolphin										
Long-beaked common dolphin										
Chinese white dolphin										
Pantropical spotted dolphin										
Beluga										
Finless porpoise										
Yangtze River dolphin										
Ginkgo-toothed beaked whale										
Ganges River dolphin										
Pygmy sperm whale										
Omura's whale										
Common minke whale										
Hippopotamus										

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144 Chapter Review: Did You Get It?

1. Test your vocabulary by matching each term to its correct definition, as identified by its preceding letter code.

common ancestor .....

cladogram .....

DNA hybridisation .....

molecular clock .....

mtDNA .....

phylogenetic tree .....

phylogeny .....

A A technique in molecular evolution that uses molecular change to deduce the time in geologic history when two species or other taxa diverged. Can be used to establish phylogenies.

B The evolutionary history or genealogy of a group of organisms.

C A diagram showing the evolutionary history of a group of organisms.

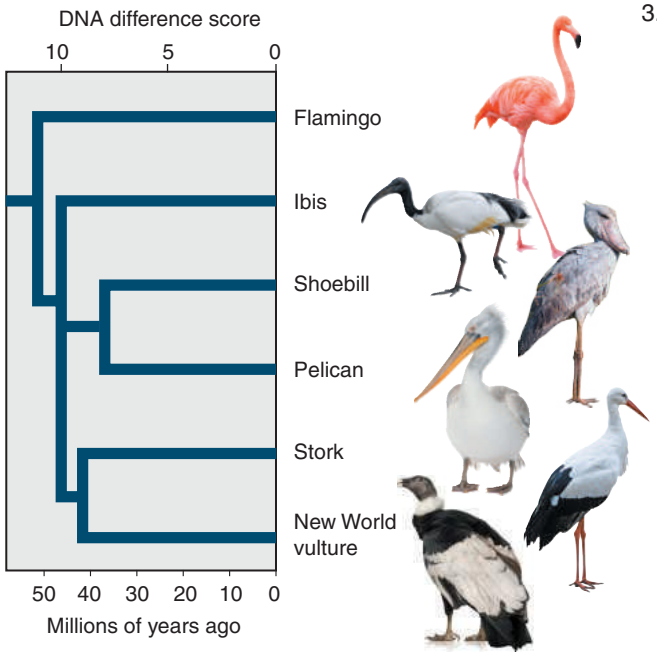
D A technique used to determine the percentage similarity between the DNA of two organisms.

E A type of phylogenetic tree that groups organisms based on their shared derived characteristics.

F DNA located in mitochondria.

G The individual from which all organisms in a taxon are directly descended.

2. Compare and contrast DNA hybridisation and DNA sequence comparison as methods for generating phylogenies:



3. The diagram left shows the evolutionary relationship of a group of birds based on DNA similarities:

(a) Place an X to the last common ancestor of all the birds:

(b) How many years ago did storks diverge from vultures?

(c) What are the most closely related birds? _____

(d) What is the difference in DNA (score) between:

i: Storks and vultures: _____

ii: Ibises and shoebills: _____

(e) Which of the birds is the least related to vultures?

4. The dewclaws found on many mammals that have evolved for running are vestigial structures. The photo (right) show the dewclaws on a deer.

What are vestigial structures and why are dewclaws an example?



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CHAPTER 13

Human Change Over Time

Key terms

A. afarensis
Ardipithecus ramidus
Australopithecus spp.
bipedal (bipedalism)
Broca's area
cultural evolution
Denisovan
gracile
hominin
hominoid
Homo
H. erectus
H. floresiensis
H. habilis
H. neanderthalensis
H. sapiens
mtDNA
primate
Palaeolithic
prehensile
prognathic
robust
valgus angle
Wernicke's area

Humans as primates

Key skills and knowledge

Activity number

☐	1 Identify primates as mammals. Appreciate that the classification of the primate order has had several revisions in recent decades to accommodate new genetic evidence.	145
☐	2 Describe the shared characteristics that define primates, hominoids, and hominins. What features distinguish hominoids and hominins?	146-148

Trends in hominin evolution

Key skills and knowledge

☐	3 Describe the evidence for the major trends in hominin evolution from the genus *Australopithecus* to the genus *Homo*. Include reference to changes in brain size and limb structure and their significance to cultural evolution. Include:	149-160 167
☐	i The distinguishing characteristics of genus *Australopithecus* and the significance of *A. afarensis* (Lucy).	149-152
☐	ii The distinguishing characteristics of genus *Homo* and the biological and cultural evolution of species of *Homo* that illustrate the trend (*H. habilis*, *H. erectus*, *H. neanderthalensis*, *H. sapiens*).	149-152
☐	4 Relate the evolution of hominins to changes in habitat and a shift in resources.	149 153
☐	5 Describe the selection pressures on early hominins and the benefits of reducing body hair and adopting bipedalism as a form of locomotion.	153 154







Interpreting the human fossil record

Key skills and knowledge

☐	6 Recognise the human fossil record as an example to illustrate why and how classifications can be questioned, refined, and reevaluated, to include at least i and ii of the following examples (iii-v optional):	160
☐	i The significance of the Denisova hominin to our understanding of human lineages and the genetics of modern populations.	162
☐	ii The evidence for interbreeding between *Homo sapiens* and Neanderthals.	163
☐	iii The importance of *Ardipithecus* to our understanding of early hominin evolution.	161
☐	iv The implications of the fossils on the island of Flores (*H. floresiensis*)	164
☐	v The significance of finds that are not easily dated (the Rising Star hominin, *H. naledi*).	165

The evidence for human dispersal

Key skills and knowledge

☐	7 Describe ways of using fossil and DNA evidence (mtDNA and whole genomes) to explain the migration of human populations around the world. What techniques can be used in the absence of fossil evidence and how can these techniques be evaluated?	162-166 168
☐	8 Describe the pattern of migration that led to the populating of Australia, including the Torres Strait Islands. Aboriginal and Torres Strait Island populations have a long history with the land. Describe the fossil and DNA evidence for their long occupation and connection to Country and Place.	169

145 Primate Classification

Key Idea: Humans are primates, with genetic evidence showing chimpanzees to be our closest living relatives.

Primates are an order of rather generalised mammals. Like other mammals they possess fur, produce milk, and give birth to live young. Primates have several distinctive features including a proportionally large brain and stereoscopic colour vision. The classification of primates has been considerably revised several times in the last few decades. Two primate suborders are recognised: the Strepsirrhini (lemurs and lorises) and the Haplorhini (tarsiers, monkeys, and apes (including humans). Based on genetic evidence, chimpanzees and gorillas are more closely related to humans than to orangutans, and chimpanzees are more closely related to humans than they are to gorillas. Within the Haplorhini are the Hominoidea (apes). Apes differ from other primates in that they have no tail and a highly mobile shoulder joint.

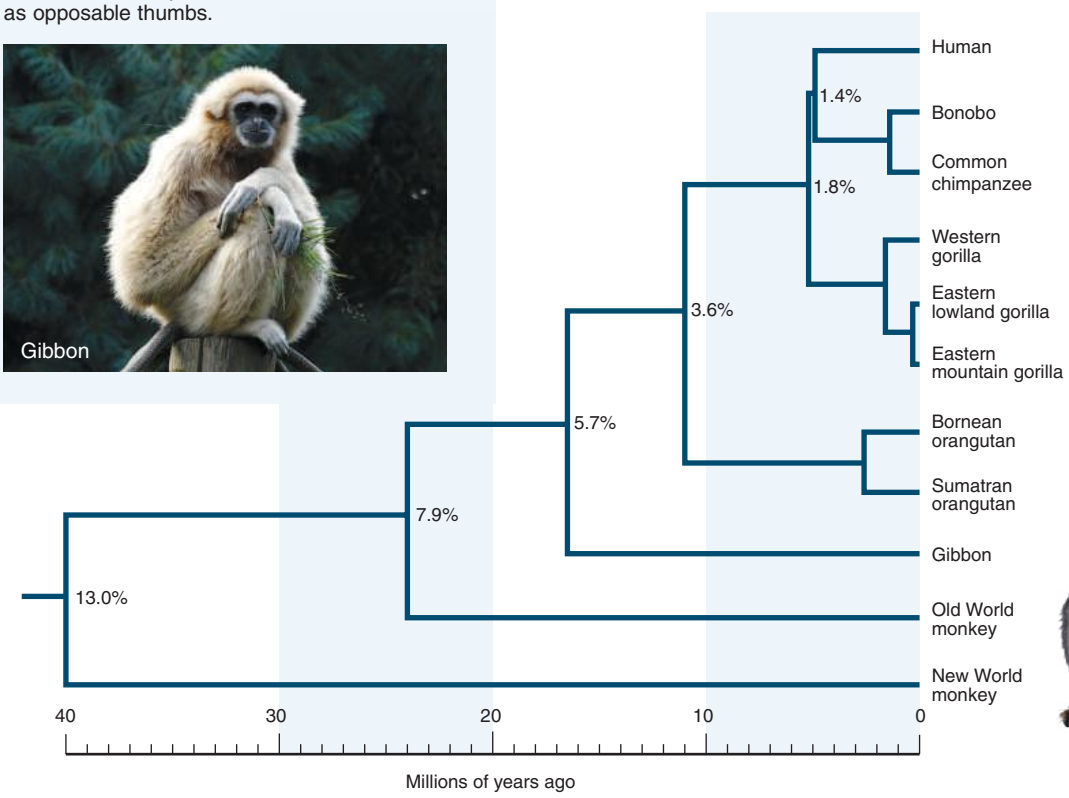
Mammals vs primates

- Primates are mammals and share mammalian characteristics of having hair, producing milk, giving birth to live young, and having three middle ear bones.
- Primate brains are (generally) proportionally much larger than other mammals, with development in the visual processing region of the brain (rather than olfactory (smell) as in most other mammals).
- While other terrestrial mammals tend to have claws or hooves, primates have nails has well as opposable thumbs.

Gibbon

Phylogenetic tree of the anthropoids

The diagram below presents a classification of the anthropoids (the hominoids and monkeys) based on genetic differences. The percentages next to each of the points where a split occurs indicates the amount of difference in the total genetic makeup (genomes) of the two groups being considered, e.g. the genome of the gibbons compared to the rest of the apes (orangutans, gorillas, chimpanzees) and humans differs by 5.7%. A large genetic difference between any two groups implies that they are distantly related, whereas small genetic differences suggest they share a recent common ancestor.




Chimpanzee


Vervet monkey

1. According to the diagram above, which shows relatedness according to genetic similarity:

   (a) Which primate group is most closely related to the two chimpanzee species? _____

   (b) Name the two chimpanzee species: _____

2. From the diagram, determine how long ago:

   (a) The two species of chimpanzee split from a common ancestor: _____

   (b) The chimpanzees split from the line to humans: _____

   (c) The African apes (and humans) split from the Asian apes (orangutans and gibbons): _____

   (d) The African (Old World) monkeys split from the American (New World) monkeys: _____

146 General Primate Characteristics

Key Idea: Primates exhibit unique, but quite generalised, morphological, physiological, and behavioural features.

Important characteristics of primates include five digits on the hands and feet and retention of the collar bone, a longer gestation than other mammals, and prolonged infant dependency and complex social behaviour.

The **gestation** (pregnancy period) in primates is longer than most other mammals. Primates typically have one young per pregnancy (below). Infancy is prolonged with longer periods of infant dependency and a large **parental investment** in each offspring. This nurturing increases the survival rate of the young and allows cultural development.


Langur


Chacma baboon


Ring-tailed lemurs

Chris Gin

Primates generally live longer relative to most other mammals and there is a greater dependency on highly flexible learned behaviour. Primates tend to be **highly sociable** (above). Unusually for mammals, adult males of many primate species often associate permanently with the group.


Chimpanzees

Chimpanzees and **gorillas** spend more time out of trees than do either of the Asian apes. The chimpanzee above shows typical **knuckle-walking** behaviour. The relatively long arms facilitate this mode of locomotion.



The **brain** is large and generally more complex than in other mammals.

Vision is important, the visual areas of the brain are enhanced. Well developed binocular, stereoscopic vision provides overlapping visual fields and good depth perception. Colour vision is probably present in all primates, except specialised nocturnal forms.

A trend towards a **reduced snout** and flattened face and reduced olfactory regions in the brain. Baboons go against this trend, with a secondary increase in muzzle length.

Primates have a generalised **dental pattern** particularly in the back teeth. Unspecialised teeth enabled primates to adopt a flexible omnivorous diet.


Gorilla

Primates have a tendency toward **erectness**, particularly in the upper body, as seen in the gorilla, above. This tendency is associated with sitting, standing, leaping, and (in some) walking.


Gibbon

Gibbons are the smallest of the apes and are specialised to use **brachiation** (a technique of under-branch swinging), in combination with rapid climbing, midair leaps, and bipedal running, to move quickly through the forest.


Moustached monkey

Monkeys walk **quadrupedally** on the palms of their hands and the soles of their feet. In the trees, they walk along branches, gripping them with their hands and feet. Retention of the collar bone in primates allows the shoulder to be more mobile.

1. On the diagram of the capuchin below, briefly describe the general physical characteristics of all primates as indicated:

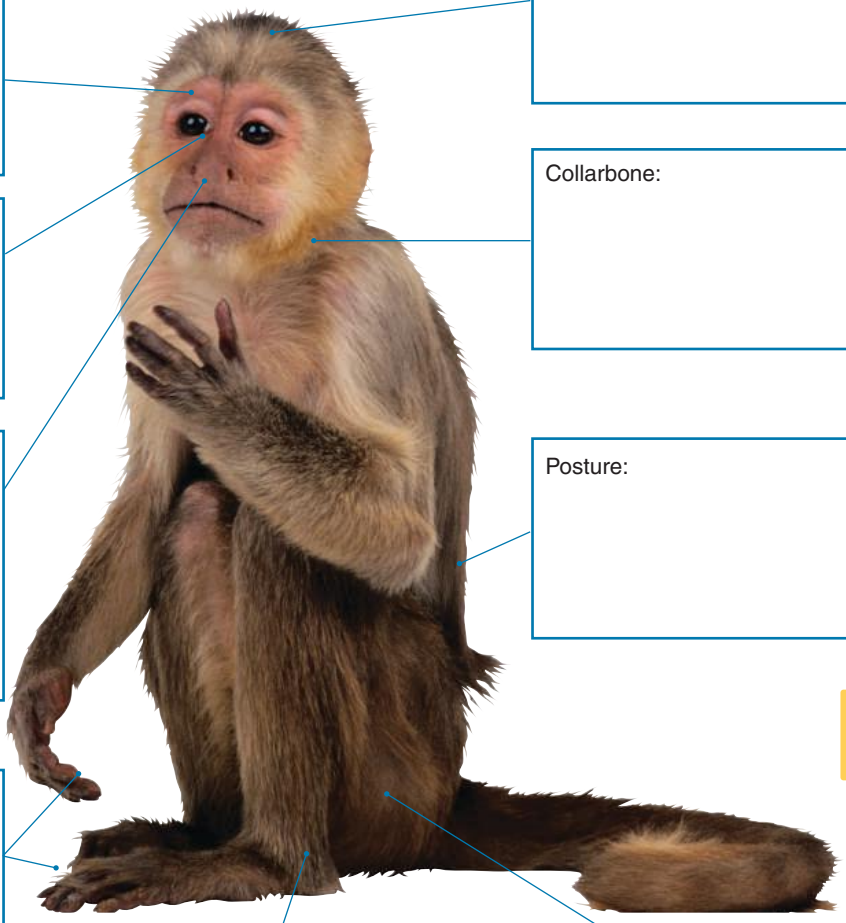
The primate pictured is a **white-fronted capuchin monkey** (*Cebus albifrons*) from northern South America. These monkeys inhabit the mid-canopy deciduous, gallery forests.

Vision:

Face shape and snout:

Teeth shape and dental arrangement:

Hands and feet:



Brain size and specialisation:

Collarbone:

Posture:

Limb joints:

Reproduction:

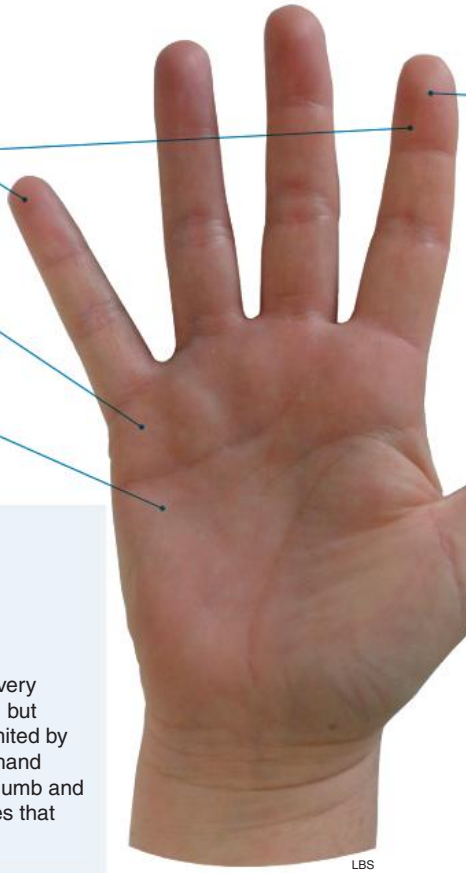
Social organisation:

2. Humans belong to the order Primates. Describe the features of humans that characterise their primate heritage:

147 The Primate Hand

Key Idea: Primates have grasping hands, but the human hand is particularly developed with respect to dexterity and the manipulation of objects.

Primates have a grasping hand. They are able to pick things up, hold, and manipulate them, although the degree to which a primate can do this depends on the species. Humans have a highly advanced ability to manipulate objects with their hands because the thumb is very long relative to the hand.



Index finger and little finger are able to pivot and move towards each other, allowing the fingers to form around small objects.

The fingers have end tactile pads that contain huge numbers of nerve endings, producing a highly sensitive surface.

Large muscles, especially around the thumb, produce a powerful grip.

Nails are found on at least some digits in all modern primates.

Flexible joints in the hand allow it to flex, increasing dexterity.

Highly mobile thumb, able to touch all other fingers on the hand.

LBS



Chimpanzees are very capable tool users, but their dexterity is limited by the length of their hand compared to the thumb and the rigid wrist bones that limit wrist rotation.

Research suggests manipulating objects may not have been the only important factor in the evolution of the human hand. The human hand shape is one of the only configurations possible that maintains dexterity while allowing the hand to form a fist. Although a punch with a closed fist produces the same force as a slap with an open palm, a punch delivers the force to a smaller area, producing a much greater impact and potential for damage to an opponent.

Grips of the human hand



Precision/ power grip



Power grip



Power grip



Precision grip

All photos LBS

1. Name two possible selective pressures acting on the human hand: _____

_____

2. Explain why being able to manipulate objects is an evolutionary advantage: _____

_____

3. Identify two features that make the human hand so dexterous and compare them to a chimpanzee hand:

_____

_____

_____

_____

148 Hominoids and Hominins

Key Idea: The hominins include modern humans and their extinct ancestors. Hominins are part of a larger superfamily, the hominoids, which also includes the apes.

The hominoids (apes as well as humans and their ancestors) are large, tailless primates, with bony eye ridges and flattened noses. Hominins (humans and their extinct closest ancestors) are a subtribe within this larger taxon identified by human features including dentition and brain size. The older taxon hominid is now a collective term encompassing the great apes and hominins and no longer refers just to humans.

Superfamily Hominoidea

Hominoid features
(lesser apes & hominids)

- No tail
- Semi-erect or fully erect posture
- Broad chest, pelvis, and shoulders
- Relatively long arms and mobile shoulder joints
- Larger brain

Family Hylobatidae



Characteristic features of the **lesser apes**:

- All found in Southeast Asia
- Long forearms with hook-like fingers specialised for brachiation
- Pads on the rump (ischial callosities)
- Arboreal; sleep on tree branches and do not build nests

Family Hominidae (hominids)

Characteristic features of the hominids:

- Large and sexually dimorphic
- Most predominately quadrupedal
- Most omnivorous
- Typical ape-like dentition but teeth large in gorillas and small in humans
- Complex social behaviour

Subfamily Ponginae



Subfamily Homininae

Features possessed to varying degrees by subfamily Homininae:

- Partially or habitually bipedal and ground dwelling
- Large cerebral cortex
- Reduced canines
- Highly sensitive skin, body hair reduced



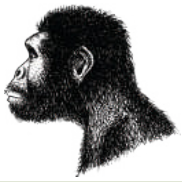
Tribe Gorillini: Gorillas

Tribe Hominini: Humans, their ancestors, and chimpanzees

Subtribe Hominina
Humans & their ancestors



Subtribe Australopithecina
Australopithecines (extinct)

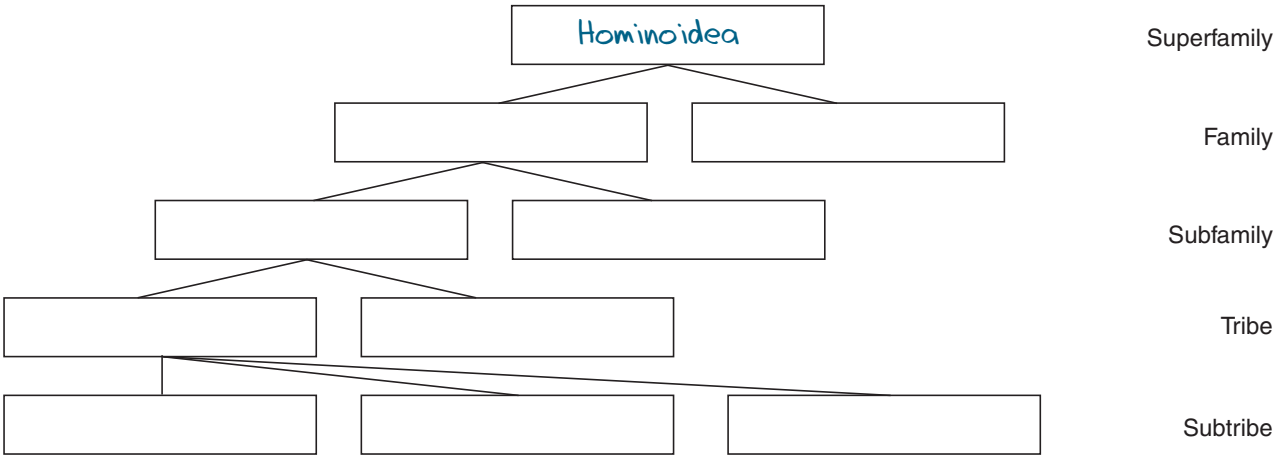


Subtribe Panina
Chimpanzees



Hominins

1. Use the information above to complete the classification of Hominoidea to show their relationships:



2. What are the differences between hominoids and hominins? _____

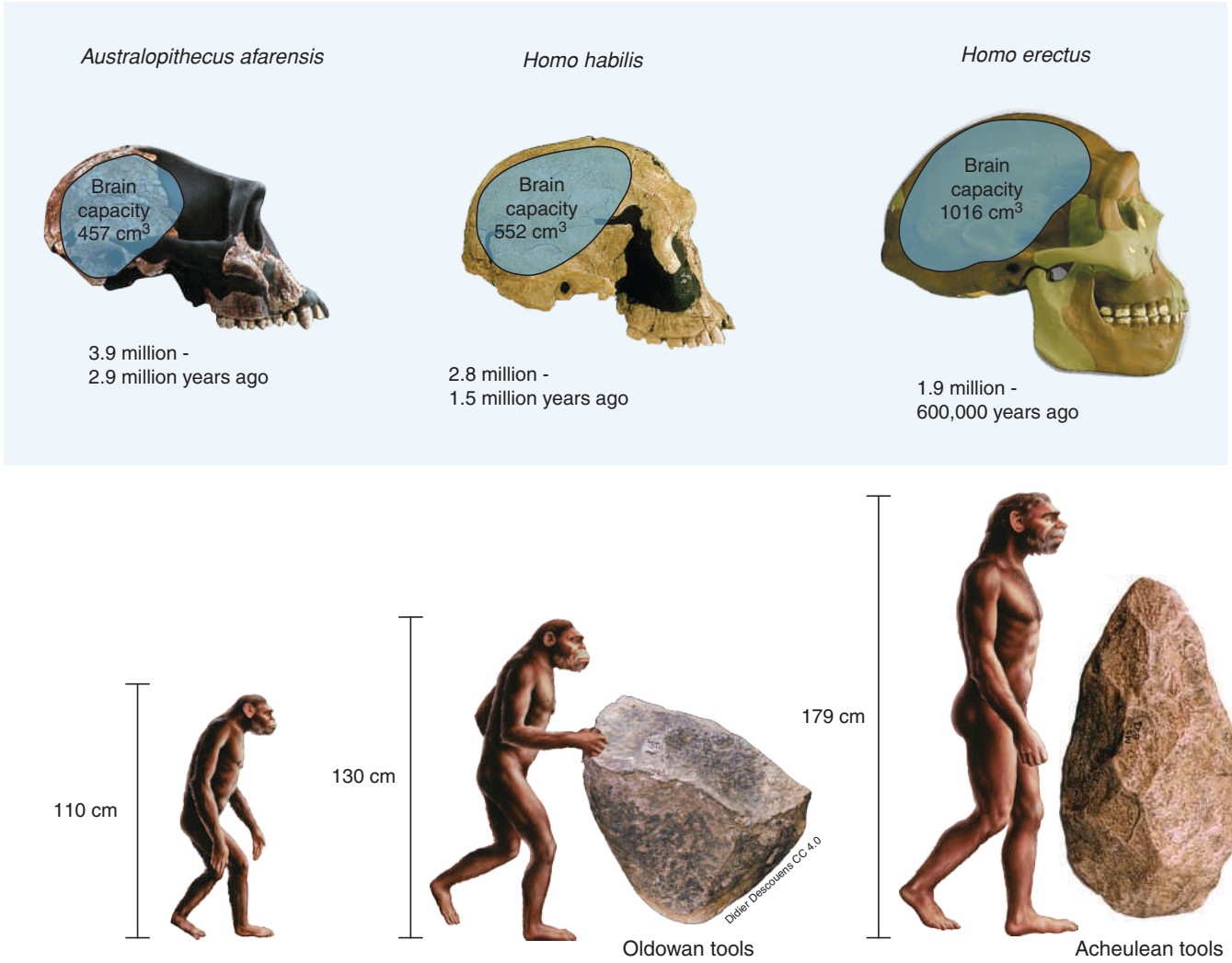
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149 Trends in Hominin Evolution: Overview

Key Idea: The hominin fossil record shows clear evolutionary trends towards bipedalism, increased brain size, increased height, and increased technical ability.

The diagram below and opposite shows a consensus view of the trends in hominin evolution over time. Only the five species representative of the general trends are shown here. The early australopithecines were almost certainly ancestral to *Homo habilis*, which was ancestral to modern humans. Some populations of *Homo erectus* migrated out of Africa, eventually giving rise to populations of *Homo* in the Middle East and Europe. Neanderthals eventually evolved in Western Europe and modern humans in Africa.



Australopithecus afarensis	*Homo habilis*	*Homo erectus*

1. Describe the general trends in the following features:

   (a) Angle of the face: _____

   _____

   (b) Size of the brain and skull: _____

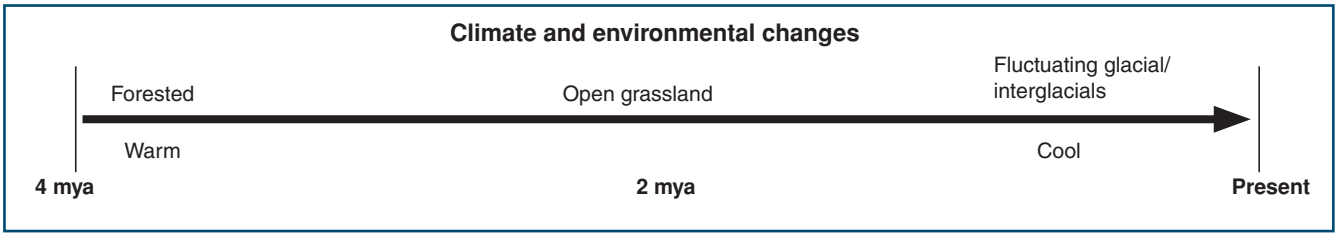
   _____

   (c) Height and stance: _____

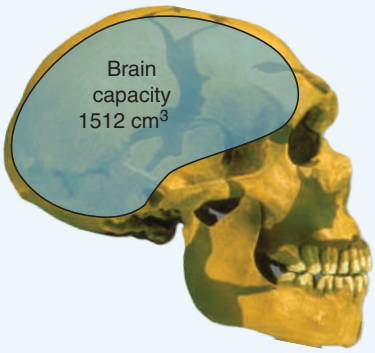
   _____

   (d) Skill at tool making: _____

   _____

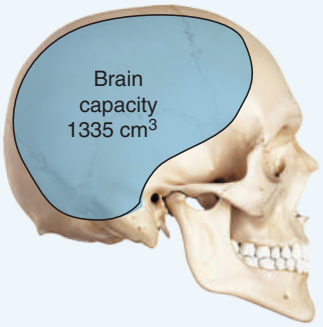


Homo neanderthalensis



500,000 - 40,000 years ago

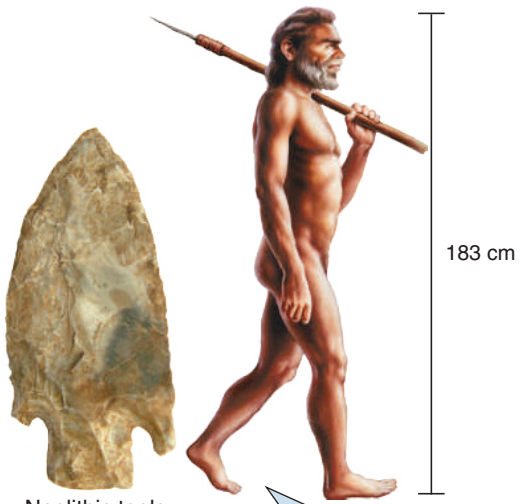
Homo sapiens



200,000 years ago - present



Mousterian tools



Neolithic tools

Homo sapiens

Homo heidelbergensis (not shown)

Homo neanderthalensis and *Homo sapiens* lived at roughly the same time. However *H. sapiens* evolved in warmer Africa and *H. neanderthalensis* evolved in cooler Europe. They may have overlapped in space for a few thousand years as *H. sapiens* migrated out of Africa.

Homo neanderthalensis

Homo neanderthalensis became extinct about 30,000 years ago.

2. What was happening to the climate and environment as human ancestors evolved?

3. The skulls of *Homo neanderthalensis* and *Homo sapiens* are similarly sized. Describe the main differences between them:

150 Trends in Skull Anatomy

Key Idea: The trend in hominin skulls has been towards a greater volume, flatter face, and more gracile features.

The shape of the modern human skull is quite different from its ancestors and that of Neanderthals. The human skull has a very high forehead and domed skull, whereas early ancestors had flatter foreheads and more elongated skulls. Modern humans have rather gracile skulls compared to their ancestors. The cheek bones and jaw are both smaller and the brow ridges are much reduced relative to earlier hominins. These changes tend to reflect a change in diet to one that requires less chewing (e.g. from tough vegetable matter to a greater amount of (cooked) meat).

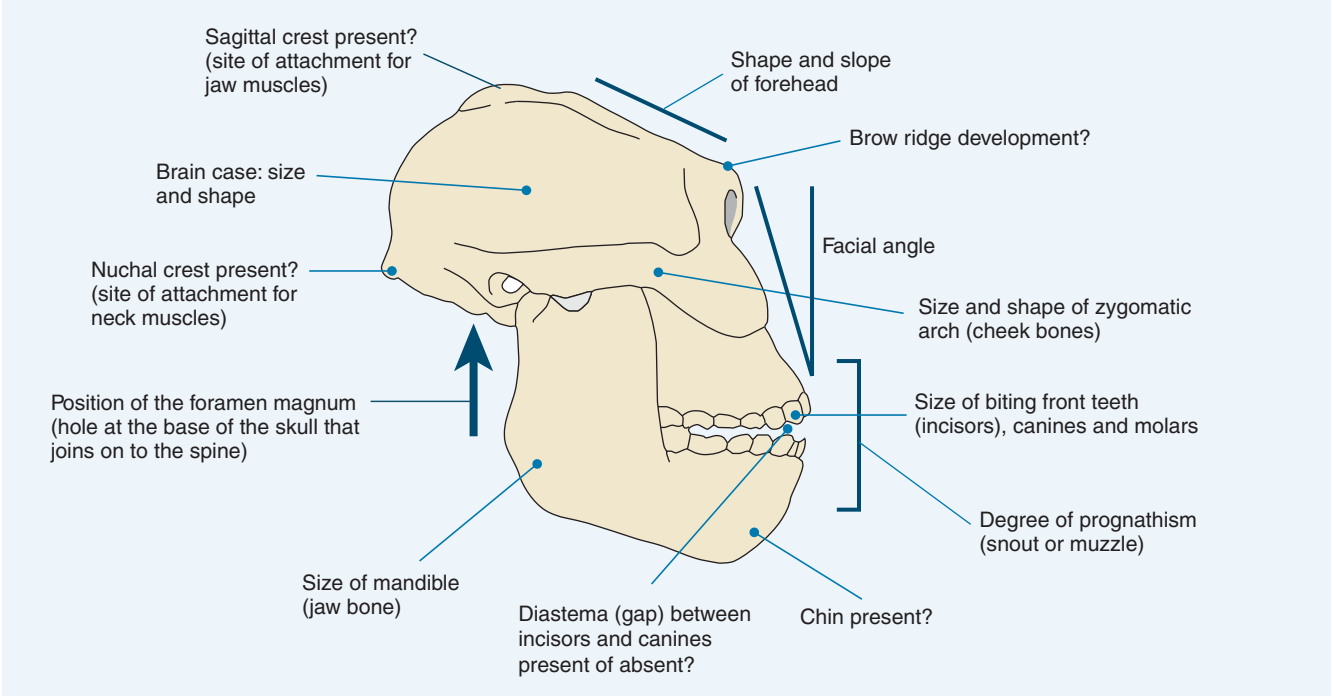
Skull features

The skulls show in this activity are representative of the many hominins both modern and prehistoric. The features mentioned below and shown in the diagram below are features that should be noted when looking at trends in skull evolution.

1. **Face**
   (a) Size of the face compared to the braincase.
   (b) Degree of prognathism (snout or muzzle development) of the jaw and mid face (mid-face projection).
   (c) Development of brow ridges (supraorbital tori): size, thickness, arching.
   (d) Size of cheek region.

2. **Jaws (mandible)**
   (a) Size and thickness of lower jaw.
   (b) Degree of curvature of dental arcade (tooth row).
   (c) Presence or absence of chin.

3. **Braincase**
   (a) Shape of forehead (slope, height).
   (b) Rear view: where is skull the widest, low down or high up? Shape: pentagonal, rounded, bell-shaped?
   (c) Presence of crests: Nuchal crest for neck muscles, Sagittal crest for jaw muscles.
   (d) Shape of occipital region (back of skull) when viewed from the side: presence of bun?
   (e) Dorsal (top) view: where is the skull widest (rear, middle)?
   (f) Position of foramen magnum (opening at base of skull connected to spine).



1. For each of the hominin species describe the features of the skull. Use the diagram above as a guide.

   (a) *Australopithecus afarensis*: _____

   _____

   _____

   (b) *Homo habilis*: _____

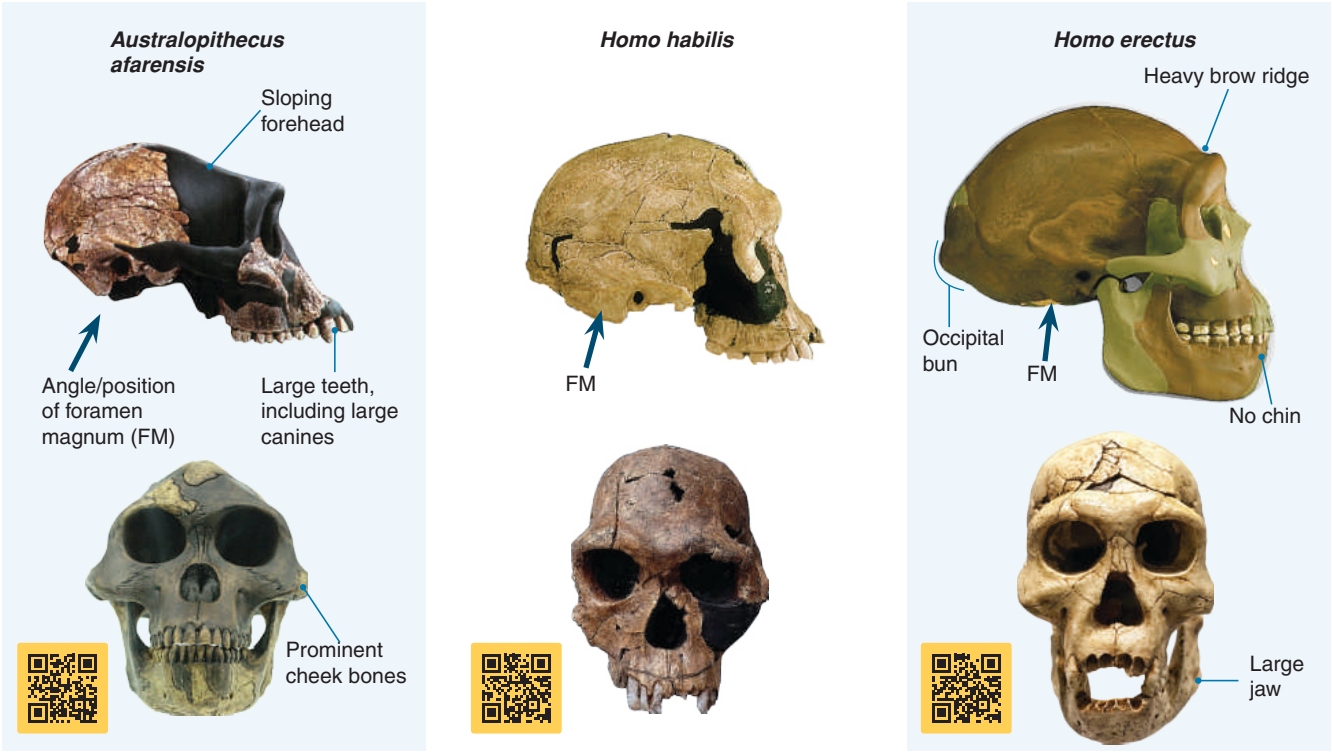
   _____

   _____

   (c) *Homo erectus*: _____

   _____

   _____





- **Neanderthals** evolved in Southern and Western Europe and are generally thought of as being adapted for the cooler conditions found there. However examination of the nasal cavity finds it does not fit the general rule in mammals, which in cold climates usually show a reduction in the size of the nose and nasal cavities. The Neanderthal nose size is therefore a bit of an oddity and may be linked simply to the degree of facial projection. Also the internal sinuses are small and do follow the rule for cold climates.
- The skull features a large occipital 'bun' which may reflect an enlarged occipital lobe. The occipital lobe is involved with visual processing. It could also reflect a larger cerebellum, which is involved in the coordination of movement and spatial information.

- The **modern human** skull has a high vertical forehead and large domed skull. This reflects an increase in the size of the frontal lobe of the cerebral cortex.
- There is no brow ridge and the facial and cheek bones have all been reduced in size. The jaw is smaller relative to the skull than in any other hominin, as are the cheek bones and teeth. This may reflect a shift in diet to food that required less chewing (less powerful musculature would be needed so muscle attachments can be less robust).
- There is also no nuchal ridge as the skull is now balanced directly above the spine so only small muscles are required to hold it upright. There is a prominent chin which acts as a buttress (support) for the small jaw.

(d) *Homo neanderthalensis*: _____

_____

_____

(e) *Homo sapiens*: _____

_____

_____

151 Trends in Brain Volume

Key Idea: The evolution of a large brain was crucial to the development of language, technology, and culture in humans.

The human brain is responsible for the unique behavioural qualities of humans. It makes up just 2% of body weight, but demands about 20% of the body's metabolic energy at rest. This makes the brain an expensive organ to maintain. The selection pressures for increased brain size must have been considerable for additional energy to be made available. The normal human adult brain averages around 1330 cc, but ranges in size between 1000 and 2000 cc. But intelligence is not just a function of brain size. There are large mammals, such as elephants and whales, with brain volumes that are greater than humans and yet they are not considered as intelligent. It appears that what is more important is relative brain size (brain size relative to body size). Modern humans have a brain volume three times larger than that predicted for an ape of the same body size. The organisation of the brain is also important. Apart from the highly developed cerebrum, two areas of the brain associated with communication have also become highly developed in modern humans: **Broca's area**, concerned with speech, and **Wernicke's area**, concerned with comprehension of language.

Growth in brain size in humans and chimpanzees

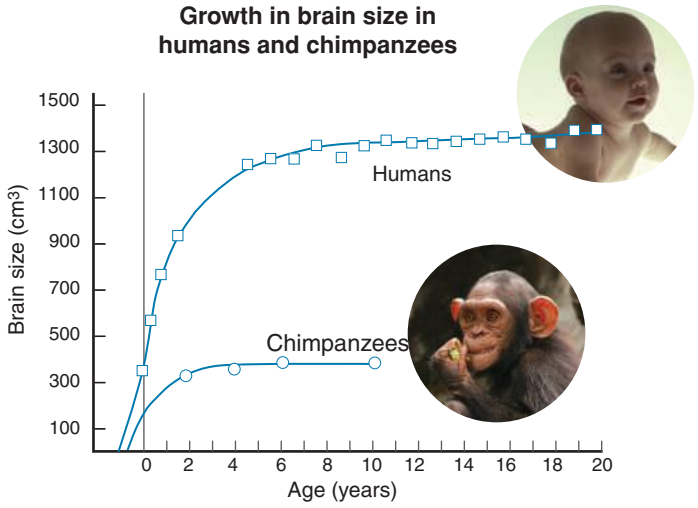


Figure above: In most primates, including chimpanzees, brain growth, relative to body size, slows markedly after birth while body growth continues. In human infants, the slowing of brain growth does not occur until more than a year after birth, which results in larger brain masses for humans than for chimpanzees at any given age (or body weight).

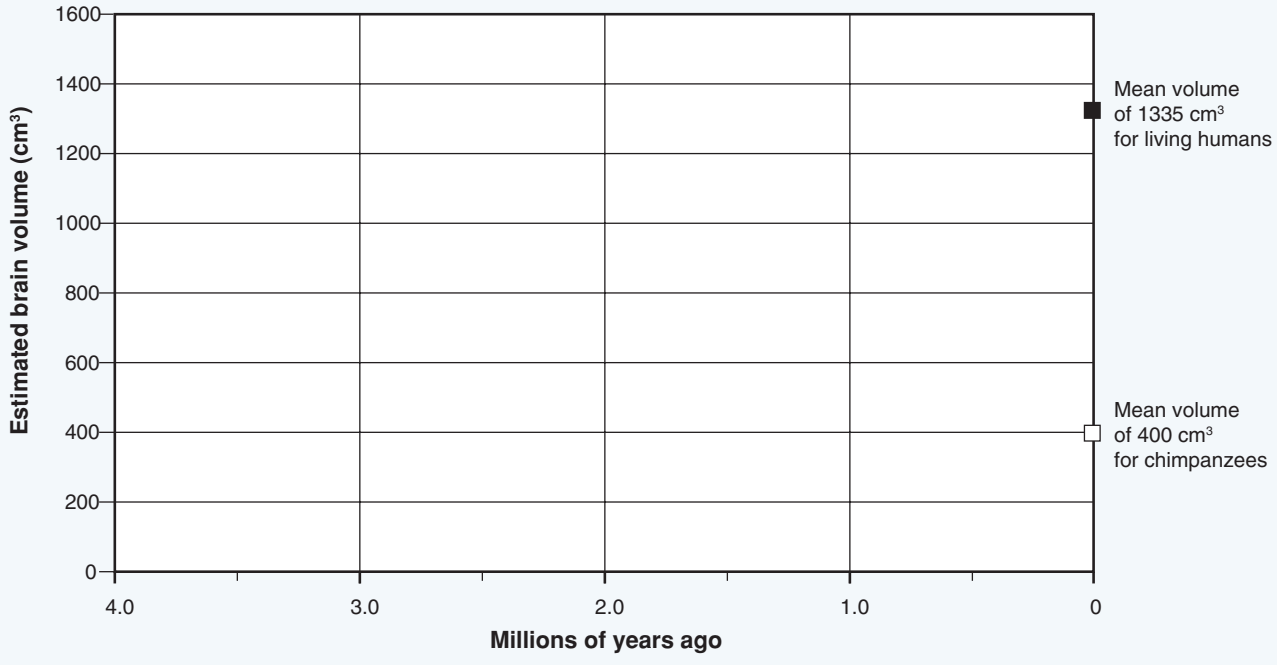
Brain volume for hominin species

Hominin species	Years ago (mya)	Average brain volume (cm³)
Australopithecus afarensis	3.5	440
Australopithecus africanus	2.5	450
Paranthropus robustus	2.0	520
Paranthropus boisei	1.5	515
Homo rudolfensis	2.0	700
Homo habilis	1.8	575
Homo ergaster	1.8	800
Homo erectus	0.5	1100
Homo naledi	0.25	560
Homo heidelbergensis	0.2	1250
Homo neanderthalensis	0.05	1550
Homo floresiensis	0.05	380
Homo sapiens	0.08	1350

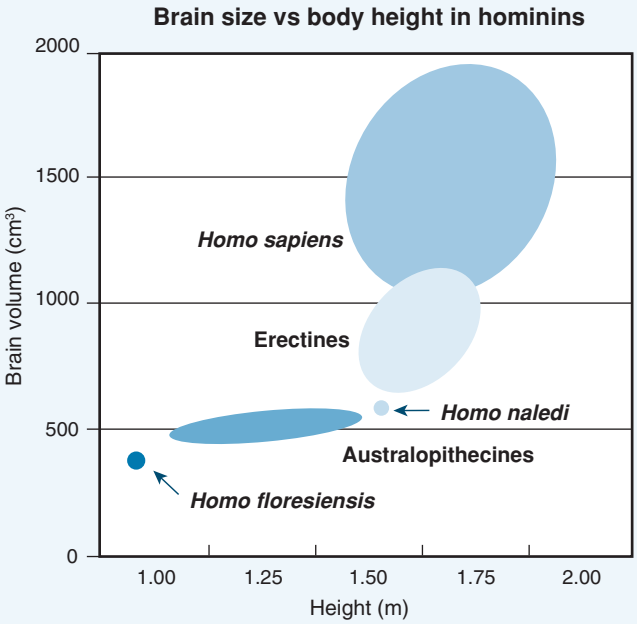
Table above: A generalised summary of the changes in estimated brain volume recorded from the fossil remains of hominins. The dates for each species are generally the middle of their time range for long-lived species or at the beginning of their time range for short-lived species.

1. Plot the data in the table above "*Brain volume for Hominin species*" on to the graph below.

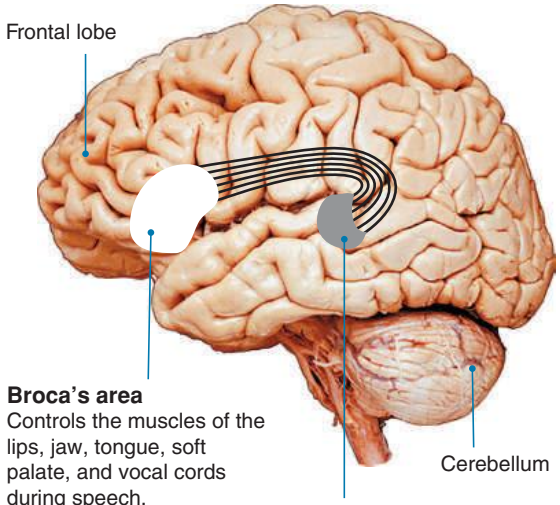
Changes in hominin brain volume over time



2. There were two 'bursts' (sudden increases) of brain expansion during human evolution. Indicate on the graph you have plotted where you think these two events occurred.



Brain size can be correlated with body height in hominins. Three distinct clusters emerge, indicating three phases of evolutionary development. *Homo floresiensis*, found on the Indonesian island of Flores, clearly falls outside these clusters. Its brain size to body size ratio is similar to that of the Australopithecines, but key aspects of its morphology, such as its small canine teeth and organisation of the brain, identify it as *Homo*. In addition, the Flores finds were associated with relatively advanced stone tools.



Modern human brain

The human brain is very large for a primate of our size, but this may not be as important as its organisation. The most important specialisation of the human brain is the capacity for language, a result of the development of **Wernicke's** and **Broca's areas**. Specific differences associated with the left and right hemispheres of the brain are associated with these specialisations.

3. Why is brain volume alone not a reliable indicator of intelligence? _____

4. Explain the significance of the high energy requirement of a relatively large brain: _____

5. Comment on the significance of the brain/body size growth curve in humans compared with other primates: _____

6. (a) With respect to the brain size: body size ratio, comment on the position of *H. floresiensis* with respect to other hominins:

   (b) Comment on the significance of the Flores finds: _____

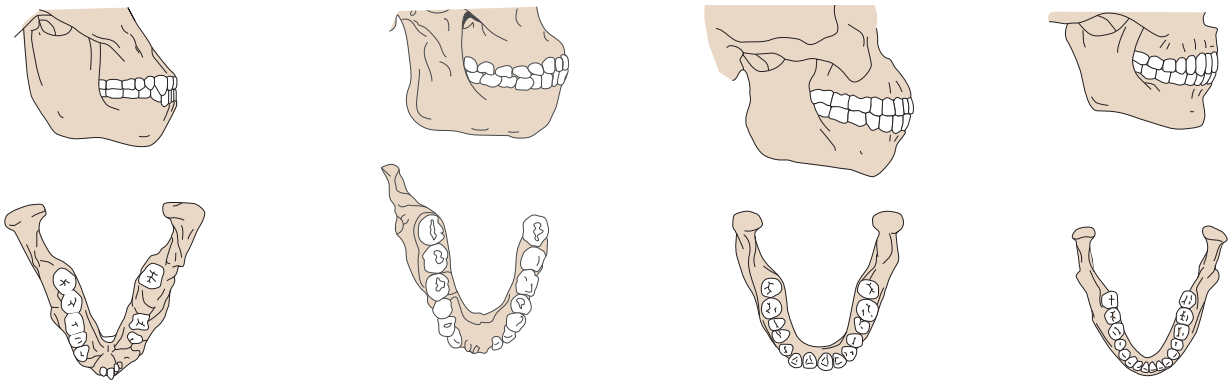
7. Describe a likely selection pressure for the evolution of increased brain size in early humans: _____

152 Trends in Dentition

Key Idea: Changes in dentition (the type, number, and arrangement of teeth) and jaw structure of hominin ancestors can reveal information about hominin evolution.

During early hominin evolution teeth (especially the molars) and jaws tended to be large. The paranthropines are the extreme example of this trend. Their diet of coarse vegetation required very large and powerful jaws and molars. During the course of hominin evolution, there was a general trend for a reduction in the size of the teeth tooth and jaw. This was a likely consequence of including a greater proportion of cooked foods, which required less chewing, in the diet. The teeth of modern humans are relatively small and generalised, reflecting an omnivorous diet of mainly processed (e.g. cooked) foods.

Early hominins → **Late hominins**



Australopithecus afarensis

- Relatively large canine teeth
- Relatively large jaw
- V-shaped dental arcade
- Thin tooth enamel
- Diet probably consisted of fruits with some tougher material

Homo habilis

- Reduced canine teeth
- Large molars and incisors
- Dental arcade more like *H. sapiens* but still intermediate
- Thick tooth enamel
- Diet probably included vegetable matter and some meat (probably from scavenging)

Homo erectus

- Thick jaw bones
- No chin
- Relatively large molars
- Parabolic dental arcade
- Thick tooth enamel
- Diet probably included vegetable material and a large proportion of meat

Homo sapiens

- Shortened jaw, allows large bite force to be generated with little effort
- Chin reinforces jaw, but leaves room for tongue muscles
- Thick tooth enamel
- Small molars adapted to chewing cooked and soft food
- Parabolic dental arcade

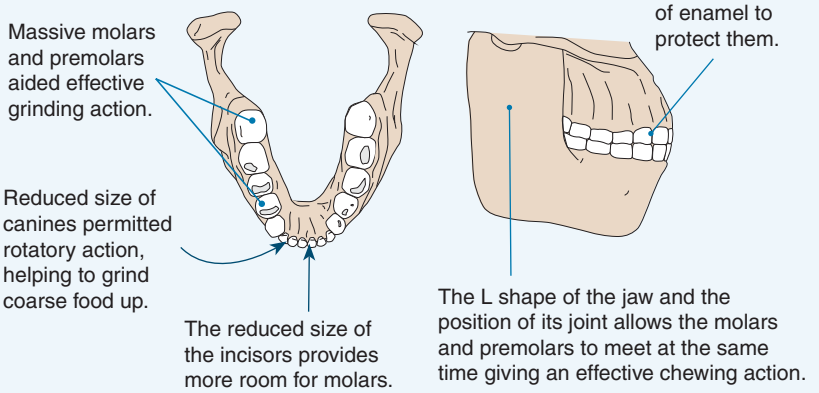
Dental formulae all follow: I-2, C-1, P-2, M-3



In many primates, the canine teeth are used in behavioural and social interactions, especially in species that show marked sexual dimorphism. Threat gestures, such as yawning (as in the baboon above) help maintain social order.

Adaptations to a coarse diet

Paranthropus boisei had jaws and teeth adapted to a diet of hard seeds and coarse vegetation. Their jaws produced a bite force of 2161 newtons, which helped to break food up. A modern human's maximum bite force is 777 newtons.



1. Describe the general trend in the evolution of hominin teeth: _____

_____

_____

2. What is one possible purpose of the chin in modern humans? _____

_____

_____

153 Bipedalism and Nakedness

Key Idea: Bipedalism provided advantages such as better provisioning, greater safety, and greater efficiencies in locomotion and thermoregulation.

The first major step in the evolution of humans as a distinct group from apes was their ability to adopt the habitually upright stance we call bipedalism. Closely linked to this shift was the reduction in body hair. A number of selection pressures for hair reduction are described below (left). Early studies suggested that bipedalism and hair reduction were both evolutionary responses to the changing climate of East Africa about 7-3 mya. However, analysis of the 4.4 mya *Ardipithecus* fossils indicates that these very early hominins were still mainly forest dwelling, so current hypotheses must account for the emergence of bipedalism in a forested environment. The *Ardipithecus* finds indicate that bipedalism was strongly associated with provisioning, and then reinforced by a move into less forested habitats as savannah became established throughout Africa in the later Miocene.

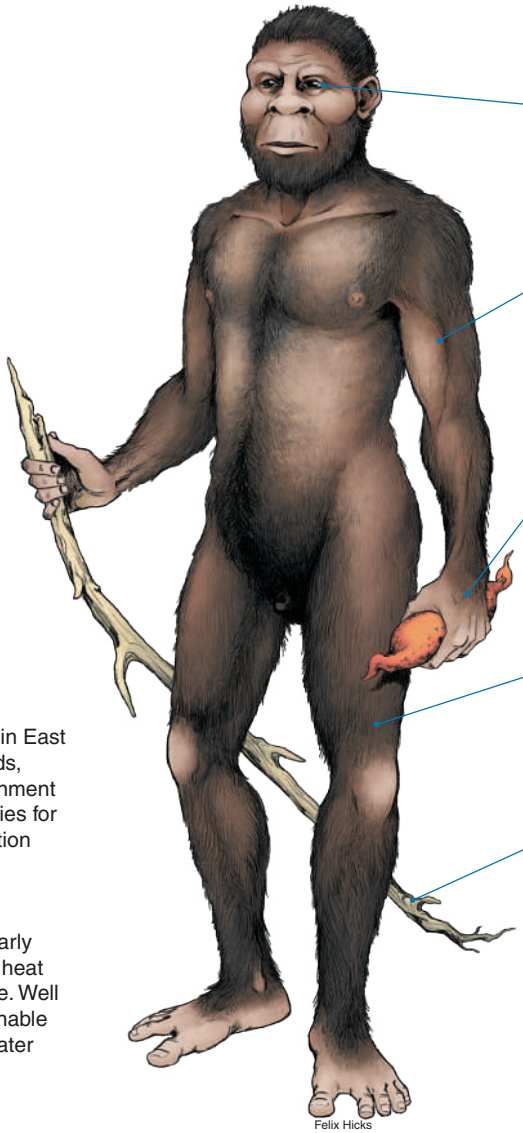
Hair reduction

Retention of head hair
Hair on the head and shoulders has been retained to reflect and radiate heat before it reaches exposed skin.

Parasite control
A reduction in body hair would have made it easier to control external parasites such as fleas and lice. This would have been increasingly important when early hominins began to use a 'home base'. Many external parasites need to complete their life cycle at a single location so that hatching eggs can reinfect their host.

Thermoregulation
About 3 mya, the vegetation patterns in East Africa began to favour open grasslands, with fewer forested areas. This environment would have provided fewer opportunities for shelter from the sun, creating a selection pressure for the refinement of several thermoregulatory mechanisms.

Shorter, finer hairs (not hair loss) in early hominins would have allowed greater heat loss via radiation from the skin surface. Well developed sweat glands in humans enable heat loss at 700 watts/m^2 of skin (greater than any other mammal).



Felix Hicks

Bipedalism

Seeing over the grass
An upright posture may have helped early hominins to see predators or locate carcasses at a distance.

Carrying offspring
Walking upright enabled early hominins to carry their offspring, so the family group could move together.

Provisioning as a selection pressure
The ability to carry food while walking seems to have been important in the initial development of bipedalism. Females would have favoured males able to provide energy-rich foods, which would improve offspring survival and increase reproductive rate. The ability to carry food from its source to a place of safety would have had a great survival advantage.

Efficient locomotion
Once bipedalism was established, changing habitats would have provided selection pressure for greater efficiency. Being able to move across the growing savannah without expending large amounts of energy would have offered a great survival advantage.

Holding tools and weapons
Tool use was probably a consequence of bipedalism, rather than a cause. Upright walking appears to have been established well before the development of hunting in early hominids.

Thermoregulation
Upright walking exposes 60% less surface area to the sun at midday and there is greater air flow across the body when it is lifted higher off the ground.

1. What advantages might an early human ancestor have gained by adopting a bipedal stance? _____

_____

_____

2. (a) What selection pressures are likely to have been important in the evolution of bipedalism initially?

_____

_____

(b) What environmental changes could have reinforced the advantages of bipedalism to human ancestors?

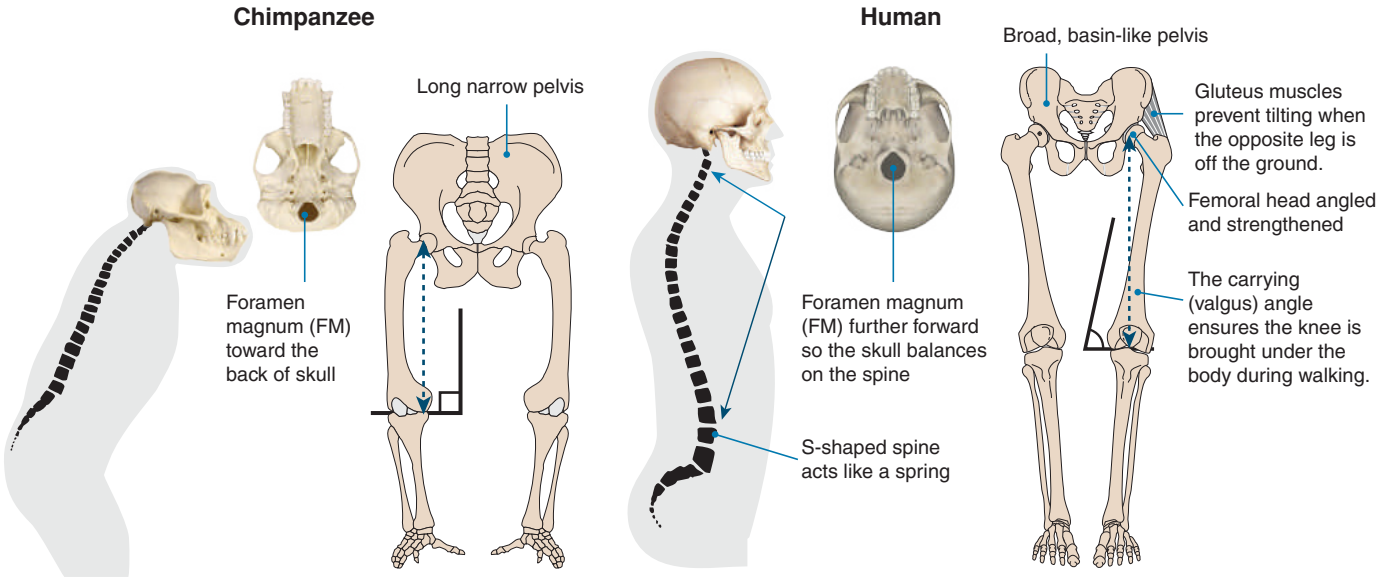
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154 Adaptations for Bipedalism

Key Idea: Important changes in the skeleton are associated with the move to bipedal locomotion in early hominins.

The reconstruction of Lucy (*Australopithecus afarensis*), opposite, shows the skeletal features of an early bipedal hominin. Lucy still possessed ape-like features but she was a fully-bipedal hominin with all the adaptations associated with bipedal locomotion. Although there is no doubt that Lucy was habitually bipedal, a number of skeletal features suggest that tree climbing was still an important part of this hominin's niche, perhaps associated with escape, security, or foraging. *A. afarensis* is an important link between the quadrupedal locomotion of apes and bipedalism in hominins.

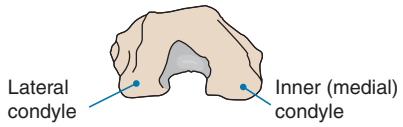


Quadruped: Straight spine and rear-ward FM, femur is at right angles to knee so an upright stance is less stable.

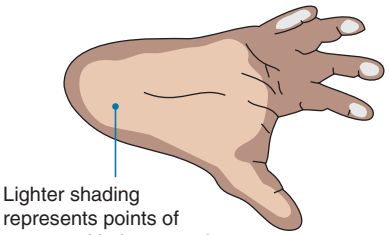
Biped: S-shaped spine and forward FM. Femur (thigh) is angled out from knee (the carrying angle). A longer femur provides a longer, more efficient stride.

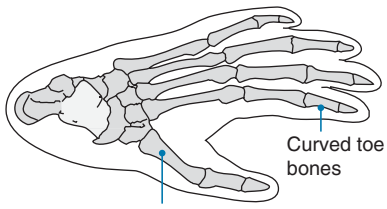
Chimpanzee

End of femur at the knee joint



Chimpanzee foot

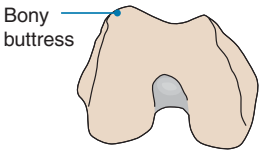




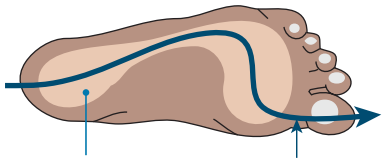
The foot of a chimpanzee has relatively long, curved toes, with an opposable big toe adapted from grasping but ill-suited to upright walking. The foot transmits weight from the heel, along the outside of the foot, and then through the middle toes.

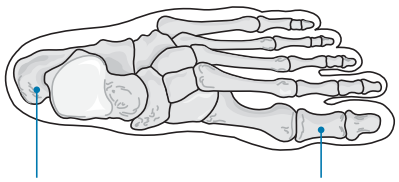
Human

End of femur at the knee joint



Human foot

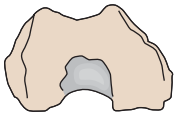




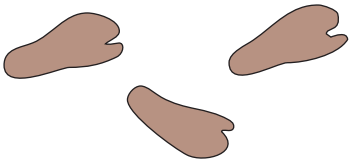
The human foot is adapted as a weight bearing platform rather than a grasping structure. The toes are reduced relative to those of chimpanzees. The foot is arched so transmits weight from the heel, along the outside of the foot, across the ball and through the big toe. This weight transference conserves energy during locomotion.

Australopithecine

End of femur at the knee joint



Australopithecine footprints





The australopithecine foot had an aligned big toe, as in humans, making it difficult if not impossible to grasp branches with the hindlimbs. The heel bones that have been found also indicate habitual bipedalism. Computer simulations suggest that *A. afarensis* could walk like humans but could not have walked like a chimpanzee.

Lucy's* ape-like characteristics

Shape of the tooth row (dental arcade) is half way between the straight-sided U-shape of an ape jaw and the more rounded, parabolic shape of a human jaw.

Shoulder joint orientated towards the head, similar to the orientation in chimpanzees and other arboreal quadrupeds.

Funnel-shaped chest (thorax).

Arms relatively long compared to legs.

Highly mobile wrist.

Curved finger bones.

Relatively short legs.

Highly mobile ankle joint.

Toes are long and curved.

Lucy's bipedal features

The foramen magnum was much further forward than in apes and much closer to the position in humans.

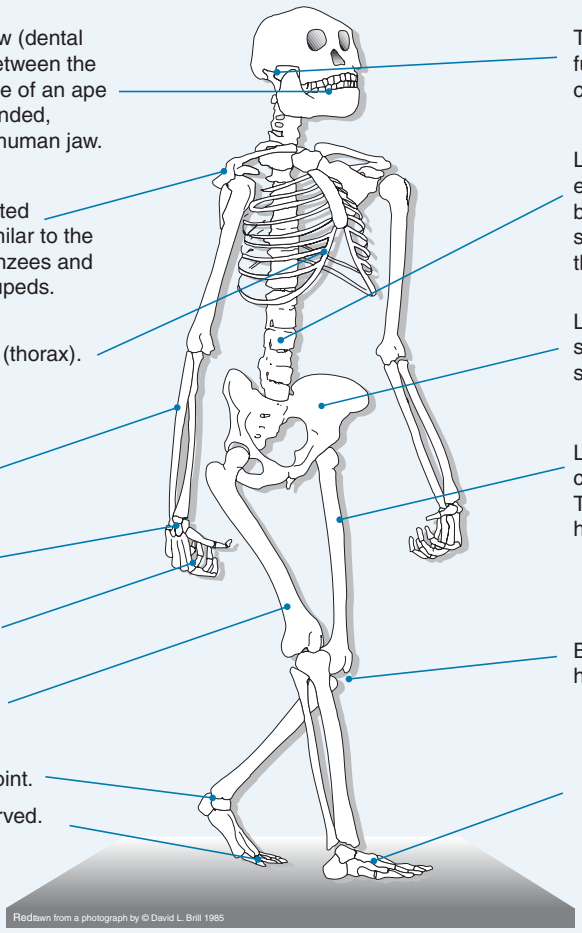
Lucy's lumbar vertebrae were broad for effective weight transmission from the upper body to the pelvis. The australopithecine spine had an S shaped curvature, similar to that of modern humans.

Lucy's pelvis was broad and basin shaped, similar to a human pelvis. It would have supported the upper body when upright.

Lucy's limbs showed human-like features consistent with bipedalism.
The femoral (valgus) angle was similar to humans, bringing the knees under the body.

Butressing of the knee was more similar to humans than to apes.

Arched feet, wide heels, and big toes aligned with the other toes and not opposable.



*Lucy is the name given to a specimen of ***Australopithecus afarensis***

1. Describe features of each of the following in *A. afarensis* and explain what they tell us about how this animal moved:

   (a) Foot: _____

   _____

   _____

   (b) Pelvis: _____

   _____

   _____

   (c) Lower limb: _____

   _____

   _____

2. What does the retention of ape-like characteristics tell us about the niche of *A. afarensis*? _____

   _____

3. Compare the position of the foramen magnum in a chimpanzee and *A. afarensis* and comment on its significance:

   _____

   _____

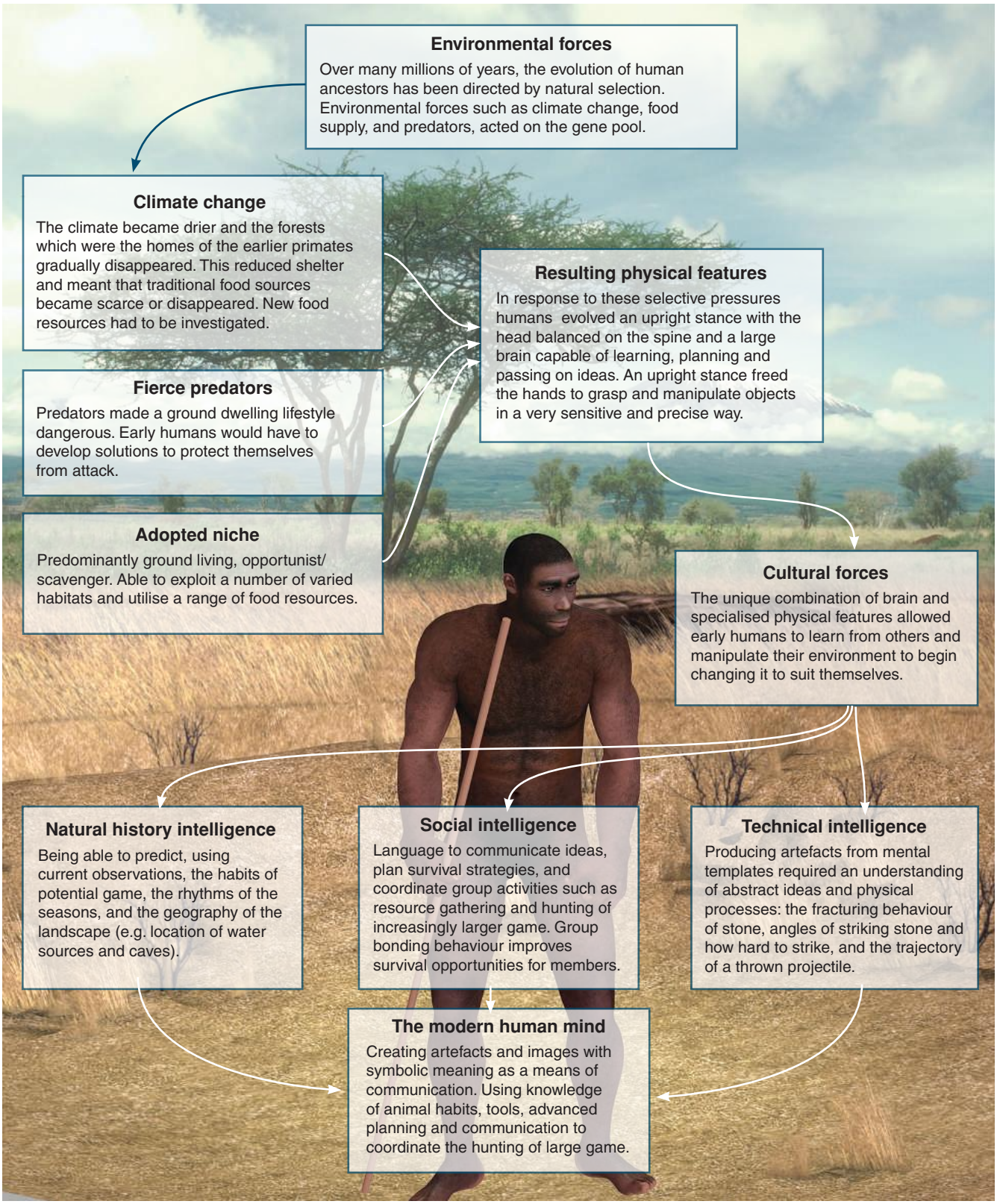
4. Imagine you are on an expedition to a well known hominin fossil site in east Africa. Describe a part of a hominin fossil skeleton that you would wish to find that would be ideal in clearly indicating bipedalism:

   _____

155 Cultural Evolution

Key Idea: Cultural evolution is a term used to describe the transmission of and changes to knowledge and ideas over successive generations.

In addition to the physical evolution of humans, ideas and behaviours also evolved as they were learned and passed on to offspring. This non-genetic means of adaptation, called cultural evolution, further enhanced the success of early humans.



1. Explain what is meant by cultural evolution: _____

_____

_____

156 Shelter and Clothing

Key Idea: The development of clothing and techniques to build shelters allowed the expansion of humans into colder climatic areas.

Human ancestors would have had a far greater amount of body hair than humans today. The move into hot, open grasslands provided a selection pressure for the reduction of body hair. However this then limited the ability to survive in cold climates as humanity migrated north out of Africa into Europe and beyond. The use of animal skins or woven plant material to make clothing provided a way of keeping warm. Building shelters allowed humans to shelter from both the weather and possible predators.

It is believed humans began wearing clothes before at least 83,000 years ago. This estimate comes from several lines of evidence. The first direct evidence of clothes-making is from a 60,000 year old bone needle. Stone hide scrapers have been dated to around 780,000 years ago. Evidence from burial sites also shows humans were adorning themselves (or at least their dead) with necklaces as far back as 35,000 years ago. Evidence from the evolution of body lice suggests human ancestors began wearing clothes some 100,000 years ago.



This finely worked bone needle (with an eyelet) is dated between 17,000 and 10,000 years old. Bone needles have been found in Australia that date to about 45,000 years ago.

Didier Descouens CC 3.0



Hide drying on rack

Human ancestors probably started using caves regularly as shelter soon after learning to control the use of fire. Bones of early australopithecines or *Homo* found in caves were likely taken there by predators or been washed in by ancient streams. Some of the first evidence of caves being used by humans is from the Atapuerca cave in Spain. It is dated at 1.2 million years old, but it is difficult to tell the exact relationship of the fossils to the cave.



Liang Bua cave, Flores Island, Indonesia.

Rosino



The first evidence of humans building purpose-built shelters dates to around 400,000 years ago. Post holes in the ground indicate poles were used as scaffolding for the shelter. Preserved bones from around 17,000 years ago in the Ukraine show humans there used mammoth bones and tusks as scaffolding, probably because there would have been few trees available to make wood scaffolding from.

1. (a) Identify the evidence for humans building purpose built shelters: _____

   _____

   _____

   (b) Identify the evidence for humans making clothing: _____

   _____

   (c) Suggest a reason why there is so little evidence of humans building shelters or making clothes: _____

   _____

   _____

2. Suggest a reason why caves where probably not regularly used by humans and there ancestors until the controlled use of fire was developed:

   _____

   _____

   _____

3. What is the main purpose of clothing? _____

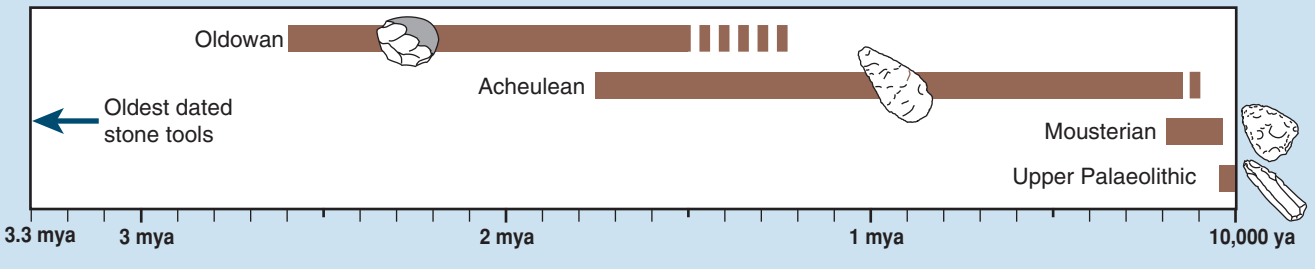
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157 Trends in Palaeolithic Tool Cultures

Key Idea: The development of stone tools is a defining characteristic of *Homo*. Particular, identifiable types can be matched with specific time periods and species.

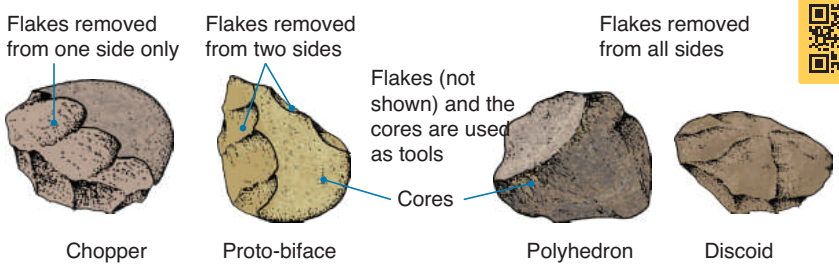
The **Palaeolithic** (Old Stone Age) is a period of early cultural development spanning the emergence of the first stone tools about 3.3 mya in eastern Africa, until the development of sophisticated tool kits in the **Mesolithic** (Middle Stone Age) about 10,000 ya. These tool cultures are known mostly by their stone implements. While other materials, such as wood, were probably also used, they did not preserve well.

Timeline of stone tool technologies through the Palaeolithic



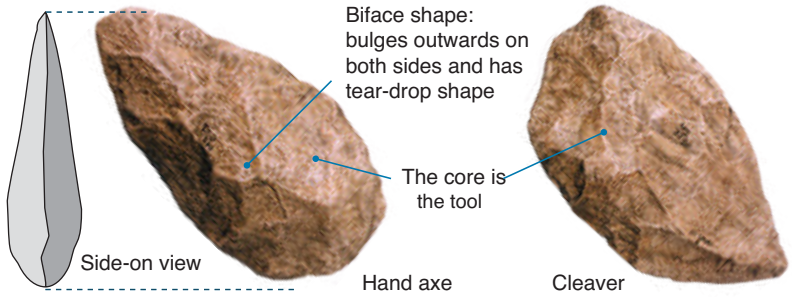
Oldowan (pebble) tool culture

Probably made by *Homo habilis*, these were crudely fashioned river-worn pebbles. A minimum number of flakes were knocked off from several angles to produce a core with a cutting edge (e.g. chopper, discoid, polyhedron). Although the cores may have been used as tools, it is known that the sharp flakes were also useful in cutting.



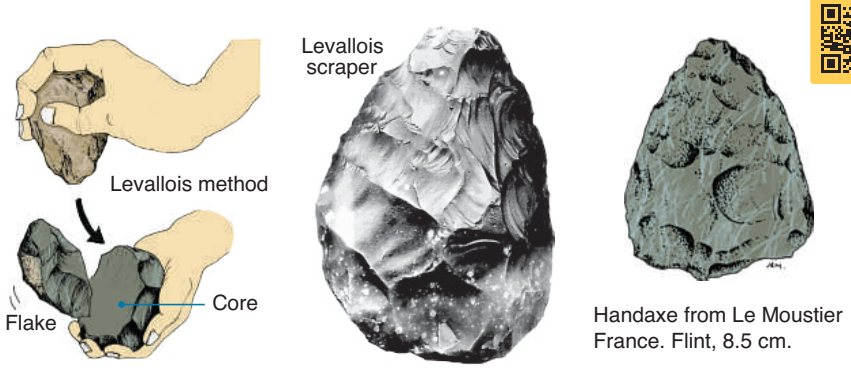
Acheulean tool culture

Made by *H. erectus* and archaic *H. sapiens*, these tools were typically 'tear drop' in shape and were crafted with a slight bulge on each broad surface (a bi-face). They ranged in size and are often referred to as hand axes although it is not clearly understood how they were used. They differ from the pebble tools in that there appears to be a standard design and each tool is manufactured using a great many more blows to remove flakes.



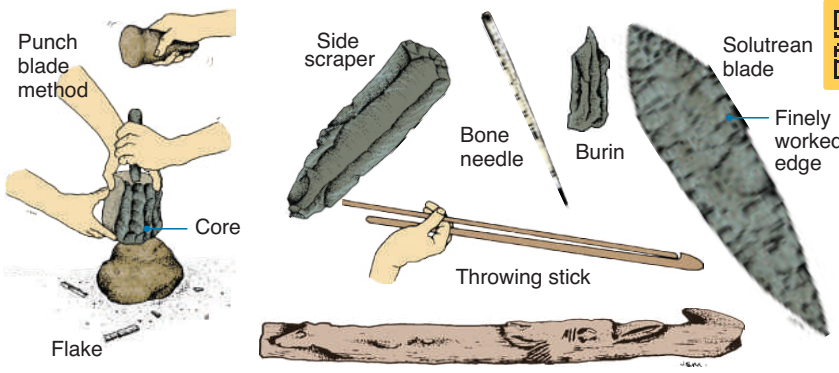
Mousterian tool culture

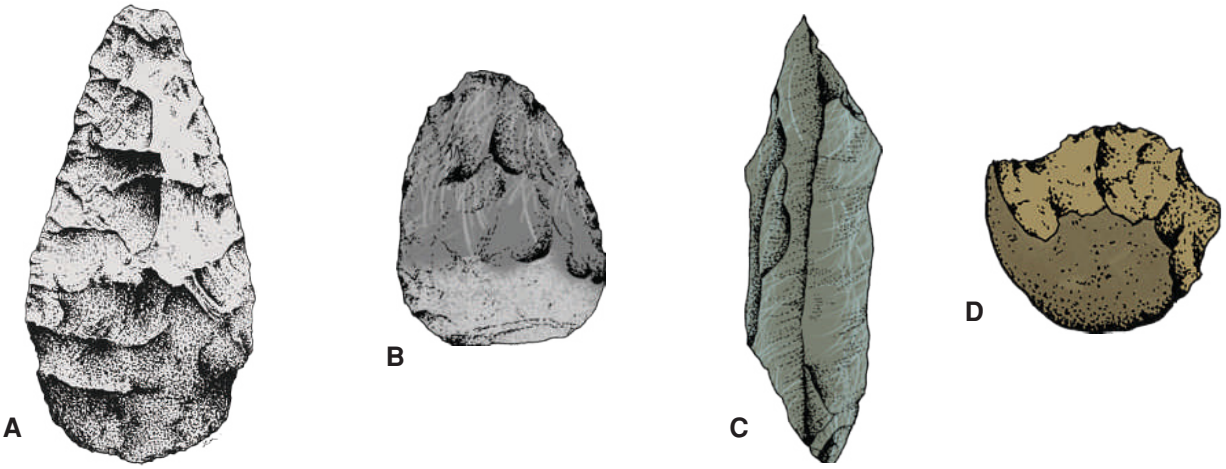
Made by Neanderthals and more refined than Acheulean tools. Flint became commonly used. This stone would chip in a predictable way when struck with another hard object so finer workmanship was possible. A particular technique from this period is known as the **Levallois method**. It involves the preparation of a core and striking off a large oval flake which is then retouched on one surface only (see the photograph on the right; the retouched surface is visible).



Upper Palaeolithic tool culture

There was a rather sudden increase in the sophistication of tool making about 35,000 to 40,000 years ago. Both the modern *Homo sapiens* and the last of the Neanderthals produced flint tools of much finer workmanship using a technique called **punch blade**. Long, thin flakes are removed and shaped into different tool types. A number of European sub-cultures, e.g. **Solutrean**, emerged. Other material such as bone, ivory and antler became increasingly utilised to produce very fine tools such as needles.





1. Name the culture associated with each of the tools above (A-D) and describe the features that help identify them:

   (a) Tool **A** culture: _____

   _____

   (b) Tool **B** culture: _____

   _____

   (c) Tool **C** culture: _____

   _____

   (d) Tool **D** culture: _____

   _____

2. Identify the hominin species associated with, and the approximate time period for, each of the tool cultures below:

   (a) Oldowan: _____

   (b) Acheulean: _____

   (c) Mousterian: _____

   (d) Upper Palaeolithic: _____

3. Describe the general trends in the design of the stone tool from Oldowan to Upper Palaeolithic cultures:

   _____

   _____

   _____

   _____

   _____

   _____

   _____

4. The tools that are recovered from early human prehistoric sites are almost invariably stone, bone or ivory. Explain why tools made from other materials are almost never recovered from these sites:

   _____

   _____

   _____

5. Name the materials used to make tools in the Upper Palaeolithic culture that were seldom used in earlier cultures:

   _____

158 Fire

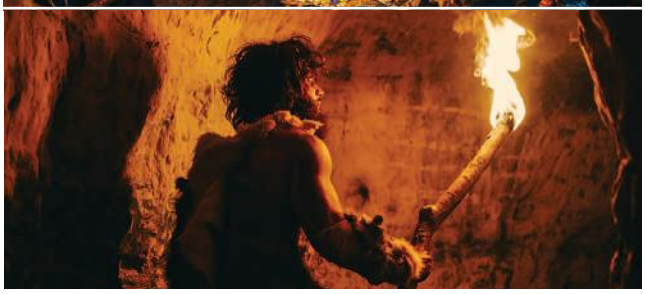
Key Idea: The controlled used of fire by early humans allowed them to eat a greater range of foods and to expand their range into darker and colder places.

Just when and how early human ancestors began using fire is debated and may never be precisely known. It is likely that early fire was "captured" from the wild, e.g. from fires started by natural events such as lightning. Whatever its origin, the controlled use of fire changed the course of both physical and cultural evolution, influencing dietary range and improving survival. Possible evidence exists for the use of fire up to a million years ago, but real evidence of controlled use of fire dates back only a few hundred thousand years.



Hand drill





Naturally set fires were the most likely source of early fire. Burning embers may have been carried back to home sites, or the fire used where it was.

The development of the hand-drill meant early humans could make fire when and where they needed it.

Cooking is the most obvious use of fire. Cooking food makes it easier to chew and digest, releasing energy more quickly for the body to use. Fire also kills parasites and pathogens in the food. Fire can also be used to preserve food. Meat can be smoked and vegetable material can be dried.

Fire may also have been used as a hunting tool by setting fire to forests to drive out game. Fire could also be used for protection to drive off predators.

Fire provides light and can be used as a torch, allowing early humans to explore dark places such as caves. It may have helped with bonding, such as sitting around a campfire. Fire provided light and warmth at night.

A less obvious, but important, use of fire, is its use in the construction of weapons. Wooden spear tips can be hardened in fire, allowing them to be made sharper and improving their penetrative power. Rocks heated in a fire become brittle and are therefore easier to shape into tools, such as blades and spearheads. The blade also holds a sharper edge.

1. List four uses of fire: _____

2. (a) Describe two important consequences of cooking food: _____

   (b) Explain how cooking food provided a selection pressure for a smaller jaw and teeth: _____

3. Discuss the influence of fire on human cultural evolution: _____

159 Art and Spirituality

Key Idea: Human art and culture can be dated to as far back as 290,000 years. However a major cultural explosion took place about 35,000 years ago.

Until recently, it was believed that art and spiritual beliefs first developed with the arrival of modern humans, particularly in Europe. However ancient artworks dating to nearly 300,000 years ago can be found outside Europe. The beginning of a period 35,000 years ago marks a dramatic cultural development occurring simultaneously over large parts of western and eastern Europe. Growing evidence suggests Neanderthals were also culturally sophisticated. The stimulus for the new cultural development was probably a need to represent ideas about the unknown, such as death, hunting success, and fertility, in a concrete way. A wide range of materials were used to do this. Ivory, bone, clay, and stone were used to create sculptures, and the walls of rock shelters and caves were adorned with drawings, paintings, and bas-relief (sculptures that stand out slightly from the rock wall).

The evolution of art


Body paint

- Art requires at least some form of abstract thought. To take an image in the mind and draw it or model it in real life first requires the formation of the idea and then the use of technical skill and imagination to shape an object or draw an image.
- Some archaeologists think that the first art was opportunistic. The Venus of Berekhat Ram is a small statue just 3.5 cm high. It is thought a rock that looked vaguely human was used as a start and then shaped further (although this is debated). The statue is dated to about 230,000 years old (the oldest confirmed Venus is 40,000 years old (right)).
- There are a number of Venus figurines. Most are small (~10 cm high) figurines of women with exaggerated breasts, buttocks, and body fat. They may have represented desirable traits in women to enhance fertility or survival.
- Rock painting may have developed in a similar opportunistic way, from simply adorning the body with pigments to using pigments on rocks. The oldest known site where ochre (a red pigment) was used is dated at 164,000 years old in South Africa.


Gerbil CC 3.00

Venus of Hohle Fels, the oldest confirmed Venus

Burial

- The oldest known intentional human burial is dated at 100,000 years, in Israel. Artefacts found with the bodies suggest a ritual of sorts. Burials became more common as ancient humans spread over the globe. Burials also became more elaborate, at least for some. Recent comparisons of burial sites show that the majority were plain with simple everyday items left with the body. Relatively few graves contained lavish or ornate materials which suggests some people had greater status than others.
- The leaving of personal items in the grave may indicate religious or spiritual beliefs of what happens after death.
- The Neanderthals of Europe and Southwest Asia buried their dead with signs of ritualisation. The position and orientation of the body are consistently the same and some graves included grave goods such as tools or animal bones (although the validity of grave goods with burials is disputed).


Mesolithic tomb, France (modern human)

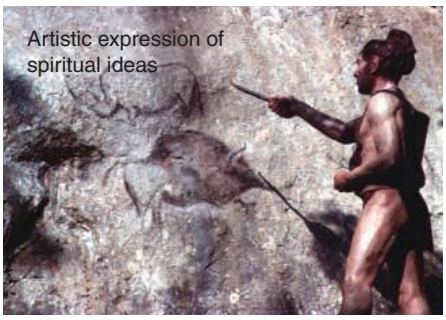
Rama CC SA 2.0

1. What might the various Venus figurines have represented? _____

2. What evidence is there that personal status may have played a part in ancient human society? _____

3. The earliest examples we have of various forms of art (e.g. figurines, painting etc) almost certainly indicate much earlier use of those art forms. Suggest why:

Art and spiritualism


Artistic expression of spiritual ideas



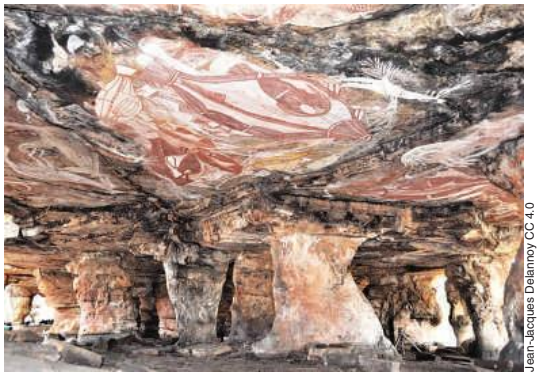


Ancient paintings were created using natural pigments such as charcoal, manganese oxide, and ochre, which were applied to grease smeared on the rock surface. Although we can never be certain of what the artist was thinking at the time, it is likely that much of the ancient art had a religious or spiritual purpose. This can be hypothesised because:

- many paintings occur in places that are difficult to access, e.g. deep in caves and often on the cave roof.
- many paintings in some way represent animals that were hunted or fertility (e.g. female genitals represented as "pubic triangles").
- many figurines represent pregnant females or attributes in women related to fertility.

Australian rock art

- Australian Aboriginal art and mythology has developed over the 60,000 years that Australia has been inhabited.
- However reliably dating art works, such as those painted on rocks and in caves, is notoriously difficult. Few sites in Australia have been reliably dated. Part of the problem is that the pigments used to create the artworks do not contain materials that are easily radiometrically dated using current techniques. Archaeologists must therefore use features around the paintings (such as layers of minerals formed over the pigments) to date them. The dates are almost always estimates and subject to interpretation.
- The oldest reliably dated rock art in Australia is found at Gabarnmung cave (right), a large rock shelter in south-western Arnhem Land, at the top of Australia's Northern Territory. The fragment of a rock painting is dated to around 28,000 years old, although human habitation there is dated to at least 44,000 years ago.
- The oldest reliably dated **intact** rock art is found in the Kimberly region. It is a painting of a kangaroo (right), dated at 17,500 years old. The painting was dated by radiometrically dating the fossilised nests of mud wasps that had been constructed over parts of the painting.
- As with other ancient rock paintings throughout the world, the ancient rock paintings in Australia are often of local animals (including fish), people or parts of the body (especially hands), and ancestral or mythological images.
- Different media have been dated to a variety of different dates. Bone ornaments have been dated to at least 40,000 years old. Some pigments (which may have been used in rock painting) have been dated to around the same time. Charcoal drawings have been dated to around 6000 years old and figures made using beeswax have been dated to 4500 years old.


Jean-Jacques Delannoy CC 4.0


17,500 year kangaroo painting. Redrawn from Pauline Heaney

4. What evidence is there that much of the ancient art found so far had a religious or spiritual purpose?

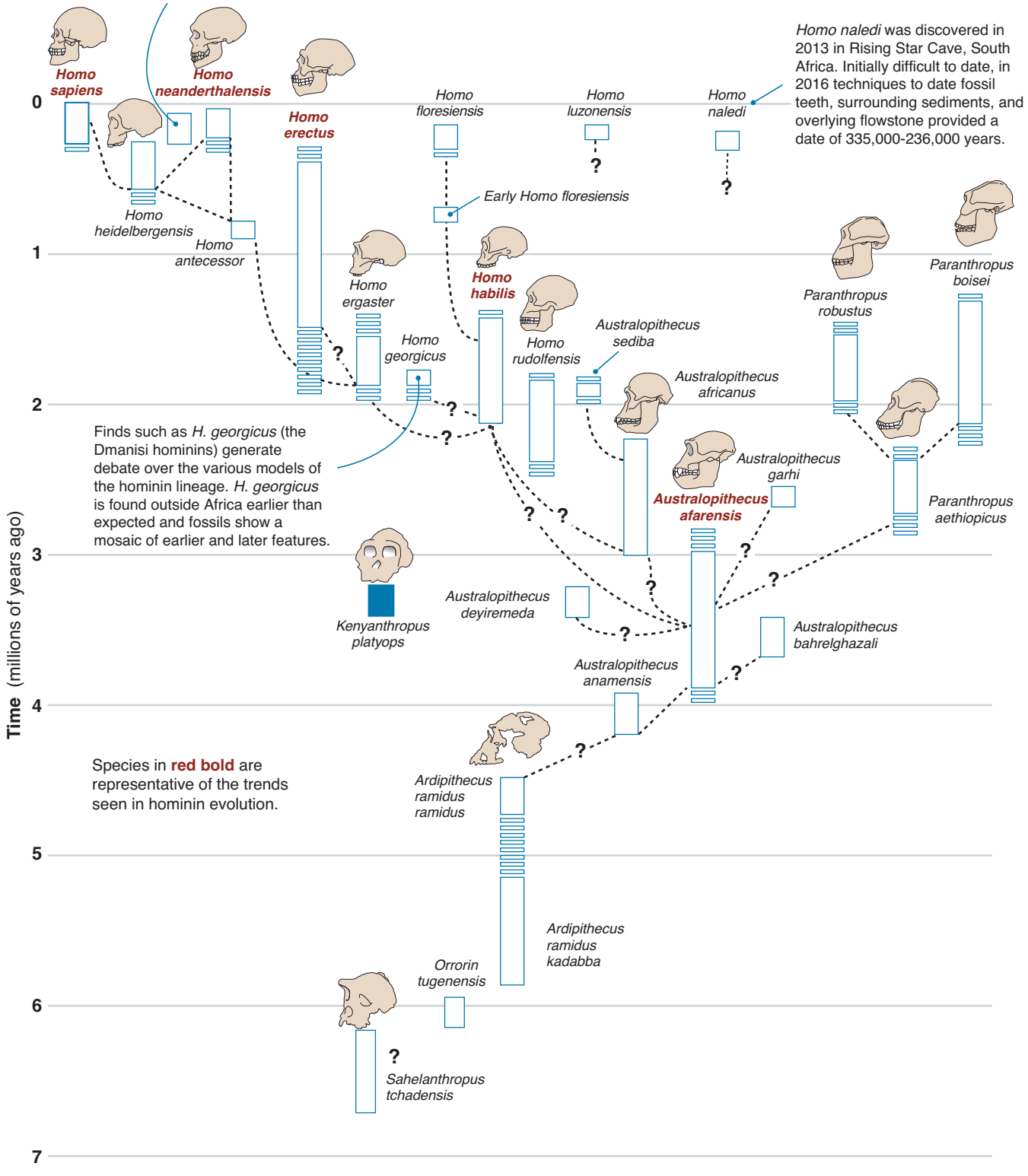
5. Clearly, early humans devoted great effort to art and ceremony. How might these skills have been adaptive?

160 Hominin Evolution: Probable Phylogenies

Key Idea: *Homo sapiens* are the last representative of a once extensive, multi-branched hominin evolutionary tree. Distinguishing the human lineage from all these species can be difficult and in some cases open to interpretation.

The diagram below shows a possible evolutionary history of hominins, demonstrating the fact that human evolution was not a linear sequence and that many phylogenies are tenable. There is much controversy over the interpretation of fossil data. The hominin lineage underwent an adaptive radiation about 3 mya, producing many different species. The genus *Australopithecus* gave rise to the genus *Homo* and the genus *Paranthropus*, which coexisted with early *Homo*, but eventually became extinct about 1 mya. The genus *Homo* is represented by many species as successive waves migrated out of Africa. *Homo sapiens*, which migrated out of Africa 80,000-60,000 years ago, is now the only living species.

It is important to remember that new hominin specimens are constantly being discovered. Their locations and relationships to other hominin species continually challenge our understanding of hominin evolution. The relationships between the various species are continually debated.



Group	Examples	
Archaic and modern humans	*Homo sapiens, Homo neanderthalensis, Homo heidelbergensis*	There have been many recent discoveries of relatively recent *Homo* species throughout Eastern Europe and Asia. How these fit into the hominin lineage and how they are related to modern humans are still debated. These specimens influence the interpretations of early human migration across the world.
Erectines	*Homo erectus, Homo ergaster, Homo floresiensis*	*Homo erectus* and closely related species show increasingly sophisticated tool cultures. *H. erectus* spread throughout Asia. There is much debate over how the different erectine species are related, with some palaeontologists suggesting that all the erectines should be labelled as one species.
Habilines	*Homo habilis*	This group shows the first signs of brain enlargement, more meat in the diet as well as the first recognisable stone tool culture. The post-cranial (below the head) skeleton remains small and slight, much like that of the australopithecines.
Paranthropines	*Paranthropus robustus, Paranthropus boisei*	These early hominins represent a group specialised for eating a bulky, low-grade vegetarian diet. They evolved large cheek teeth, powerful chewing muscles and a generally robust skull (large crests for muscle attachment, heavily buttressed face).
Australopithecines	*Australopithecus afarensis, Australopithecus africanus, Australopithecus sediba*	The earliest australopithecines were among the first apes to achieve bipedalism. They possessed a gracile body form and were probably opportunistic omnivores, scavenging meat from carcasses and exploiting a range of resources.
Very early hominids	*Ardipithecus ramidus, Orrorin tugenensis*	Essentially chimpanzee-like animals that have begun to show some human characteristics in their locomotion (bipedalism) and in the shape and arrangement of their teeth. *Sahelanthropus tchadensis* may be a common ancestor of chimpanzees and humans.

1. On the diagram on the previous page, colour in the blue outlined species boxes so that all species in the following groups are coloured the same: archaic and modern humans (orange), erectines (green), habilines (red), paranthropines (yellow), australopithecines (black), and very early hominins (blue).

2. Hominin evolutionary relationships are open to interpretation. The diagram on the previous page allows us to construct various phylogenies as hypotheses because the exact lineages are unknown. Starting at *Australopithecus afarensis*, complete three likely phylogenies for the evolution of *Homo sapiens* by writing the species in order of appearance. You can use the dotted lines to help you but other paths may be possible. The first one has been started for you:

   (a) Australopithecus afarensis , Homo habilis, Homo ergaster...

   (b) _____

   (c) _____

3. How might finds such as *Homo naledi* and *Homo georgicus* make us rethink our understanding of how and where humans evolved:

4. Which hominin existed for the longest length of time? _____

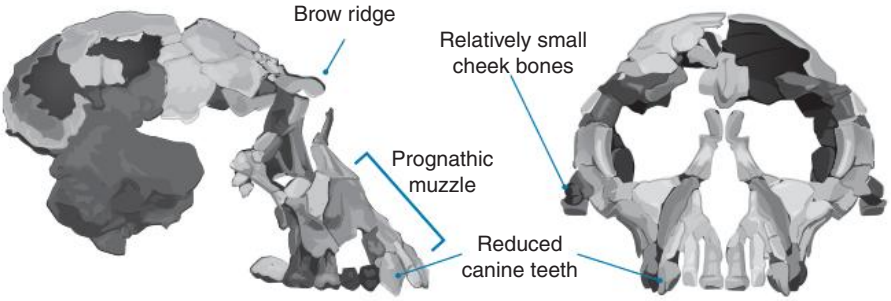
5. Which hominin is the probable most recent ancestor of *Homo sapiens*? _____

161 The Importance of *Ardi*

Key Idea: Analysis of the skeleton of *Ardipithecus ramidus* has produced findings that suggest bipedalism and a manipulative hand are very ancient features.

The first fossils of *Ardipithecus ramidus* were discovered in the Middle Awash region of northeastern Ethiopia in 1994. After many years of excavation, a partial skeleton was unearthed. Studying the skeleton of *Ar. ramidus* is beginning to change our understanding of hominin evolution. Until recently, it had been theorised that our earliest ancestors moved about very much like the chimpanzees of today. However the evidence from the *Ar. ramidus* skeleton shows that this is not the case and that bipedalism developed in quite a different way to what was once thought. Moreover, a dextrous hand developed early and is also an ancient trait.

Skull: The skull of *Ar. ramidus* shares certain features with *Australopithecus*, including a reduction in the size of the canine teeth in both male and females. This implies a reduction in aggression between males. The orientation of the base of the skull on which the brain stem rests suggests that the parts of the brain involved in visual and spatial perception were already beginning to develop.

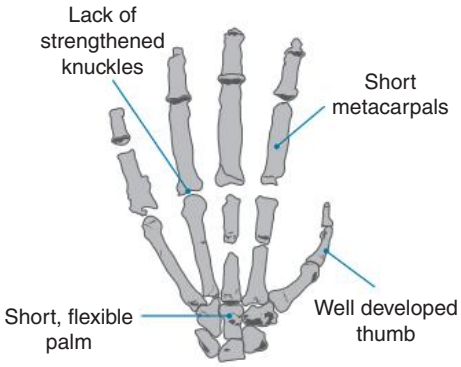


***Ardipithecus ramidus* skull features**

Hand: Analysis of the hand of *Ar. ramidus* shows that it is similar to our own, and that human hands are therefore close to the primitive form and not as greatly modified for tool use as was previously thought. *Ar. ramidus* had a flexible wrist and the opposable thumb was well developed. By contrast, chimpanzees move on the ground by knuckle walking, a motion that requires strengthening of the wrist and knuckle bones and lengthening of the palm, making the hand less flexible and not as dextrous.



Human hand



The primitive features of the ***Ardipithecus* hand** that are shared with *Homo*.

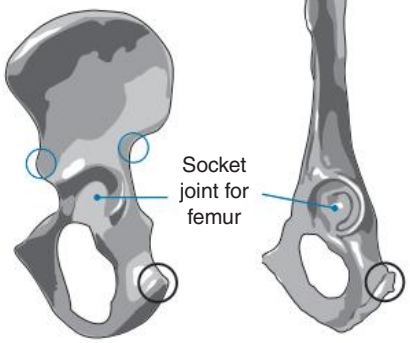
Pelvis: The pelvis of *Ar. ramidus* indicates that the modern pelvis, evolved for bipedal locomotion, began its evolution in the trees. Although several features of the upper pelvis strongly indicate bipedalism, features of the lower pelvis show that muscles associated with tree climbing were still well developed.

Features associated with bipedalism that are shared by *Ardipithecus* and *Homo*

Feature associated with tree climbing that is shared by *Ardipithecus* and *Pan*



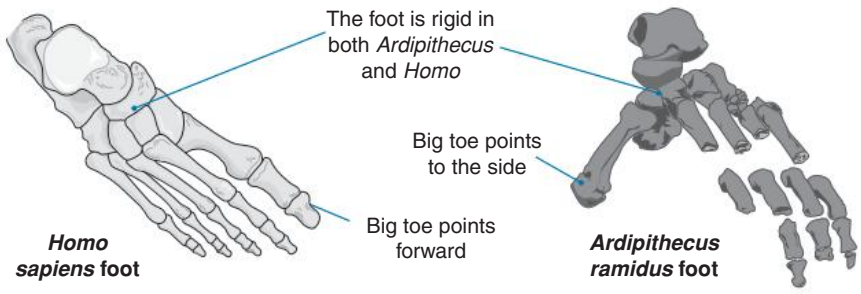
Human pelvis



***Ardipithecus ramidus* pelvis**

Chimpanzee pelvis

Foot: The foot of *Ar. ramidus* is a generalised one, with some human-like features, such as a rigid foot, as well as some modern ape-like features, such as an opposable big toe. These features indicate that *Ar. ramidus* spent considerable time climbing in trees.

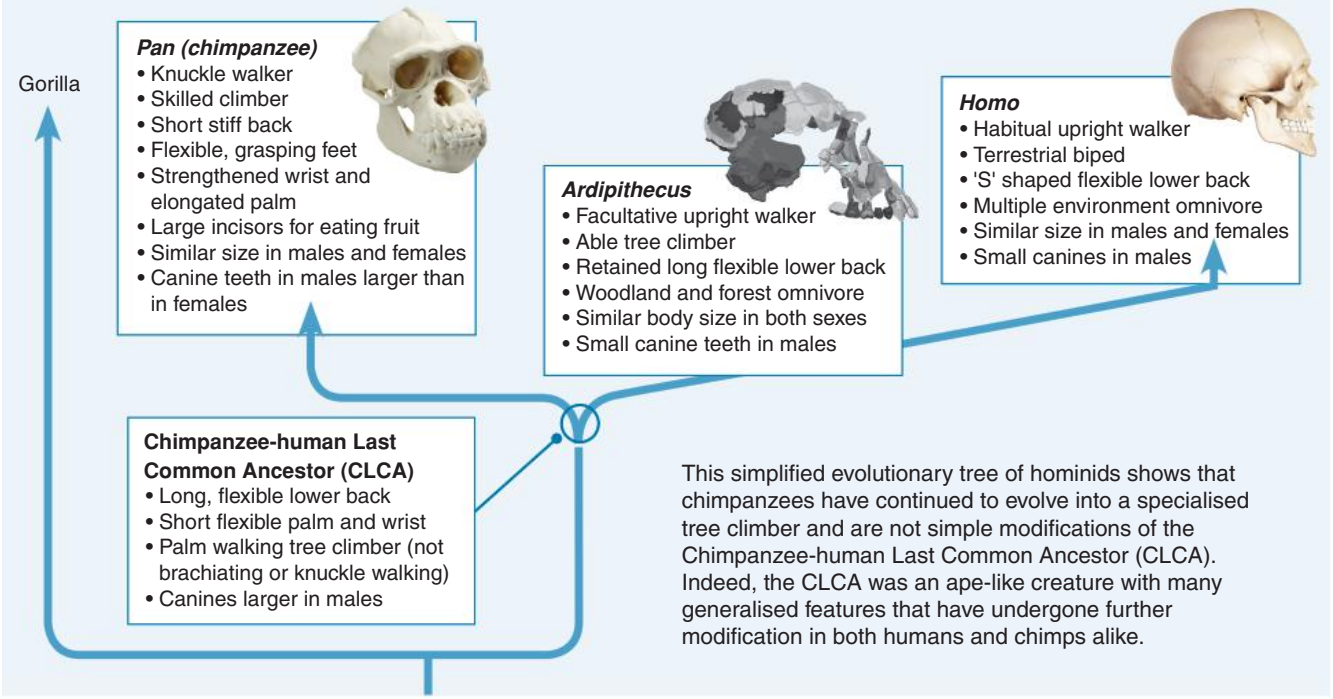


***Homo sapiens* foot**

***Ardipithecus ramidus* foot**

Images redrawn from C. Owen Lovejoy, et al Science, vol 326, 2009

Proposed evolutionary tree with *Ardipithecus*



Modified from C Owen Lovejoy, et al Science, vol 326, 2009

This simplified evolutionary tree of hominids shows that chimpanzees have continued to evolve into a specialised tree climber and are not simple modifications of the Chimpanzee-human Last Common Ancestor (CLCA). Indeed, the CLCA was an ape-like creature with many generalised features that have undergone further modification in both humans and chimps alike.

1. Describe the evidence for reduced aggression between *Ar. ramidus* males: _____

2. Explain why the human hand might now be viewed as the primitive type: _____

3. Describe the evidence for bipedalism developing in a primarily arboreal (tree-dwelling) ancestor rather than in a knuckle walking, terrestrial ancestor:

4. How does having a generalised body plan increase possible evolutionary pathways? _____

162 New Findings: Denisovans

Key Idea: DNA evidence suggests that early modern humans interbred with a previously unknown *Homo* species.

Fragments of bone and teeth found in a cave in Siberia indicated that a previously unknown *Homo* species once lived in Asia. The Denisovans, (after the cave in which the fossils were found) have yet to be assigned a species name.

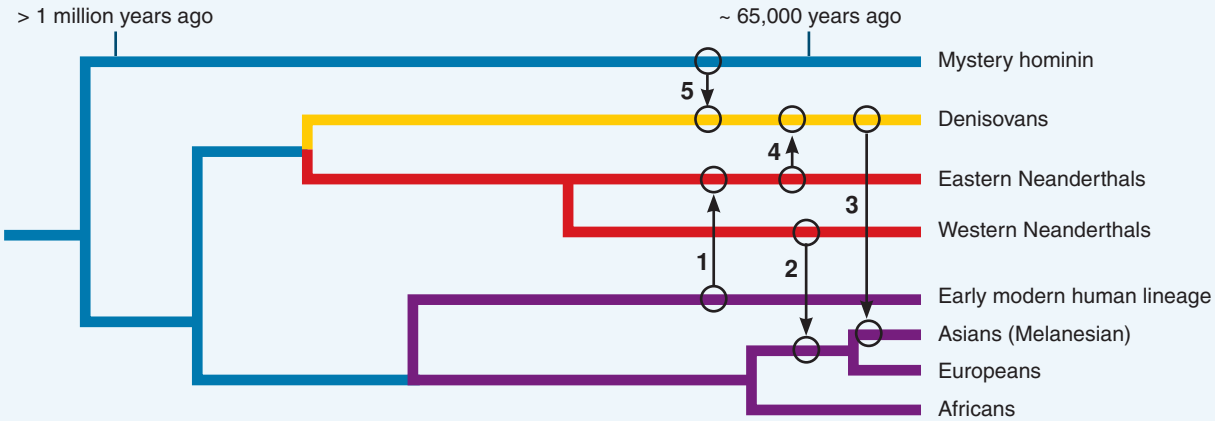
The Denisova cave finds

- In 2008, archaeologists discovered a fragment of finger bone in the Denisova cave, in Siberia. The bone fragment belonged to a juvenile female (named X-woman).
- Artefacts, such as a bracelet, were found at the same level as the finger bone.
- In 2010, a molar tooth was found at a different level to the finger bone, indicating it belonged to a different individual. A toe bone found in 2011 was at the same level as the tooth.
- The molar found in the Denisova cave has unique characteristics, which are not present in the molars of Neanderthals or modern humans.
- Carbon dating estimates the age of the artefacts and bone fragment at 40,000 years.
- Fossil DNA degrades quite rapidly with increasing temperature and in acidic soil conditions. The cool temperatures within the Denisova cave preserved the DNA in the fossil fragments. The fossils contained very low levels of DNA contamination from other organisms.



The Denisova cave, in the Altai mountains, Siberia, Russia

Obersachse



Source: Nature 2016

Probable interbreeding events

1. Early modern humans and Neanderthals
2. Humans and Neanderthals
3. Humans and Denisovans
4. Neanderthals and Denisovans
5. Denisovans and a "ghost" population of hominins

Nuclear DNA analysis suggests the Denisova fossils belong to a hominin species that existed at the same time as modern humans and Neanderthals, but was genetically distinct from them. The fossils (the **Denisovans**) have not yet been formally classified.

Nuclear genome analysis suggests the Denisovans were a sister group to the Neanderthals. They probably shared a more recent common ancestor with Neanderthals (~300,000 years ago) than with present day humans (~400,000 years ago).

The Denisovans interbred with the ancestors of present day Melanesians (right), and possibly with Neanderthals. There is also molecular evidence that the Denisovans interbred with an unknown hominin species known only from DNA analysis.



A Melanesian woman

1. Why are the Denisovans difficult to classify? _____

_____

2. (a) What modern human lineage appears to have interbred with the Denisovans: _____

   (b) What percentage of DNA does this lineage appear to share with the Denisovans? _____

3. Why was the Denisovan DNA in remarkably good condition? _____

_____

163 New Interpretations: The Neanderthals

Key Idea: New, more complete analysis of Neanderthal DNA is revealing multiple interbreeding events with early humans.

Neanderthals appeared about 400,000 years ago, and disappeared 25,000-30,000 years ago. They lived in Europe and parts of western and central Asia. Neanderthals are the closest relative to modern humans, so there is considerable interest in analysing the Neanderthal genome. By comparing the Neanderthal genome to the genome of present-day humans, it may be possible to identify genes in modern humans that have been influenced by positive selection.



NIH DNA being extracted from a Neanderthal fossil



Front view Neanderthal skull Image: Bone clones



La Ferrassie Neanderthal skull, France

Difficulties in analysing Neanderthal DNA

- The DNA is often degraded to small fragments less than 200 base pairs long. This makes it difficult to obtain sequence overlaps (critical for assembly of the complete genome).
- The DNA is often of poor quality because it has been chemically modified and degraded by the environment.
- Samples are often contaminated with the DNA of other organisms. Between 95-99% of the DNA obtained from the Neanderthal fossils analysed was from microbes that colonised the bone after the Neanderthal died. Researchers must be careful not to contaminate the sample with their own DNA.

What has been found?

The continuing analysis of Neanderthal (and Denisovan) DNA has found that there were at least five interbreeding events between humans (*H. sapiens*), Neanderthals, and Denisovans.

- **Between archaic humans and Neanderthals**. Analysis in 2016 shows there may have been an interbreeding event around 100,000 years ago when an early wave of humans migrating out of Africa met a group of Neanderthals migrating from Europe to Asia.
- **Between humans and Neanderthals**. Analysis shows that between 1-4% of the genomes of people outside of Africa is derived from Neanderthals (more than for Africans). It is thought these encounters may have occurred as humans migrated out of Africa around 50,000 - 60,000 years ago and met Neanderthal populations already in the Middle East.
- Some human populations that migrated east across Eurasia interbred with the Denisovans. Evidence of this in found in Melanesian DNA (see opposite).
- Denisovans also interbred with Neanderthals, probably about 50,000 years ago (see opposite).
- Denisovans interbred with an unknown group of hominins, possibly an offshoot of *H. erectus*, about 100,000 years ago.

The Neanderthal legacy

Analysis of Neanderthal DNA published in 2016 suggests that they carried various mutations that made them up to 40% less reproductively fit than modern humans. When interbreeding occurred with humans, some of these mutations would have been passed to the human gene pool. Over time, most of the harmful mutations were discarded through natural selection, but some have remained. Other genes that may have been beneficial also entered the gene pool. However the benefits that these genes once conferred may no longer exist as the human lifestyle becomes more sedentary and diets change.

Studies matching health problems to Neanderthal DNA have found that genetic variants inherited from Neanderthals are linked to an increase in the risk of heart attacks, depression, skin disorders, and nicotine addiction. However, the Neanderthal DNA may not necessarily be causing the health problem. It might just be associated with human DNA that is.

Some genes that were possibly inherited from Neanderthals or Denisovans have provided benefits. Tibetans appear to have inherited Denisovan genes that enabled high altitude adaptation. Humans may also have inherited genes associated with immunity to new diseases found outside of Africa, but already encountered by Neanderthals.

1. What percentage of Neanderthal DNA is present in modern humans? _____

2. In which group of modern humans is Neanderthal DNA mostly found and why? _____

_____

3. Describe some possible positive and negative effects of Neanderthal DNA in modern humans: _____

_____

_____

_____

164 Problems With Interpretation: *H. floresiensis*

Key Idea: The discovery of *Homo floresiensis* has proved highly controversial with several hypotheses proposed to explain its position in hominin evolution.

In 2003, hominin fossils, including an almost complete skeleton, were discovered on the island of Flores, Indonesia. The fossils were assigned to a new species, *Homo floresiensis*, thought to have lived on the island as recently as 18,000 years ago. However, revised dates in 2016 indicate that *H. floresiensis* lived ~190,000-50,000 years ago. The new date is close to the time that modern humans reached the area, suggesting that encounter with *H. sapiens* may have contributed to the demise of the Flores population.

Homo floresiensis was very small and fully bipedal. Although the brain was very small, its organisation was advanced and stone tools associated with the skeleton indicated well established hunting technology. In contrast to these features, aspects of the skeleton showed primitive features found only in apes and early hominins. *H. floresiensis* probably lived 190,000 to 50,000 years ago, making it one of the latest surviving hominins along with the Neanderthals and the Densiovans. Its discovery caused widespread controversy and several opposing hypotheses were put forward to explain its place in human evolution. Since its discovery, earlier fossils dating to 700,000 years old have been found.


Rosino

The fossils were discovered in Liang Bua, a limestone cave on Flores Island, Indonesia. The cave contains 12 m of stratified deposits. The remains of modern humans, as well as *Homo floresiensis*, have been found in the cave.

Different interpretations of the fossils

The discovery of *Homo floresiensis* caused a storm of controversy, not helped by the fact that the specimens were removed from their repository by one palaeontologist, kept from other scientists for three months, and returned damaged. The Indonesian government also denied scientists access to the cave where the fossils were found for two years. Several hypotheses were eventually put forward to explain how such a diminutive hominin evolved or survived. These included:

- *H. floresiensis* evolved a result of island dwarfism. Island dwarfism is a relatively common occurrence where large animals become smaller over time when isolated, e.g. the extinct pygmy elephants on Flores showed this adaptation.
- *H. floresiensis* is not a new species but instead an individual with a disease or disorder. Some scientists put forward the idea that the small skull was a result of microcephaly, a neurodevelopment disorder. Others thought that perhaps *H. floresiensis* suffered from congenital hypothyroidism.
- Detailed examination of the bones showed no overlap with any features expected from individuals with the diseases or disorders listed above. A study of the bones and joints of the arm, shoulder, and lower limbs concluded that *H. floresiensis* was more similar to early humans and apes than modern humans. Small brain size coupled with more advanced brain organisation indicate possible parallel evolution of sapiens-like features.



Brain size: Very small, only 380 cc.
3D scans showed expansion of the prefrontal cortex and temporal lobes, brain regions associated with complex thought.

Facial features: Relatively modern dentition, but teeth are large relative to the rest of the skull.

No chin present.


Scallyna CC 4.0

Homo floresiensis *Homo sapiens*

1. Explain the effect that the Flores finds had on the hypothesis that hominins continually evolved larger brains and bodies:

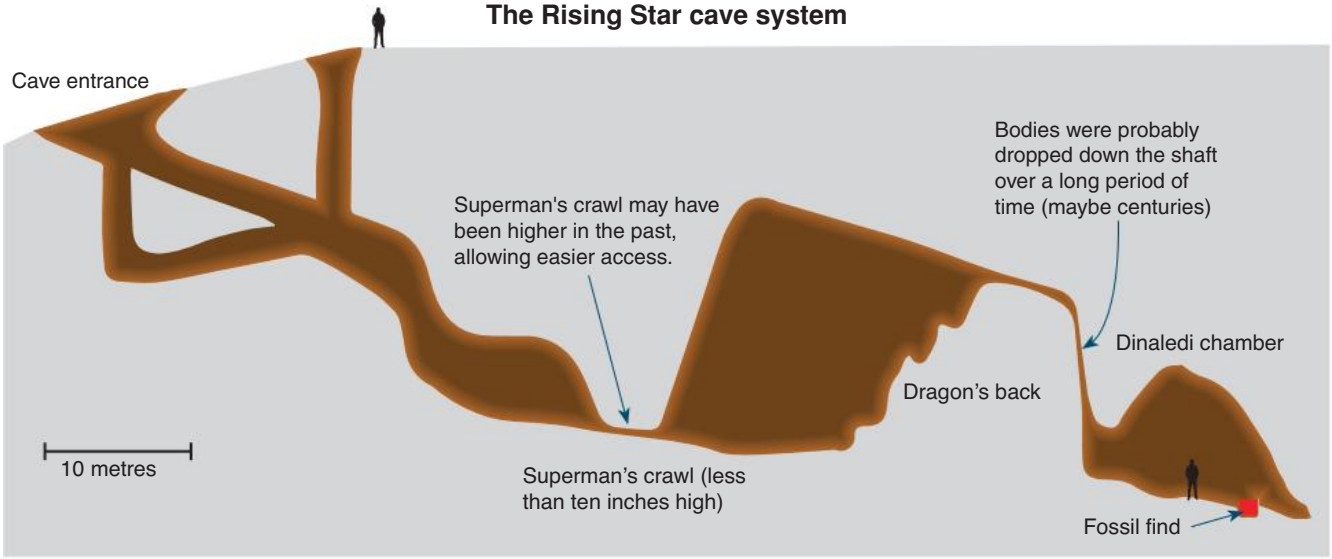
2. Describe the different interpretations of the *H. floresiensis* bones:

165 Problems with Dating: *H. naledi*

Key Idea: The discovery of *Homo naledi* presents problems. The placement of the bones appears deliberate, which seems unlikely for an ancient hominin, and they are difficult to date because there are no dateable sediments around them.

In 2013 two cavers exploring the Rising Star cave system near Johannesburg in South Africa found a passage that led to a chamber containing the bones of a new hominin species. Excavation has found more than 1500 specimens from at least 15 individuals including ribs, skulls, jaws, and dozens of teeth. Interestingly, no other types of animal or plant have been found in the cave and there is no indication of water flowing in the past. This has led the investigators to hypothesise that the bodies were deliberately placed there by other *Homo naledi*. Also the lack of other flora or fauna and sediments (other than cave dust) has made it very difficult to date the fossils. They have both advanced and primitive features, which some think puts them at the cusp of the transition between *Australopithecus* and *Homo*.

The Rising Star cave system



Dating *Homo naledi*

- Dating the *Homo naledi* bones is problematic because they were found deep in the cave. Ordinarily, fossils can be dated by relative dating. Other fossils in the sediment can be used to date the unknown fossils. For example, if the bones of a predator species of a known age had been found in the cave, then it may imply that *H. naledi* lived at the same time. However, only *H. naledi* bones have been found, expect for a few small birds on the surface. Radiocarbon dating cannot be used because it only dates accurately to 50,000 years of age and *H. naledi* is likely to be much older than that.
- If the bones had been washed into the cave by a river system it may have been possible to use the sediments deposited or other bones that had been washed in to provide a date. The excavation team has yet to find any evidence of a river or water flow. One other way of dating the bones is by dating the flowstones found in the cave. Flowstones are sheets of calcium carbonate built up by water flowing down cave walls (similar to stalactites). However the flowstones do not cover much of the cave floor and fossils.
- Various anatomical ways of dating the fossils were tried, initially providing a date of ~1-3 my. However, more recent radiometric dating of specimens and the overlying flowstones has provided a much more recent date of 335,000- 236,000 years.



Lee R Berger CC 4.0

1. Describe one of the problems in explaining the deliberate placement *Homo naldei* bones in the Dinaledi chamber.

_____

_____

2. Describe two reasons why dating the *H. naledi* bones is difficult: _____

_____

_____

_____

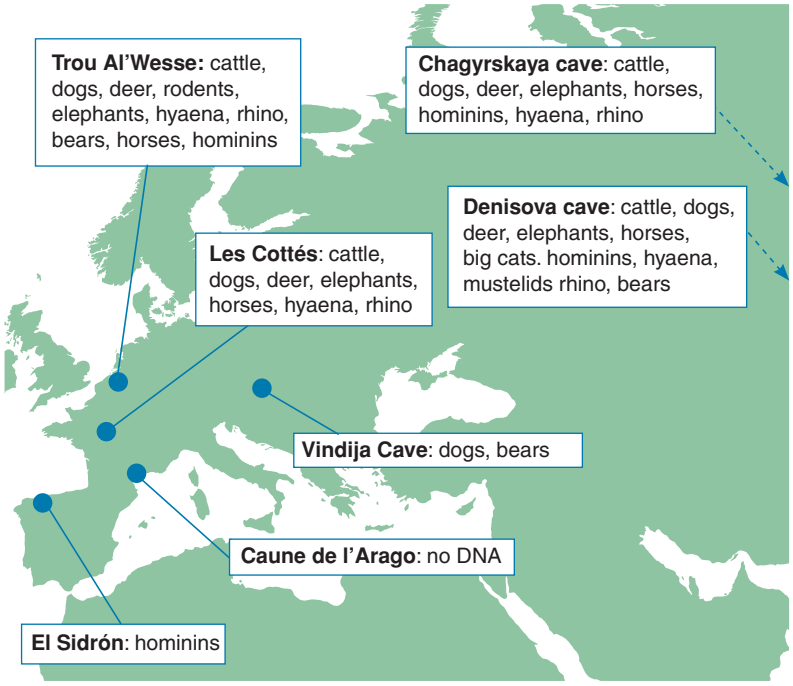
166 New Techniques: Ancient DNA

Key Idea: DNA surviving in sediments can help us understand more about the evolution of hominins and their environment.

For most of the time people have studied hominin evolution they have had to rely on finding physical evidence of hominin presence. This might include fossil bones, or evidence of occupation, including tools and fire pits. In the late 1990s, techniques were developed that allowed DNA to be extracted from fossil bones. This helped reconstruct the first Neanderthal DNA. Recently techniques have been developed that have allowed researchers to extract DNA directly from sediments. This means the presence of hominins can be found even without physical fossil evidence.

Finding ancient DNA

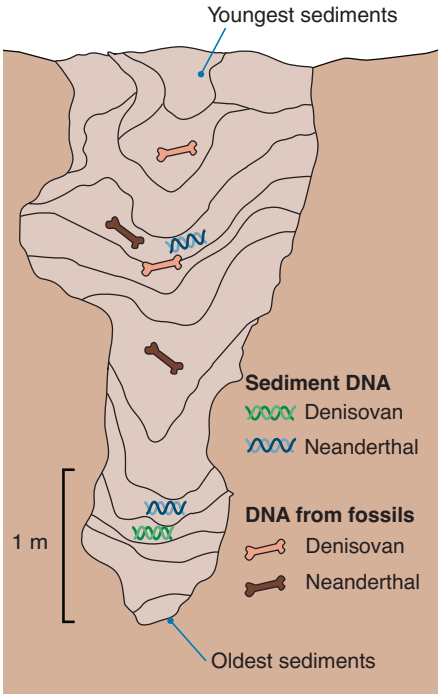
- In 2017 researchers collected sediment samples from a number of caves around Europe where ancient hominins were known to have lived. The evidence of occupation at some of these sites consisted only of stone tools of specific design, such as Mousterian tools at Trou Al'Wesse cave in Belgium.
- The sediment samples were treated to release any DNA. mtDNA was targeted because this is much more abundant than nuclear DNA.
- Because only a very small fraction of the DNA was going to be from ancient hominins (or ancient mammals of any kind) the DNA was identified using hybridisation capture. RNA or DNA probes designed to bind to mammalian or hominin DNA identify its presence.
- To check the DNA found was actually ancient, DNA researchers looked for specific types of damage that accumulate in the DNA over thousands of years.
- Between 10% and 0.05% of the samples were found to be mammalian DNA and (on average) only 7.5% of that was hominin DNA.



DNA from hominins and many different groups of mammals have been found at many sites. Mammals may have lived or been butchered at these sites.

Adding to the Denisovan cave

- The Denisovans are known only from fragments of fossils from a few occupation horizons in the Denisova cave. Using the technique above researchers were able to identify DNA belonging to both Neanderthals and Denisovans in the sediments of occupation horizons where no fossils or tools had previously been discovered.
- The diagram on the right shows the layers of sediments in the Denisova cave. Ancient DNA in the sediments has shown occupations extend maybe tens of thousands of years.



1. The technique for extracting and identifying ancient was first carried out at sites known to have been occupied by ancient hominins. Why test these sites when occupation was already known to have occurred?

2. Ancient DNA found the DNA of many types of mammals in these caves tested. What is the most likely explanation for finding these mammals?

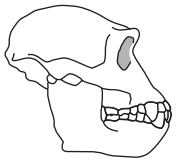
3. How has using ancient DNA added to our knowledge of hominin occupation in the Denisovan cave?

167 A Summary of Trends in Hominin Evolution

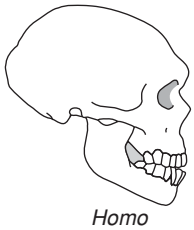
Key Idea: Specific trends can be seen in human physical (biological) and cultural evolution.

Use this activity to revise your knowledge about trends in human evolution. Cut out the images on page 289 and place them in their correct place on the timeline of human evolution (below and on page 291). Add notes about significant developments and trends. These may include information about the tool technology (including the tool user), skull features and brain size (represented by blue circles), bipedalism, how food and diet influenced dentition, and hominin distribution.





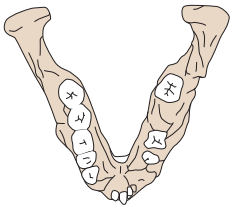
Australopithecus afarensis



Homo neanderthalensis

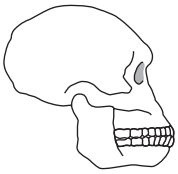
1350 cc



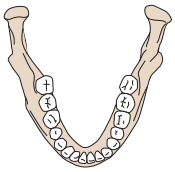


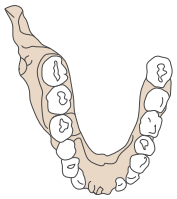






Homo habilis



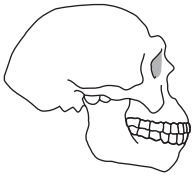


1200-
1700 cc



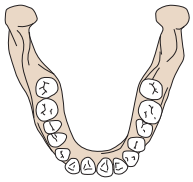


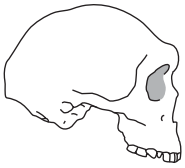
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Homo erectus

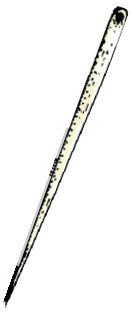






Archaic *Homo sapiens*



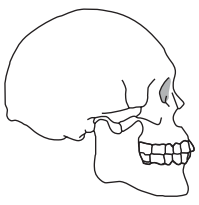


375-
550 cc

500-
800 cc

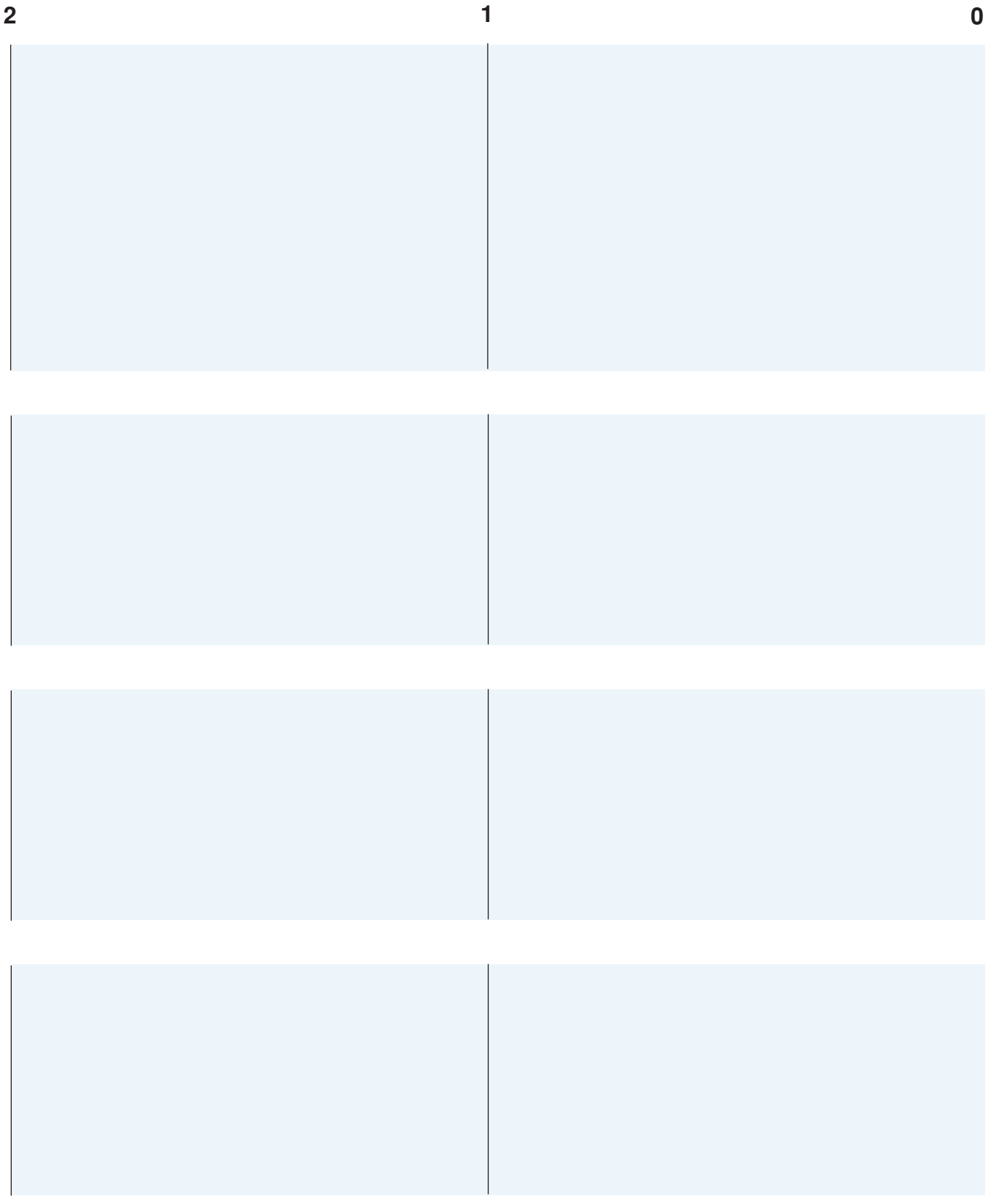
1000-
1250 cc





Homo sapiens

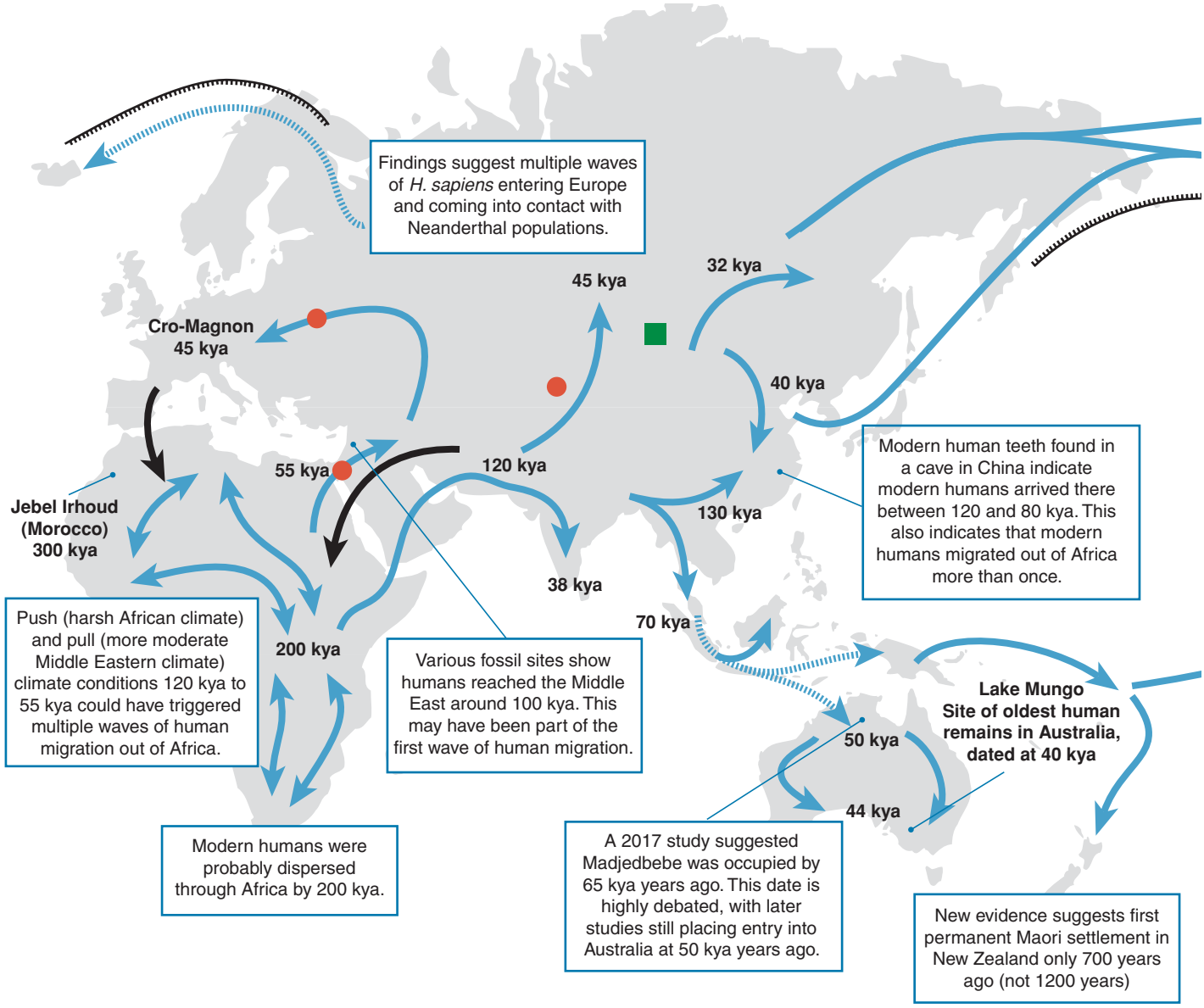
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168 The Dispersal of Modern Humans

Key Idea: Hominins evolved in Africa and dispersed throughout the globe. Waves of migrations of *Homo sapiens* probably occurred from as early as 180,000 years ago.

The map below shows a suggested probable origin and dispersal of modern humans throughout the world. An African origin is almost certain, with south eastern Africa being the most likely region. Evidence generally supports continual waves of migrations of *H. sapiens*, perhaps from as early as 180,000 years ago. The dispersal was influenced at crucial stages by the last glacial, when ice sheets covered much of Europe and 'land bridges' formed as a result of the associated drop in sea level. Recent evidence suggests that island-hopping and coastal migration may also have been important, e.g. for the movement of people into Indonesia. The late development of boating and rafting technology slowed dispersal into Australia and the Pacific. New Zealand was one of the last places on Earth to be populated. (On the map, **ya** = years ago.)



1. (a) Suggest why modern humans reached Europe much later than they reached East Asia: _____

_____

_____

_____

(b) Suggest why New Zealand was one of the last land masses populated by humans: _____

_____

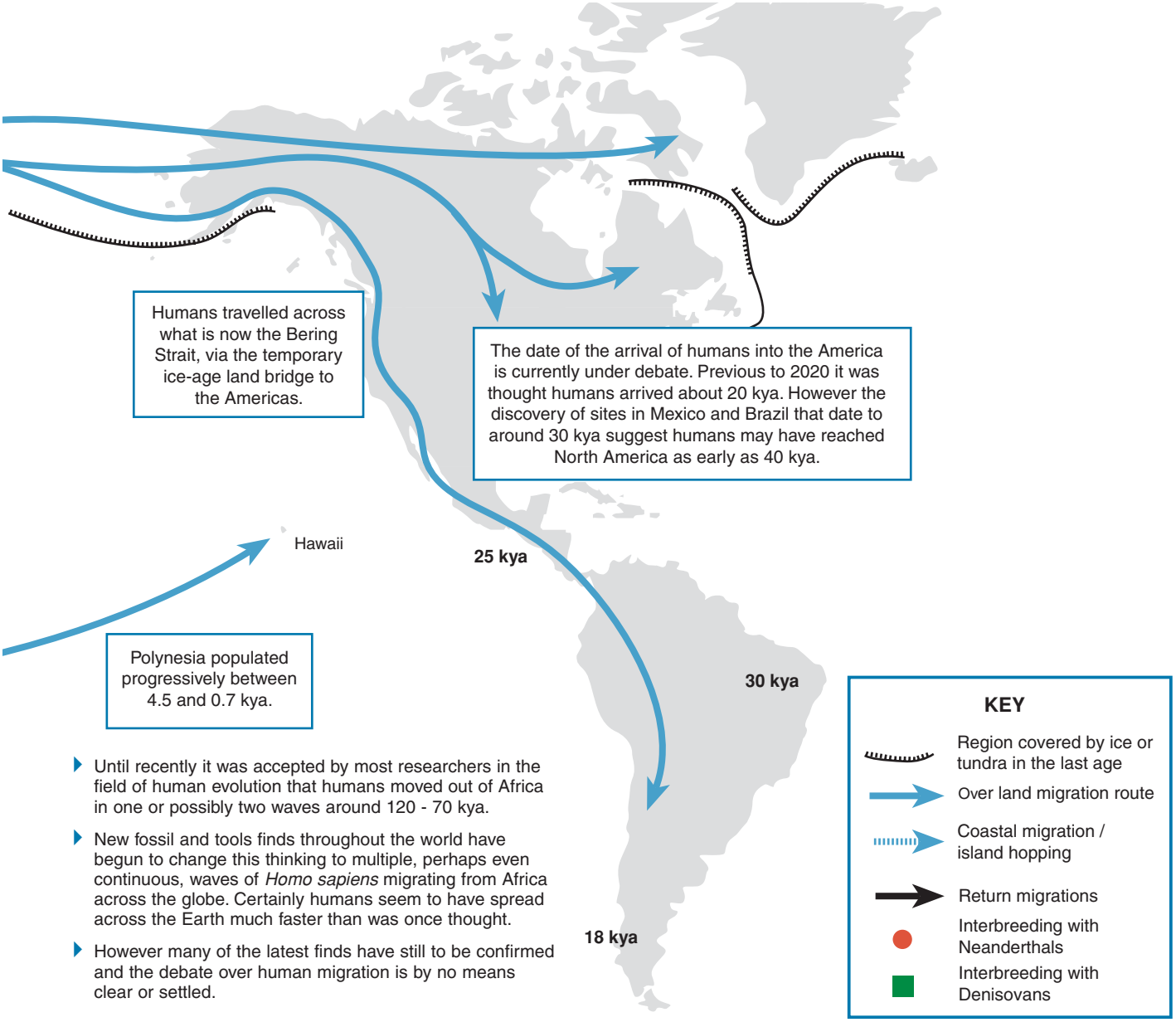
_____

_____

When humans arrived in Australia around 50,000 years ago, giant marsupials still roamed the land. In fact there is speculation that some of the fabled monsters in various Aboriginal oral histories may have been based on some of these megafauna (e.g. was the Yamuti based on *Diprotodon* (right)). Rock art in the Kimberly appears to show large marsupials such as the marsupial lion.



For a long time it was assumed that humans caused the extinction of this marsupial megafauna. However, a 2020 study suggests this was not the case. It argues that climate change caused the extinction of the megafauna as they disappear from the fossil record about the same time as widespread changes in available water and vegetation, as well as an increase in fire frequency.



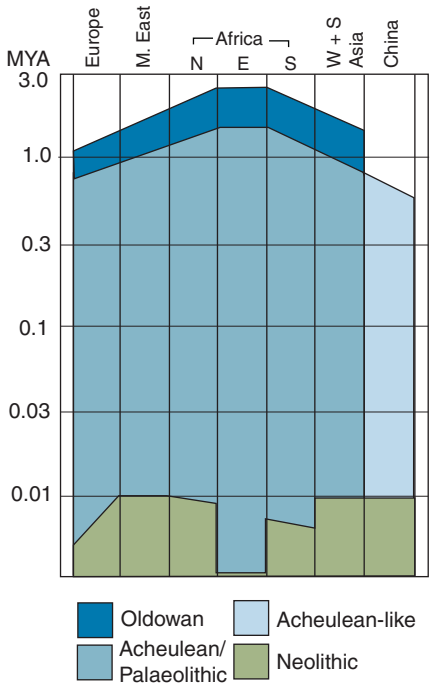
- Until recently it was accepted by most researchers in the field of human evolution that humans moved out of Africa in one or possibly two waves around 120 - 70 kya.
- New fossil and tools finds throughout the world have begun to change this thinking to multiple, perhaps even continuous, waves of *Homo sapiens* migrating from Africa across the globe. Certainly humans seem to have spread across the Earth much faster than was once thought.
- However many of the latest finds have still to be confirmed and the debate over human migration is by no means clear or settled.

2. Using examples, discuss the importance of land bridges, glaciations, and island hopping in the global dispersal of modern humans:

Evidence for dispersal

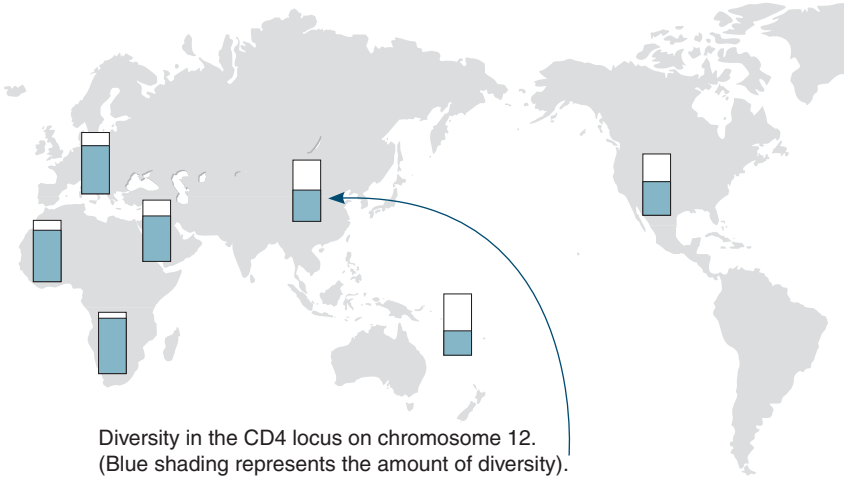
There is little doubt that humans evolved in Africa. Fossils of ancient hominins such as *Australopithecus afarensis*, *A. africanus* and *Homo habilis* are all found in Africa. Analysis of fossils and genetic evidence supports theories that *Homo erectus* migrated out of Africa about 1.8 mya. Populations of *H. erectus* remaining in Africa gave rise to *H. heidelbergensis*, which also migrated out of Africa about 650,000 years ago but remained in Europe and Western Asia. Those populations gave rise to *H. neanderthalensis* and Denisovans. *Homo heidelbergensis* in Africa eventually gave rise to *H. sapiens. H sapiens* began to migrate out of Africa between 120,000 and 70,000 years ago but the movement into Europe was slowed by a period of cold climatic conditions.

Acheulean stone tools are found throughout Africa, Europe, and South and Western Asia, but appear to be largely absent from Eastern Asia. Tool cultures develop first in Africa then move to other parts of the world.



Analysis of various loci in the human genome show humans have very little genetic diversity. The analysis shows that outside of Africa, human genetic diversity is a sub-set of African genetic diversity.

	Diversity (0 = low)		
Locus (measurement)	Africa	Asia	Europe
30 microsatellites	0.807	0.685	0.730
Xq 13.3 (short arm X chromosome)	0.035	0.025	0.034
50 autosomal sequences	0.115	0.061	0.064
mt DNA control region	2.08	1.75	1.08



Diversity in the CD4 locus on chromosome 12.
(Blue shading represents the amount of diversity).

3. What does the evidence above say about hominin dispersal across the globe? _____

4. When did modern humans appear in Europe? _____

5. How did humans manage to migrate into the Americas? _____

6. How did tool development change from 3 million years to the end of Neolithic times? _____

7. What push and pull factors might have driven human migration out of Africa: _____

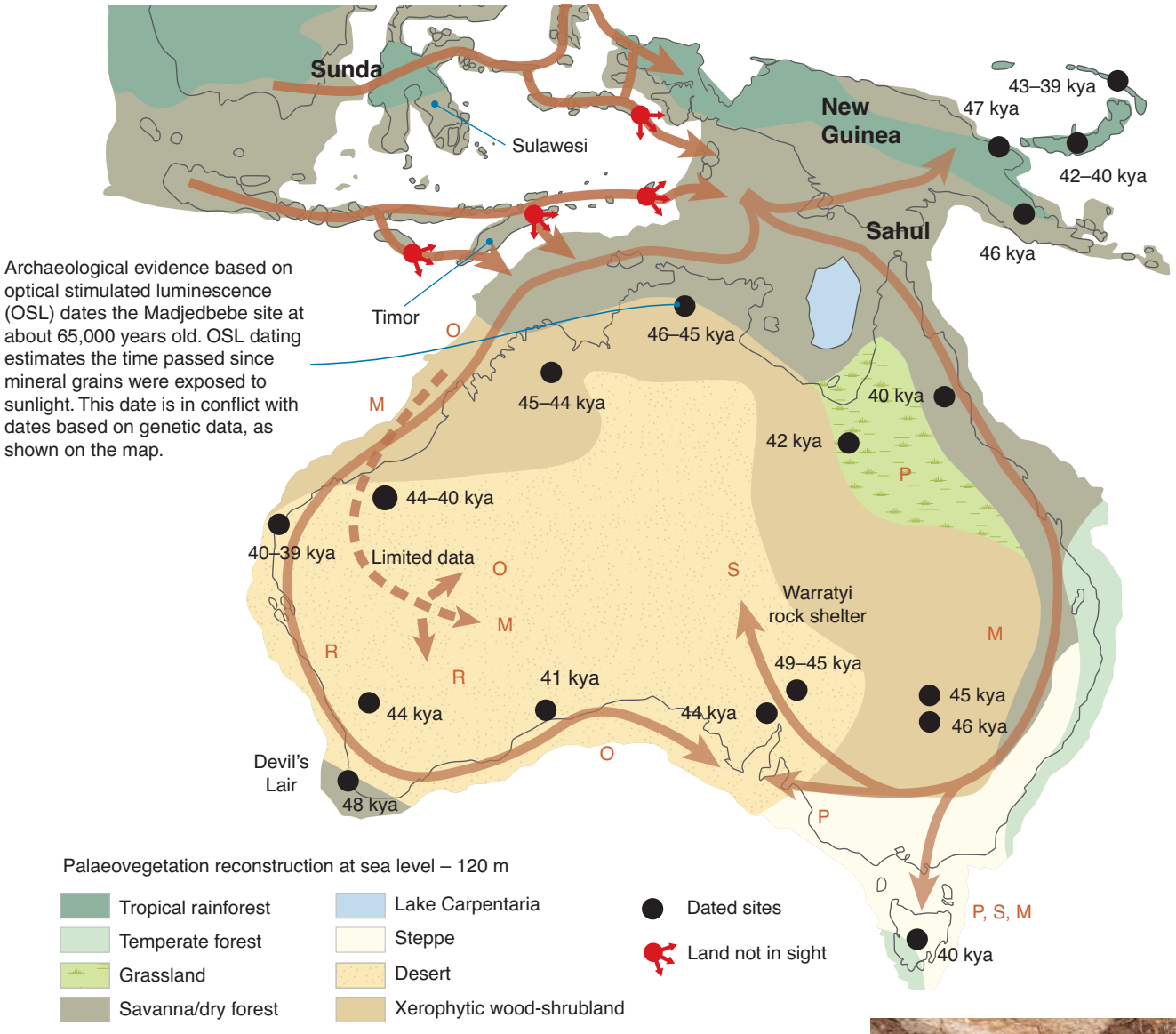
169 The Populating of Australia

Key Idea: The populating of Australia began about 50,000 to 60,000 years ago via New Guinea. Humans had spread across Australia by 40,000 years ago.

After it split from Gondwana, the Australian continent (technically known as Sahul) has remained disconnected from the rest of the world. Low sea levels 70–60,000 years ago saw a land bridge form between mainland Australia and the island of New Guinea to the north, but there has been no connection of Sahul to the other continents. Therefore, to reach Sahul, humans must have travelled across open water by some kind of watercraft. The exact route taken is unknown but it is likely people first reached Australia via island hopping either from Sulawesi to New Guinea (then walking to Australia) or from Timor to Australia. They then followed coastal routes around Australia.

Populating Sahul

- The map below shows the possible routes taken to reach Sahul. Even by island hopping, people would have had to take a route across open water at some point along the journey where they could not see land on the horizon. Some reasonable form of seafaring transport would have been needed.



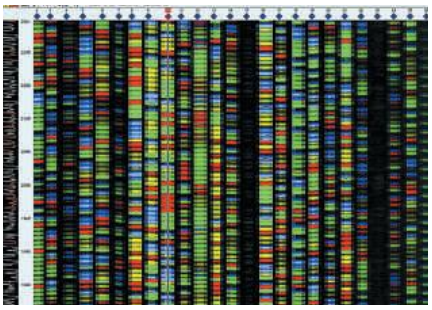
Archaeological evidence based on optical stimulated luminescence (OSL) dates the Madjedbebe site at about 65,000 years old. OSL dating estimates the time passed since mineral grains were exposed to sunlight. This date is in conflict with dates based on genetic data, as shown on the map.

Adapted from Tobler *et al* Nature, 2017. See credits for full reference.

- There is much debate over exactly when the ancestors of Aboriginal Australians actually arrived in Sahul. Some archaeological sites suggest dates as early as 65,000 years ago, whereas other sites and DNA evidence suggest about 50,000 years.
- Either way, the latest and most comprehensive mitochondrial DNA analysis has shown the modern Aboriginal Australians are indeed directly related to the first humans to populate Australia. Mitochondrial haplotypes* fall into five major groups labelled S, O, M, P and R. The movement of these groups is shown on the map above. **A haplotype is a group of genes that was inherited together from a single parent.*
- Results are consistent with the hypothesis that a wave of modern humans migrated out of Africa 80–70,000 years ago. Some of those humans arrived in Australia around 50–60,000 years ago. Interestingly, Aboriginal Australians diverged from Papuans about 40,000 years ago, long before the physical separation of the two countries 10,000 years ago.
- The diagram above shows the estimated routes taken during the populating of Sahul based on the five mitochondrial haplotypes.



Rock shelters are sources of much archaeological evidence of Australia's settlement.



Like other modern human groups outside of Africa, Aboriginal Australians carry about 2.1% Neanderthal DNA. This indicates they were part of the latest human migration out of Africa and not an earlier separate dispersal.



A recent study on a 45,000 year old human leg bone found in Siberia showed it too had 2.1% Neanderthal DNA. However the DNA was in large chunks rather than dispersed as in modern humans. Using this as a marker, scientists were able to date the migration out of Africa at around 55,000 years ago.



Whatever the date of humans arriving in Australia, it is clear Aboriginal Australians have been there a very long time with no later migrations or major contacts. Animal arrivals such as the dingo may have arrived by way of traders, but these people did not leave any evidence of their contact.

1. Describe routes taken by humans to Sahul (Australia): _____

2. Looking at the dates on the map, what can be said about the rate at which Australia was populated?

3. Looking at the locations where the major haplotypes are found, what can be said about migration within Australia after the initial population event?

4. Describe the routes taken when populating Australia and suggest why these routes were taken:

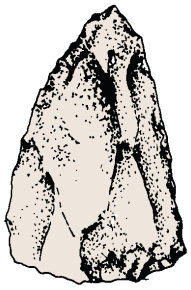
5. Describe the evidence of humans populating Australia. Where does the evidence conflict and agree?

6. What evidence is there that ancestral Aboriginal Australians were part of the latest wave of human migration out of Africa rather than an earlier wave?

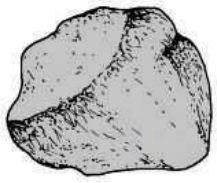
170 Chapter Review: Did You Get It?

1. Identify the following tools as Oldowan, Acheulean, Mousterian, or Upper Palaeolithic:

A 

B 

C 

D 

(a) A: _____ (c) C: _____

(b) B: _____ (d) D: _____

2. *Homo erectus* is believed to be the first user of fire. Describe the benefit the use of fire would have had for them:

_____

_____

_____

_____

_____

3. Draw lines to match up the hominid name with its meaning, then match the name to the correct skull number (1-8 below).

Name:	**Skull number:**	**Meaning:**
Australopithecus afarensis	_____	Man from the Neander Valley.
Australopithecus africanus	_____	Handy man
Homo neanderthalensis	_____	Southern Ape from Afar, Ethiopia
Homo sapiens	_____	Southern Ape from Africa
Homo habilis	_____	Man from the island of Flores or Flores man
Homo erectus	_____	Knowing, or wise man
Homo floresiensis	_____	Upright man

1 

2 

3 

4 

5 

6 

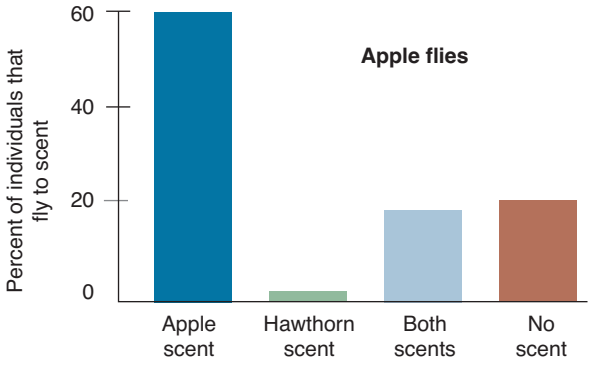
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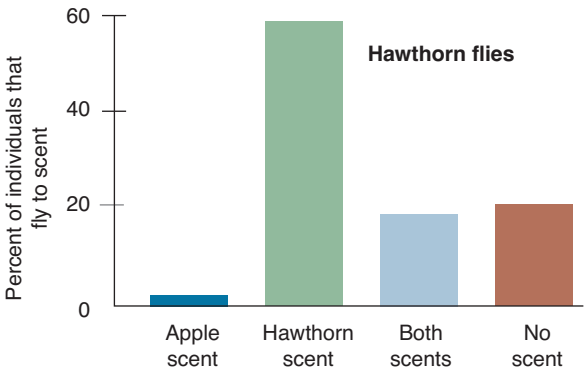
171 Synoptic Questions: Unit 4, Area of Study 2

1. Apple maggot flies are native to North America. They infest the fruit of apple trees, laying eggs in the fruit, which develop into maggots that burrow into and eat the fruit. However, apple trees are not native to North America and were introduced less than 300 years ago. The apple maggot fly is also known to infest hawthorn fruit, which is native to North America.

   Some apple maggot flies prefer the scent of apple fruit, while other prefer the scent of hawthorn fruit. This is shown in the graphs below.

   It appears that flies that develop from maggots infesting hawthorns prefer to mate and lay eggs on hawthorns (hawthorn flies). Flies that develop from maggots that infest apples prefer to mate and lay eggs on apples (apple flies). Only 6% of matings take place between flies from different fruits.





   (a) What plant did the apple maggot fly infest before apple trees were introduced to North America?

   _____

   (b) What kind of natural selection is occurring in the apple maggot fly? _____

   (c) Explain the mechanisms that are causing this selection to occur: _____

   _____

   _____

   _____

   _____

   (d) How might these mechanisms affect the future evolution of the apple maggot fly? _____

   _____

   (e) Apple fruits tend to drop earlier in the season that hawthorn fruits. How might this enhance the separation of apple flies and hawthorn flies?

   _____

   _____

   _____

   _____

2. The colour of the exoskeleton of a population of bugs is controlled by a single gene with the alleles B (black) and b (brown). The B allele has a frequency of 0.72 in the population. The beetles are active at night but so are their predators.

   (a) What is the frequency of the b allele? _____

   (b) Suggest why the frequency of the B allele is much higher than the b allele in the population:

   _____

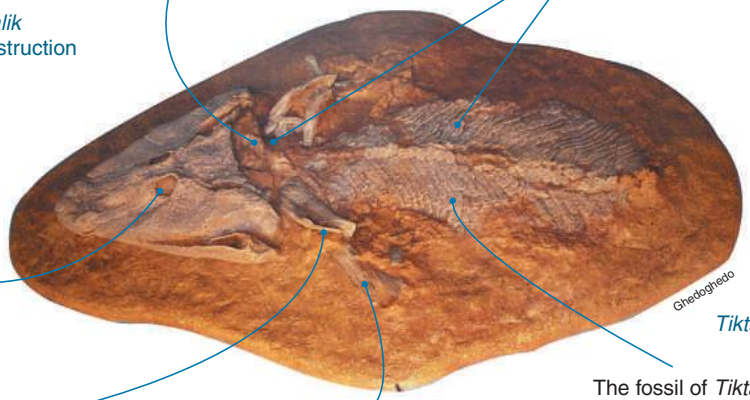
   _____

   _____

3. In 2004, a fossil of an unknown vertebrate was discovered in northern Canada and subsequently called *Tiktaalik roseae*. The *Tiktaalik* fossil was quite well preserved and many interesting features could be identified. These are shown on the photograph of the fossil below. A reconstruction is also shown.



Tiktaalik reconstruction



Tiktaalik fossil

The shoulder bones are not attached to the skull, allowing its neck to turn independently of the body.

Rod-like bones that help pump water over gills are present, but the presence of ribs indicates that lungs were also present.

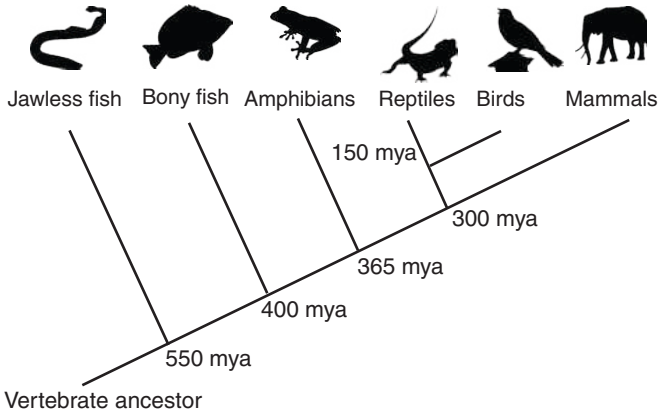
Tiktaalik's head is flattened dorsally, like that of a crocodile with the eyes on top.

The bones of the limbs have a primitive pentadactyl arrangement, similar to tetrapods, which allowed it to support its body weight.

Fish-like fins are clearly visible.

The fossil of *Tiktaalik* was covered with scales much like those of fish.

Use the information above to place *Tiktaalik* on the time line of vertebrate evolution. Discuss the evidence for your decision.



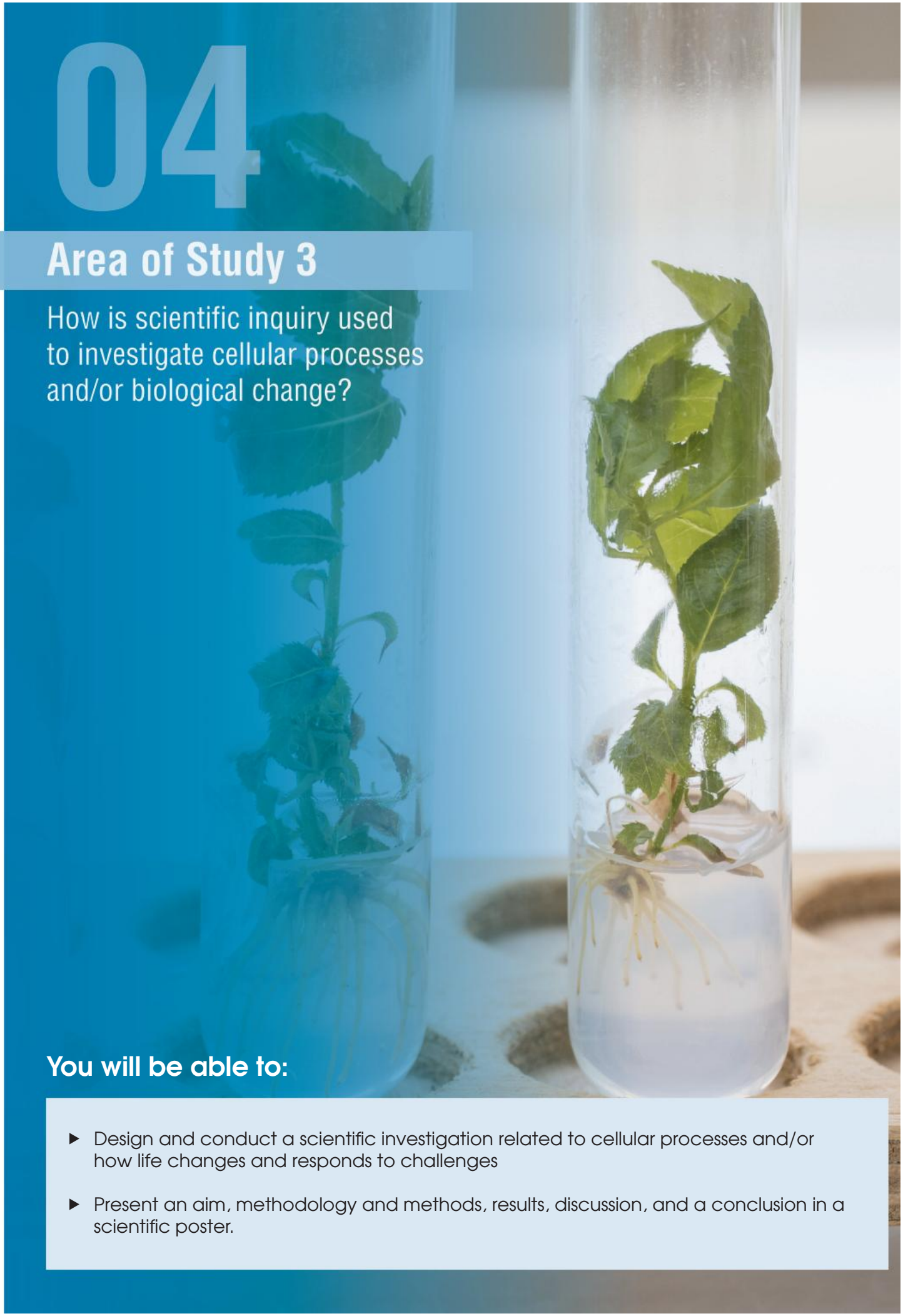
4. *Homo sapiens* societies changed from being hunter-gatherer societies to agricultural societies about 10,000 BC. The development of agricultural skills brought many advantages to *Homo sapiens*, but there were disadvantages too.

Describe one advantage and one disadvantage of developing an agricultural society:

04

Area of Study 3

How is scientific inquiry used to investigate cellular processes and/or biological change?



You will be able to:

- Design and conduct a scientific investigation related to cellular processes and/or how life changes and responds to challenges
- Present an aim, methodology and methods, results, discussion, and a conclusion in a scientific poster.

CHAPTER
14

Investigating Cellular Processes or Biological Change

Key terms

accuracy
aim
assumption
control
controlled variable
dependent variable
ethics
fair test
hypothesis
independent variable
observation
precision
prediction
primary data
quantitative data
qualitative data
reliability
sample
scientific poster
validity (of data)
variable

Experimental design

Key knowledge

Activity number

TASK: Design or adapt a lab or field-based investigation related to cellular processes and/or biological change and continuity over time. You are required to identify an aim, develop a question, formulate a hypothesis, and plan a course of action to answer the question, complying with safety and ethical guidelines. **175**

☐ 1 Show an understanding of the biological concepts and key terms as relevant to your investigation. **174 175**

☐ 2 Demonstrate an understanding of the characteristics of scientific research methods and techniques for collecting primary data as relevant to your investigation, be it laboratory work (e.g. biochemistry, microbiology) or fieldwork. Determine your aim and hypothesis, ask questions, and make predictions that can be tested. **175 176**

☐ 3 Identify dependent, independent, and controlled variables. Explain your choice of a control, and show awareness of assumptions in your investigation. **174 175**

☐ 4 Show understanding of precision, accuracy, reliability, and validity when collecting your data. Precision, accuracy, and reliability are features of the assessment or measurement tools used, whereas validity is a feature of design. **174 175**

Precision: How close your measurements are to each other.

Accuracy: How close your measurements are to the true value of the variable.

Reliability: How much your assessment tool provides consistent, stable results.

Validity: The extent to which the study measures what it is intended to measure.

Explain how you will minimise bias, ensure accuracy (e.g. through calibration of equipment, and maximise precision of measurements.

☐ 5 Demonstrate an ability to carry out an investigation safely and ethically. **172**



Pasco





Analysis and presentation of research

Key skills and knowledge

☐ 6 Use appropriate means to organise, analyse, and evaluate primary data to identify patterns and relationships. Include reference to sources of error and limitations of data or methods. **173 174**

☐ 7 Understand how models, theories, and classification keys are used to organise and explain observations and biological concepts and recognise their limitations. **175**

☐ 8 Make clear, accurate scientific drawings as appropriate to your investigation. **175**

☐ 9 Present and explain the key findings of your investigation as a scientific poster presentation to include a title, introduction, methodology, results, discussion, conclusion, and references and acknowledgements. Include appropriate biological terminology and representations, standard abbreviations, and units of measurement, and acknowledge all sources of information. **176**

172 Ethics and Safety Issues of Research

Key Idea: When designing an investigation all potential health and safety risks must be first identified and steps taken in the design process to reduce or eliminate them.

When designing an experiment it is important to identify any potential hazards to health and safety and take steps to eliminate or reduce the risks they pose. A failure to do this could result in harm to yourself or others around you. Ethical considerations also need to be addressed. These include bioethics (the moral implications of new discoveries), animal welfare issues, and your own behaviour in recording reporting results accurately and honestly and acknowledging the work of others.

Heath and safety considerations

Safety must be considered in all investigations as there is always potential for harm, even from seemly unlikely sources. Minimising hazards is always important, as is protecting yourself from those potential hazards. Hazards might come from:

- **Chemical sources**: The various chemicals in laboratories can cause severe harm if not used carefully. They are often reactive and are thus hazardous (e.g. disinfectants, stains, reagents etc). Protection from contact, inhalation, or ingestion must be considered.
- **Biological**: All biological material can be potentially hazardous. It may carry pathogens or toxins that can cause harm. Efforts must be taken to avoid contamination. Examples include microbial samples, animal tissue, fluid samples, and plant samples.
- **Physical**: Physical hazards exist as a physical part of the lab (or field) environment. Even everyday objects in the lab (e.g. a lab stool) can be potentially hazardous if not placed out of the way. Common hazards include equipment (e.g. razors used for cutting samples) and cluttered working spaces.





Reducing heath and safety risks

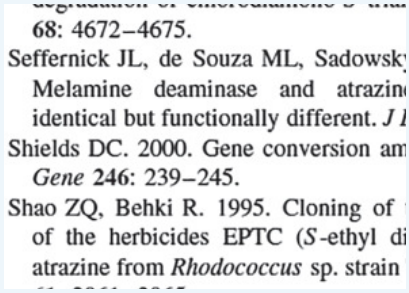


- Identify potential hazards before you start and become knowledgeable about their risks.
- Wear appropriate safety gear (lab coat, gloves, safety glasses, ear protection, covered shoes, and a mask as necessary).
- Ensure all chemicals and solutions are clearly labelled for easy identification.
- Know how to correctly use all equipment and machinery before you begin.
- Maintain clean work spaces and floors to reduce the risk of slips and spills. Keep access ways to exits and emergency equipment clear.

Ethical considerations



Report your true data and findings, even if they are not the results you were expecting. Changing results to fit your hypothesis is misleading and unethical.



68: 4672–4675.
Seffernick JL, de Souza ML, Sadowsky
Melamine deaminase and atrazine
identical but functionally different. *J*
Shields DC. 2000. Gene conversion am
Gene 246: 239–245.
Shao ZQ, Behki R. 1995. Cloning of
of the herbicides EPTC (*S*-ethyl di
atrazine from *Rhodococcus* sp. strain

It is very important to acknowledge the work of others (e.g. photographs, data, reference material). Failure to do so is plagiarism.



Janet Stephens

Get a teacher to review your experimental design for ethical approval prior to beginning. Minimise the impact of your research on the environment.

1. Identify a health and safety issue associated with a stream field study: _____

_____

2. (a) Identify a health and safety issue associated with investigating microbial contamination of spaces around your school:

_____

(b) Describe how you could reduce this risk: _____

_____

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173 Maintaining a Logbook

Key Idea: A logbook records your ideas and results throughout your scientific investigation. It provides a record of work and proof that you have carried out that work.

A logbook provides a complete record of the ideas and work you have carried out during your investigation. Each entry must be dated and it should show in detail how you carried out your experiment or research project. The logbook provides proof that you have carried out certain activities on specific dates, and records the results of your work. Logbooks can be used to verify the authenticity and originality of your ideas.



1. Find a notebook to use that will suit your purposes (e.g. if the logbook is to be used in a field a waterproof book is useful). A hardback A4 lined exercise book is a good choice, anything smaller will make it difficult to include photos or extra pages later on.

2. Name your logbook in a prominent location. Number the pages so you can create a good table of contents. Creating sections in your logbook with tabs helps you keep track of ideas, methods, and results easily.

3. Date and sign **every entry**. Entries should be concise, but contain enough information that you can understand them later on. Short notes and bullet points are often used.

4. You must be able to read your entries at a later date, but don't worry too much about neatness. Logbooks are a record of your work, not the final report. It is more important to accurately record information during lab trials or field studies than to have a nice looking logbook!

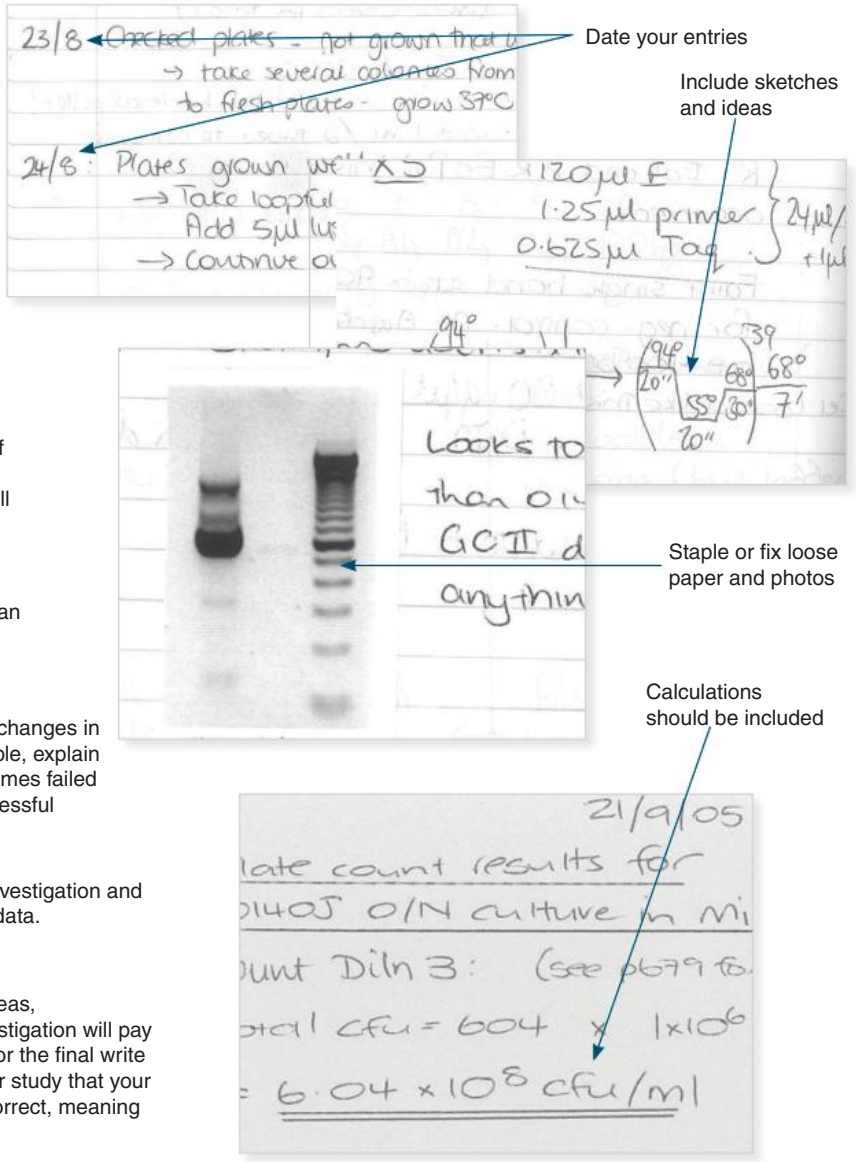
5. Your logbook should be used in all phases of your investigation, from planing to write up. Record ideas on methods or analysis, as well as results.

6. Glue, staple, or tape any loose paper or photos into your logbook. Loose papers are an annoyance, both for you to keep track of and your teacher to sort through.

7. Include any mishaps, failed experiments, or changes in methodology in your logbooks. Where possible, explain the reasons for the failure or change. Sometimes failed experiments can be just as valuable as successful experiments in understanding a result.

8. Include all observations made during your investigation and any calculations and transformations of the data.

9. Remember, systematically recording your ideas, observations, and analyses during your investigation will pay off when you have to organise the material for the final write up. It will also help to clarify any parts of your study that your teacher or marker may find confusing or incorrect, meaning you could still get credit for your work.



1. Why is it important to keep a detailed logbook during a scientific investigation?_____

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174 Analysis and Interpretation

Key Idea: Once you have collected your data you must select an appropriate form of analysis to establish if your hypothesis is supported and identify any trends or patterns in the data.

Data analysis involves examining and processing the data you have collected to identify trends and patterns and establish whether or not the data support your hypothesis and help to answer the questions you posed in your investigation.

There are many ways to analyse and present data and your choice must be appropriate for the data you have collected. A statistical analysis is sometimes necessary but for simple, well designed experiments basic descriptive statistics (e.g. means) may be all that is needed. Finally you must choose the best way to display your results (e.g. table or graph) so that you can present your data in an organised way.

Review your initial data

- After you have collected your first set of data (or your preliminary data) it is a good idea to spend a short period of time analysing it.
- You may discover that you need to collect your data differently to how you first planned (e.g. taking more measurements or changing the way you collect your data, such as automation for rapidly occurring changes or prolonged time series data).
- Take some time to plot the data or calculate summary statistics as these will allow you to see trends and patterns more easily than when the data is recorded in a logbook. Once you are satisfied that your methods of data collection are adequate you can continue with your investigation.



How do I analyse my data?

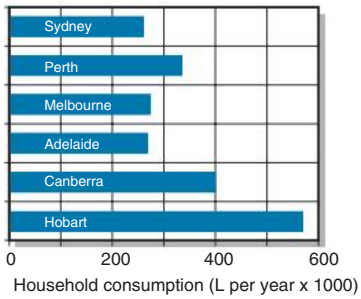
- Check your data to see that it makes sense. Do the results seem logical? Are there any outliers? If so, you must decided whether to include them in your analysis.
- Raw data may need to be transformed to see trends and patterns. Recall that these transformations are often quite simple (e.g. percentages, rates, ratios). Other transformations are used to normalise the data so that it can undergo further analysis (e.g. log transformations when working with large numbers).
- Descriptive statistics (e.g. mean and standard deviation) provide a way to summarise your data, and provide results that can easily be presented and compared across groups. Summary statistics are also useful in identifying trends and patterns in the data.
- Sometimes an appropriate statistical analysis is required to test the significance of results. However, with simple experiments, if the design is sound, the results are often clearly shown in a plot of the data.



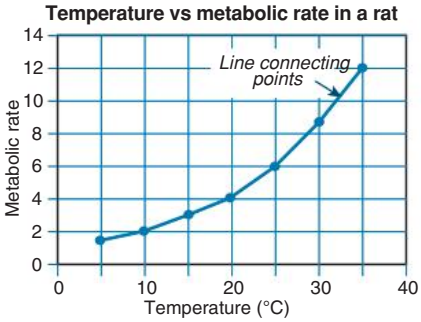
Presenting your data

Tables and graphs provide a way to organise and visualise data in a way that helps to identify trends. Each has a different purpose. Tables provide an accurate record of numerical values and allow you to organise your data so that relationships and trends are apparent. Graphs provide a visual representation of trends in the data in a minimum of space and are an excellent choice for displaying results in a poster. Histograms, line graphs, and scatter graphs are common ways to graphically display data.

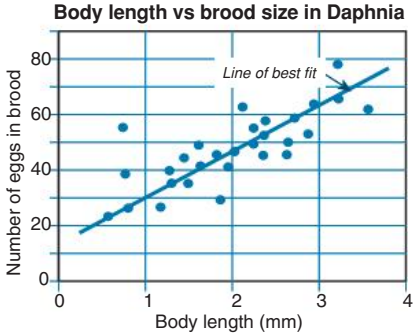
Average household water consumption in Australian cities



Temperature vs metabolic rate in a rat



Body length vs brood size in Daphnia



Bar and column graphs

- One variable is a category
- One variable is continuous data (measurements)

Line graph

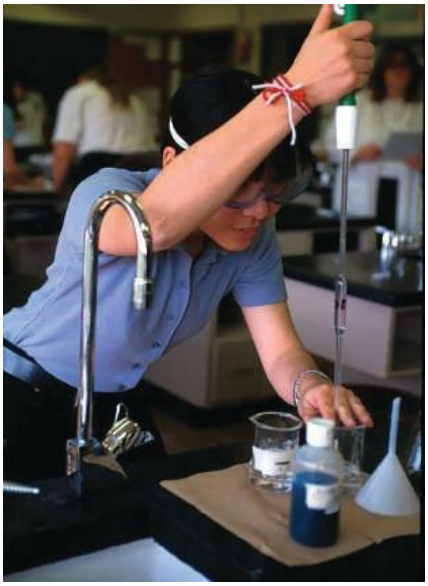
- Both variables are continuous
- The **dependent** (response) variable is dependent on the **independent** (manipulated) variable

Scatter plot

- Both variables are continuous
- The two variables are interdependent but there is no manipulated variable

A-1

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A student carried out an experiment to measure the activity of enzyme A at three temperatures. They measured the amount of product formed to determine the enzyme activity at each temperature. They performed one trial (n = 1) for each temperature. The results are shown below.

Effect of temperature on volume of product formed by enzyme A (mg/mL)

Time (min)	2°C	40°C	80°C
0	0	0	0
2	2	19	0
4	3	29	0
6	5	45	0
8	6	37	0
10	6	39	0
12	8	40	0
14	10	42	0
16	11	42	0

The student noticed an outlier in their data.

1. Why is it a good idea to review your preliminary data before proceeding with the investigation? _____

2. The student noticed they had an outlier in the data for enzyme activity at 40°C.
   (a) They decided not to include the outlier in their results. Do you agree with their decision? _____
   (b) Why or why not? _____

3. (a) The student tested enzyme activity at each temperature once. What are the limitations of carrying out a single trial?
   (b) What additional data analysis could the student carry out if they ran multiple trials? _____
   (c) How would multiple trials increase the reliability and validity of their findings? _____

4. (a) Why are data often presented as tables or graphs? _____
   (b) How would you recommend the student present their data on their scientific poster? _____
   (c) Explain your choice: _____

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175 Designing a Practical Investigation

Key Idea: If designed well and executed carefully, a well researched practical investigation will provide reliable, valid data to test a hypothesis.

This activity will help you to design and undertake a practical investigation of your own related to a cellular process or biological change and continuity over time. In this activity you will use the background information provided and your own scientific knowledge to design an experiment to investigate the effect of light colour (wavelength) on the rate of photosynthesis in an aquatic weed, *Cabomba aquatica*. You must formulate your hypothesis and then plan a scientifically robust experiment to test it. Remember that ethical and safety issues must be taken into consideration as you design your experiment. Think carefully how you will collect and analyse your data so your results are meaningful and allow you to make valid conclusions about your findings. You can use this as a template for your required practical or use it as a practice run before designing your own experiment.

Background information

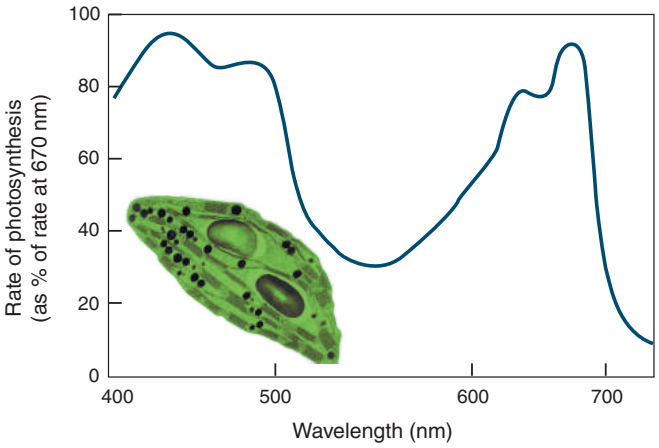


Piotr Kuczynski cc 3.0

- *Cabomba aquatica* (above) is an aquatic plant native to South America, but is commonly found in aquaria all over the world.
- *Cabomba* is very easy to grow and maintain. *Cabomba* is classified as a restricted weed in Victoria because of its highly invasive nature.
- *Cabomba* will produce a stream of oxygen bubbles from its cut stem when illuminated. The oxygen bubbles are a waste product of photosynthesis.
- Stems are snipped just before the experiment is performed to allow the oxygen to escape freely.
- The rate of oxygen production provides an approximation of photosynthetic rate.

Action spectrum for photosynthesis

(Effectiveness of different wavelengths in fuelling photosynthesis)



- There are two categories of photosynthetic pigments: **Chlorophylls** absorb red and blue-violet light. **Carotenoids** absorb blue-violet light.
- The effectiveness of different wavelengths in powering photosynthesis can be determined by measuring oxygen production.
- The equation for photosynthesis is given below.

$$6CO_2 + 12H_2O \xrightarrow{Light} C_6H_{12}O_6 + 6O_2 + 6H_2O$$

Equipment list



Boiling tubes



Sodium bicarbonate (CO_2 supply)



Timer



Red, green, and blue cellophane



Lamp with 60 W bulb



Piotr Kuczynski cc 3.0

Cabomba



Scissors

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Based on the aim of this experiment "To investigate the effect of light colour on photosynthesis rate in *Cabomba*", the background information provided, your own knowledge about photosynthesis, and the equipment list provided, set up your own experiment using the questions below to guide you.
Remember you want to design a **fair test** where only one variable is changed and all other variables are kept constant.

1. Carry out background research before you begin your experimental design so you have good knowledge of the topic. In the space below note down any information sources you have used. You will need to acknowledge these in your poster.

2. State an aim and a hypothesis for your experiment: _____

3. In the space below summarise your method as step by step instructions and draw your experimental set up:

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4. (a) Identify any safety issues associated with your experiment and note the steps required to reduce the risk (if any):

(b) Identify any ethical issues associated with your experiment:

5. (a) What data will you collect?

(b) How often will you collect it?

(c) In the space below draw a template to record your data:

6. (a) Identify any sources of error and limitations in the methods and/or results:

(b) What changes would you make to improve the experiment?

7. Write your conclusions here:

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176 Presenting Your Findings

Key Idea: A well designed scientific poster summarises key information in a clear, easy to follow format.

At the conclusion of your practical investigation you will present your findings as a scientific poster. A poster is a visual summary of your research. Every piece of information on the poster provides key information to your audience so that they have an overview of your findings. Getting the right balance of information is crucial. Too much information can make the poster busy and hard to read, but too little information leaves your audience with a poor understanding of the work. The example below shows an effective poster presentation, although deliberately on a topic you would be unlikely to choose. The message is focussed, it uses graphics with minimal text, and presents material in a clear sequence.

Adapted from a poster by
Brendan J. Hicks & J. Lee Laboyrie, Dept of Biological Sciences, University of Waikato, NZ. Modified with permission.

Leaf breakdown in streams

Introduction

Fallen leaves can provide a major energy source for forest stream ecosystems, but decomposition by microbes is necessary to lower the C:N ratio and increase the food value of leaves to aquatic invertebrates.

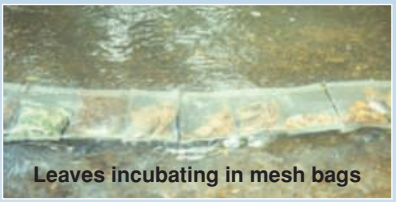
The aims of this investigation were to:
(1) compare rates of mass loss between leaves of different tree species,
(2) determine changes in C:N ratio during the conditioning process,
(3) make preliminary estimates of invertebrate colonisation.

If leaf litter provides food and habitat for stream invertebrates, they will colonise in-stream leaf bags. Breakdown rates vary, so colonisation will be influenced by leaf type.

Methods

1. Fallen leaves were collected from the forest floor, and placed in mesh bags after drying and weighing.

2. The leaf bags were strung onto a wire and left in the Mangoatama Stream, Waikato. The mean water temperature was 14.5°C.



Leaves incubating in mesh bags

3. The leaf bags were retrieved after 28 days. The leaves were gently washed, the aquatic insects were sorted, and the leaves and insects were dried and weighed.

4. The carbon and nitrogen content of the leaves were determined before and after incubation to determine the change in food quality.

Results

1. Food quality of the leaves was increased. Mean C:N ratio fell from 45:1 to 35:1 with incubation.

2. These aquatic insects were commonly found in the leaf bags:



Inset photos: Stephen Moore

Mayfly larva, *Deleatidium* *(collector-browser)*



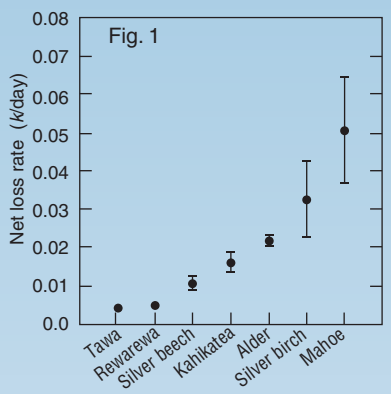
Beetle larva (Elmidae) *(collector-browser)*

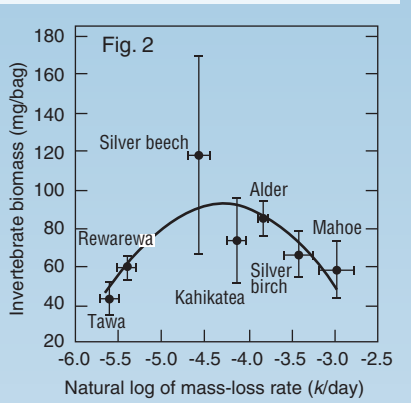


Caddisfly larva, *Olinga* *(generalist feeder)*

3. Mahoe and silver birch leaves broke down fast compared to rewarewa and tawa (Fig. 1).

4. There were more aquatic insects on the leaves with intermediate rates of breakdown than on those with very fast or very slow rates (Fig. 2).





Conclusions

1. Leaves of different tree species showed a range of breakdown rates. Progression of leaf breakdown determined the colonisation of leaves by aquatic insects. Slower breakdown provides habitat. Faster breakdown provides energy quickly.

2. The food quality (C:N ratio) of leaves was improved by breakdown.

3. Leaves of trees planted in riparian zones should have a range of breakdown rates to give energy that is quickly available (e.g. mahoe) as well as to provide habitat structure (e.g. silver beech).

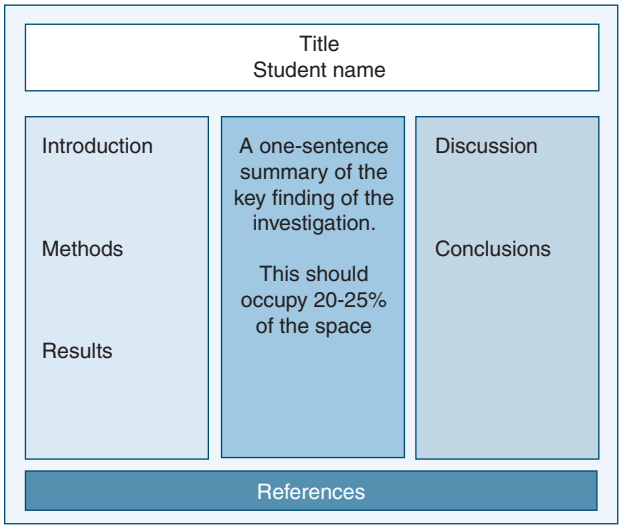
The hypothesis was supported. Further research will investigate suitable mixes of tree species to maximise invertebrate community diversity.

References

Hicks, B.J.; Laboyrie, J.L. 1999. Preliminary estimates of mass-loss rates, changes in stable isotope composition, and invertebrate colonisation of evergreen and deciduous leaves in a Waikato, New Zealand, stream. NZ Journal of Marine and Freshwater Research 33.

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Poster format



- The key to an excellent poster presentation is to provide all the required information in a simple format and not to overload the poster with too much text. Bulleted or numbered text blocks present information in an easy-to-read format.
- Use graphics where possible to communicate information in a space efficient manner. Where possible, use graphs to present results as they are easier to read than large tables and provide a quick summary of your results at a glance.
- Choose a simple background that does not detract from your message. Choose text font, size, and colours that can be easily read and that do not blend into your background.
- A scientific poster has a particular structure, which leads the reader through the study and presents and explains its findings. Key section headings and a checklist are provided below to help you plan your own scientific poster.
- Story boarding your poster (left) before you start laying it out can help to produce a good poster.

Poster content

Title

A clear, brief description of the investigation. A well constructed title make a clear statement about the content of the poster.

Introduction

Includes the aim and hypothesis, and background information to the project. Not all scientific posters have an introduction, but it is an expectation for student posters at this level.

Methods

A description of the materials used and the experimental procedures involved. Where appropriate, photos or diagrams can be used to explain your experimental set up.

Results

A statement or description of the results. This section should not discuss the results, but can state trends. Raw data should not be included, but processed and presented in tables and/or graphs. Tables and graphs should be identified, e.g. Fig. 1, so that they can be clearly identified in the text.

Conclusion

A clear statement describing whether or not the results of the investigation support the hypothesis.

Acknowledgments

A list of sources of information, including citations of written material (e.g. journals, texts), web pages, and practical and advisory help. It is important that entries are consistent within your report. Your teacher will advise you as to the format preferred.

Purpose and checklist

- ☐ Clearly indicates the type and extent of the study
- ☐ Includes species scientific and common name if this is appropriate

- ☐ Gives a clear statement of the aims and hypothesis
- ☐ Provides relevant background and a logical rationale for the study
- ☐ Explains specialised terminology

- ☐ The study design is complete and clearly outlined.
- ☐ Provides the information to enable the procedures to be repeated
- ☐ The reason for each step in the methodology is evident or explained
- ☐ Factors important in the outcome are mentioned and explained
- ☐ Includes relevant details of data collection and analysis (e.g. preparation of materials, controls, sample sizes, methods of measurement)

- ☐ Provides the reader with the findings of the investigation and allows them to evaluate it themselves
- ☐ Clearly presents the findings of the study or experiment. General statements are supported with reference to the data.
- ☐ Major results are presented in tables and figures and briefly described
- ☐ Each figure or table is self-sufficient and includes title and caption
- ☐ Figures and tables are numbered sequentially

- ☐ The conclusions are clearly stated
- ☐ You can discuss continuing research (if applicable)

- ☐ Acknowledges the work and expertise of others
- ☐ Allows your work to be assessed in the light of other work in the area.
- ☐ Full citations are provided for every reference cited in the report
- ☐ There are no references included that are not cited in the report.
- ☐ Each citation is correctly and consistently formatted.

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A-1 Appendix 1: Glossary

A

accuracy
The correctness of a measurement; how close a measured value is to the true value.

acetyl coenzyme A, acetyl coA
A coenzyme that adds acetyl groups to biochemical reactions.

activation energy
The minimum amount of energy needed to initiate a chemical reaction.

active immunity
Immunity resulting from the production of antibodies in response to an antigen.

active site
The region of an enzyme where substrate molecules bind and undergo a chemical reaction.

adaptation (*verb*)
The process by which populations become more suited to their environments.

adaptive immune response
The antigen-specific immune response, responsible for immunological memory.

adaptive radiation
A process in which organisms diversify from an ancestral species into many species.

adenine
One of the nucleobases in nucleic acids. Pairs with thymine in DNA and uracil in RNA.

alcoholic fermentation
A biological process carried out by yeasts, in which simple sugars (e.g. glucose) are converted to ethanol and carbon dioxide.

allele frequency
The frequency of an allele relative to other alleles at a particular locus in a population.

allopatric speciation
Speciation that occurs when biological populations become geographically isolated.

anabolic reaction
A chemical reaction that constructs large, complex molecules from simpler molecules.

anaerobic fermentation
The chemical breakdown of carbohydrates by cells or microorganisms to extract energy when oxygen is not available.

anaerobic metabolism
Biological processes that produce energy for an organism without using oxygen.

annealing *(as used in PCR)*
The process of joining two pieces of denatured DNA.

antibiotic resistance
The ability of bacteria to resist the effects of an antibiotic they were once sensitive to.

antibody
A Y-shaped protein produced by B cells in response to an antigen (*see immunoglobulin*).

anticodon
A sequence of three adjacent nucleotides in tRNA that binds to a corresponding codon in mRNA during protein synthesis (*cf. codon*).

antigen
Any substance foreign to the body that induces an immune response.

antigenic drift
A mechanism for genetic variation in viruses, involving the accumulation of mutations within the antibody-binding sites.

artificially acquired immunity
Any form of immunity produced by deliberate exposure to an antigen (such as a vaccine).

assumption
A statement that is assumed to be true but is not (or cannot be) tested.

ATP
An organic compound that serves as an energy source for metabolic processes.

ATP synthase
A protein that catalyses the formation of ATP from ADP and inorganic phosphate.

***Australopithecus* spp.**
An extinct genus of early hominins, the ancestors of modern humans.

autoimmune disease
A condition arising as a result of the immune system attacking its own tissues.

B

B cell
An antibody-producing lymphocyte responsible for humoral immunity.

base-pairing rule
The way in which nucleobases pair in DNA and RNA. Guanine pairs with cytosine, and adenine pairs with thymine (or uracil in RNA).

biofuel
A fuel produced from renewable resources, particularly plant, algal or animal biomass.

biogas
A type of biofuel produced by bacterial degradation of organic matter, made up of a mixture of methane and carbon dioxide.

biogeographical evidence
Evidence of evolution from the distribution of related species.

bipedal (*adjective*); **bipedalism** (*noun*)
Having two feet; the habit of standing and walking on two feet.

Broca's area
A region of the frontal lobe of the brain, associated with speech production.

C

C_3 plant
The majority of plants, with no physical separation of photosynthetic pathways.

C_4 plant
Plants that fix carbon dioxide in two physically separated steps.

Calvin cycle
The light-independent phase of photosynthesis during which chemical reactions convert carbon dioxide into sugars.

CAM metabolism
Arid-adapted plants in which carbon dioxide is fixed in a 4C compound at night and released to the Calvin cycle during the day when stomata are closed.

catabolic reaction
The breakdown of large, complex molecules into smaller, simpler molecules.

catalyst
A substance that modifies and increases the rate of a chemical reaction without being consumed in the process.

cellular immunity
Immunity involving T cells, which attack invading organisms on a cellular level.

cellular respiration
The series of metabolic reactions that produce ATP via oxidation of organic molecules.

chlorophyll
A green photosynthetic pigment found primarily in the chloroplasts of algae and plants, essential to photosynthesis.

chloroplast
An organelle within the cells of plants and green algae that contains chlorophyll and is the site of photosynthesis.

chronometric dating
A method of dating that uses a known measurement (e.g. the decay of unstable elements) to produce definitive times and dates for the age of an object.

cladogram
A diagram used in cladistics to show relationships between groups of organisms.

clonal selection
An immunological process that determines which B and T lymphocytes will be produced in response to a specific antigen.

coding strand
The strand of a DNA molecule with the same nucleotide sequence as the mRNA.

codon
A sequence of three adjacent nucleotides in a DNA or mRNA sequence that is part of the genetic code (*cf. anticodon*).

coenzyme
A non-protein compound that binds to an enzyme to initiate or aid in its function.

common ancestor
An ancestor that two or more descendant species have in common.

competitive inhibition
A form of enzyme inhibition in which an inactive molecule reversibly binds to the active site, preventing the actual substrate from binding (*cf. non-competitive inhibition*).

control
A sample in an experiment that is not exposed to the experimental factor/treatment.

controlled variable
Any factor that is constant or unchanged throughout the course of an experiment.

CRISPR-Cas9
A prokraryotic gene sequence used as a tool in the editing of genomes.

cristae
One of the inward projections or folds of the inner membrane of a mitochondrion.

cultural evolution
An evolutionary theory of social change, describing the change of culture over time.

cytosine
One of the nucleobases in nucleic acids. Pairs with guanine.

D

denaturation
The alteration of a protein shape resulting in a loss of cellular function.

dendritic cell
An antigen-presenting cell of the mammalian immune system that processes antigen material and initiates an immune response.

dependent variable
The variable being tested in a scientific experiment. The response variable.

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directional selection
A mode of natural selection in which an extreme phenotype is favoured over others, causing the allele frequency of a population to shift towards that phenotype.

disruptive selection
A natural selection mechanism in which extreme values for a trait are favoured, creating trait variance that drives divergence.

divergent evolution
The accumulation of differences between closely related populations within a species, resulting in the formation of new species.

DNA (deoxyribonucleic acid)
A large molecule composed of two polynucleotide chains that carries the genetic code and enables cells to function.

DNA profiling
The process of determining an individual's DNA characteristics.

DNA amplification
The production of multiple copies of a sequence of DNA.

DNA hybridisation
The process of combining two complementary single-stranded DNA or RNA molecules to form a single double-stranded molecule through base pairing.

DNA ligase
An enzyme that joins DNA strands by catalysing phosphodiester bond formation.

DNA ligation
The joining of two DNA fragments through the action of the enzyme DNA ligase.

DNA polymerase
An enzymes that catalyses DNA synthesis from nucleoside triphosphates.

double-helix
The shape of the molecular structure of DNA.

E

electron transport chain
A series of protein complexes that transfer electrons from donors to acceptors across a membrane via redox reactions.

emerging disease
Outbreaks of previously unknown diseases; a known disease whose incidence or range has increased in the past 20 years.

endemic disease
A disease that is constantly present in a specific population or region.

enzyme
Globular proteins that act as biological catalysts for specific reactions.

enzyme inhibition
A reaction between a molecule and an enzyme that blocks the action of the enzyme, either temporarily or permanently.

epidemic
The rapid spread of disease to a large number of people in a given population within a short period of time.

ethanol
A simple alcohol made from sugar, starch and other carbohydrates by fermentation.

ethics
A set of moral obligations that define right and wrong in scientific practices and decisions.

evolution
The change in the heritable characteristics of populations over successive generations.

exon
A protein coding region of a gene.

extracellular
Located or occurring outside a cell or cells.

F

FAD/$FADH_2$
A redox coenzyme that can accept or donate electrons. H_2 is the oxidised version.

feedback inhibition
A cellular control mechanism in which enzyme activity is inhibited by the end product.

fermentation
An anaerobic metabolic process by which a carbohydrate, such as starch or a sugar, is converted into an alcohol or an acid.

fibrous protein
A class of insoluble proteins that form strong filaments and constitute the main structural elements of the body in vertebrates.

fitness
A mathematical measure of an organism's genetic contribution to the next generation.

fixation *(of alleles)*
The process through which an allele becomes fixed (100%) within a population.

forensics
The analysis of body fluids, stains and other bodily materials to help solve a crime.

fossil
The preserved, mineralised remains of an organism, or of an organism's activity, from an earlier geological period.

fossil record
The history of life as documented by fossils.

founder effect
The loss of genetic variation that occurs when a new population is established by a very small number of individuals.

G

gel electrophoresis
The separation and analysis of protein molecules of varying sizes by moving them through a block of gel using an electric field.

gene
A unit of hereditary information consisting of a specific nucleotide sequence in DNA.

gene expression
The transcription and translation of a gene.

gene flow
The exchange of alleles between two or more populations.

gene pool
The collective genetic information within a population of interbreeding organisms.

genetic bottleneck
The reduction in a population's genetic diversity caused by a sharp decrease in population size.

genetic code
The set of rules used by living cells to translate information encoded in genetic material into proteins.

genetic drift
The random changes in allele frequency in a population over generations.

genetic equilibrium
Allele frequencies in a population that are static, or unchanging, over time.

genetic modification
The process of making changes to an organism's genes, e.g. to give it new traits.

genome
The genetic material of an organism, and all the heritable traits encoded in its DNA.

genotype
The genetic makeup of an organism.

geologic time scale
A system of chronological dating that classifies geological strata in time.

globular protein
A very common type of spherical and somewhat water-soluble proteins.

glucose
A simple sugar that functions as the main source of metabolic energy in living things.

glycolysis
The metabolic pathway that converts glucose into pyruvate.

GMO (genetically modified organism)
Any organism whose genetic material has been altered using genetic engineering.

granum (*pl., grana*)
Stack of thylakoids found in the stroma of chloroplasts, where the light-dependent reactions of photosynthesis take place.

guanine
One of the nucleobases in nucleic acids. Pairs with cytosine.

H

herd immunity
A form of indirect protection from infectious disease that occurs when a significant proportion of the population are immune.

heterozygous advantage
The higher fitness of heterozygotes for a condition relative to either homozygote.

hominin
A member of the taxonomic tribe Hominini, which includes *Homo sapiens* as well as extinct humans and immediate ancestors.

hominoid
A member of the superfamily Hominoidea, which consists of the lesser apes, the great apes, and hominins.

Homo
The genus of primates that includes modern humans and several extinct related species.

homology
Similarity between two different species of organisms due to shared ancestry.

humoral immunity
Immunity involving antibodies, which circulate through extracellular fluids.

hydrogen bonding
A dipole-dipole interaction, where one atom is hydrogen and the other may be any electronegative atom.

hypothesis
A tentative explanation for a phenomenon, capable of being tested by experimentation.

IJK

immunised (*adjective*)
Having been rendered not susceptible to a disease, as through vaccination or exposure.

immunity
Inherited, acquired, or induced resistance to infection by a specific pathogen.

immunoglobulin
See **antibody**

immunological memory
The ability of the immune system to recognise and respond to an antigen that it has previously encountered.

immunotherapy
Treatment of a disease by adjusting the body's immune response.

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in-vitro
Outside the living body or cell.

in-vivo
Within the living body or cell

independent variable
The variable that is changed or controlled in a scientific experiment, and is assumed to have an effect on the dependent variable.

induced fit model
A model for the interactions between an enzyme and substrate involving conformational change in the enzyme.

infection
The invasion of bodily tissue by pathogenic microbes, their multiplication, and the reaction of host tissues to the pathogens.

intracellular
Occurring or situated within a cell or cells.

intron
A segment of DNA that does not code for a protein and is removed before translation.

isolation
Removing an infected person from any contact with others in the population.

Krebs cycle
A cycle of aerobic catalysed reactions in respiration occurring within mitochondria. Generates ATP and reducing power.

L

lactic acid fermentation
A metabolic process by which glucose is converted into cellular energy and lactate.

leucocyte (white blood cell)
A type of blood cell that protects against pathogens and can move under its own power. A component of the immune system.

light dependent phase
The phase of photosynthesis during which light energy is converted into chemical energy through chemical reactions.

light independent phase
See **Calvin cycle**

limiting factor
A resource or environmental condition which limits the growth, distribution or size of a population within an ecosystem.

link reaction
The stage in respiration that converts pyruvate into acetyl CoA, linking glycolysis to the Krebs cycle.

lymph
A clear fluid containing white blood cells that flows through the lymphatic system.

lymph node
Small masses of lymphoid tissue that filter bacteria and foreign particles from lymph.

lymphocyte
T and B white blood cells responsible for the adaptive immune response.

M

macroevolution
Large-scale evolution involving the formation of new species and higher taxa.

macrophage
A large phagocytic leucocyte, which engulfs and devours invading cells.

marker gene
A gene with a known location used to track the insertion of DNA into organisms.

matrix *(of mitochondria)*
The space within the inner membrane of a mitochondrion.

metabolic pathway
A linked series of chemical reactions occurring within a cell.

metabolism
The chemical processes occurring within a living cell/organism that sustain life.

MHC (major histocompatibility complex)
A tightly linked cluster of genes that code for cell surface proteins essential for the adaptive immune system.

microevolution
The change in allele frequencies in a population over time.

microsatellite
A segment of DNA consisting of multiple repetitions of short sequences of base pairs.

mitochondrion (*pl., mitochondria*)
An organelle in eukaryotic cells that serves as the site of cellular respiration.

molecular clock
The mutation rate of DNA or amino acid sequences over time, from which the dates of taxonomic divergences can be deduced.

monoclonal antibody
A specific antibody produced by cloning a unique white blood cell.

monophyletic (taxon)
Organisms descended from a common ancestor. That ancestor and all its descendants.

mtDNA
DNA in mitochondria of eukaryotic cells.

mutation
A change in the nucleotide sequence of an organism's DNA (or RNA).

N

NAD/NADH
Electron carriers in cellular respiration (oxidised/reduced forms).

NADP/NADPH
Electron carriers in photosynthesis (oxidised/reduced forms).

natural selection
The differential survival and reproduction of favourable phenotypes.

naturally acquired immunity
Immunity developed through exposure to an infectious agent or other antigen.

non-competitive inhibition
Enzyme inhibition where the inhibitor binds to the enzyme at a region other than the active site (*cf. competitive inhibition).*

nucleic acid
A polymer (polynucleotide) consisting of many nucleotide monomers; serves as a blueprint for proteins and all cellular activities. The two types are DNA and RNA.

nucleotide (pl., nucleotides)
An organic molecule that is the building block of DNA and RNA.

O

operator
A segment of DNA that is the binding site for proteins that initiate transcription.

operon
A cluster of functionally-related genes in prokaryotes controlled by a shared operator.

optimum *(enzyme)*
The conditions under which a particular enzyme is most active.

outbreak *(disease)*
A sudden increase in the occurrence of a disease in a particular time and place.

oxidative phosphorylation
The process in which ATP is formed as a result of the step-wise transfer of electrons to a final acceptor, oxygen.

P

Palaeolithic
A period in human prehistory distinguished by the original development of stone tools.

palaeontological evidence
Evidence of evolution through the study of prehistoric life and the fossil record.

pandemic
Epidemic occurring globally or across multiple continents, affecting a large proportion of the population.

passive immunity
Immunity acquired by the transfer of antibodies from another individual.

peptide bond
A covalent bond between two amino acids; the primary linkage in all protein structures.

phagocyte
A cell that protects the body by engulfing and ingesting harmful foreign particles, bacteria, and dead or dying cells.

phenotype
The observable physical, behavioural, and physiological traits of an organism.

photolysis
Chemical decomposition induced by light or other radiant energy.

photosynthesis
A process used by green plants, algae and some bacteria to convert light energy into chemical energy (carbohydrate).

photosystem
Membrane-bound pigment complexes that capture light in photosynthesis.

phylogenetic tree
A branching diagram showing evolutionary relationships among organisms.

phylogeny
The evolutionary history of a taxon.

plasmid
A circular extra-chromosomal segment of DNA capable of self replication.

polymerase chain reaction (PCR)
An *in-vitro* technique for rapid synthesis of a given DNA sequence.

polypeptide
A chain of amino acids linked together by peptide bonds.

polyploidy
A condition in which the cells of an organism have more than two paired sets of chromosomes.

population
A group of interbreeding organisms of the same species, found in the same area.

postzygotic isolating mechanism
A mechanism preventing interbreeding by blocking development of viable, fertile young.

precision
The consistency of results when measurements or tests are repeated.

prediction
What is expected to happen if an experimental hypothesis is true.

prehensile
Able to seize, grasp, or hold by wrapping around an object.

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prezygotic isolating mechanism
A mechanism that prevents interbreeding by blocking the fertilisation of eggs.

primary data
Data collected from first-hand sources.

primary response
The response that the immune system displays when first exposed to an antigen.

primary structure
The linear sequence of amino acids in a polypeptide chain.

primate
Any of various mammals in the order Primates, which includes lemurs, monkeys and apes (including humans).

primer
A short single-stranded nucleic acid used by all living organisms to initiate DNA synthesis.

producer
An organism that produces its own food using materials from inorganic sources.

prognathic
Having protruding jaws or mouthparts.

promoter
A sequence of DNA to which RNA polymerase binds to initiate transcription.

protein
A biologically functional molecule consisting of one or more polypeptides folded into a specific three-dimensional structure.

proteome
The entire set of proteins expressed within a cell, tissue or organism at a certain time.

purine
Two-ringed nucleobase in DNA and RNA.

pyrimidine
Single-ringed nucleobase in DNA and RNA.

pyruvate
A versatile biological molecule that is the end product of glycolysis, and may be metabolised to lactate or to acetyl CoA.

Q

qualitative data
Non-numerical data that describes qualities.

quantitative data
Numerical data expressing a certain quantity, amount, or range.

quarantine
Disease control mechanism to isolate exposed individuals that *may* be infected.

quaternary structure
The structure formed by the association of two or more polypeptides.

R

radiometric dating
A method for determining the age of an object based on the proportion of a particular radioactive isotope within it and the half-life of that isotope.

re-emerging disease
An infectious disease that has reappeared after having previously been in decline.

recognition site
A specific nucleotide sequence in DNA recognised by restriction enzymes.

recombinant DNA
Genetically engineered DNA made by recombining DNA from different organisms.

recombinant plasmid
A circular piece of DNA comprising DNA from two sources.

regulatory gene
A gene that produces a repressor substance to control the expression of other gene(s).

relative dating
A method of determining the age of an artefact, fossil, or stratigraphic layer relative to another. Does not give specific dates.

reliability
The degree of consistency of a measurement.

repressor
A protein that binds to an operator, blocking transcription of an operon and the enzymes for which the operon codes.

reproductive isolation
A set of mechanisms and conditions that prevent breeding between different species.

restriction enzyme
An enzyme that can cleave DNA strands at recognition sites.

ribosome
A complex of rRNA and protein molecules that function as a site of protein synthesis in the cytoplasm.

RNA (mRNA, rRNA, tRNA)
A long (generally) single-stranded nucleic acid, essential in various biological roles including gene expression.

rock strata
Stacked-up layers of sedimentary rock.

RuBisCO
A plant enzyme involved in fixing atmospheric CO_2 during photosynthesis.

S

secondary response
Immune response that occurs as result of the second (and subsequent) exposure to a particular antigen.

secondary structure
The folding of a polypeptide chain into an alpha helix, beta sheet, or random coil.

selection pressure
Any reason for organisms with certain phenotypes to have either a survival benefit or disadvantage.

selective breeding
The practice of modifying the characteristics of an organism through breeding to develop particular phenotypic traits.

semi-conservative replication
The normal mechanism of DNA replication, where each strand acts as a template for a new double helix.

speciation
The formation of new biological species through the process of evolution.

stabilising selection
A mode of natural selection that favours retention of the median phenotype.

STR (Short Tandem Repeat)
A short DNA sequence, repeated many times.

stroma
The fluid surrounding the membranous stacks within the chloroplast.

structural gene
A gene that encodes the amino acid sequence of a protein.

substrate level phosphorylation
The synthesis of ATP by the transfer of a phosphate from a substrate directly to ADP.

sympatric speciation
The evolution of a new species from an ancestral species while both continue to inhabit the same geographic region.

T

T cell
A type of lymphocyte that plays a central role in the adaptive immune response.

T killer cell
A type of T lymphocyte that kills infected cells, cancer cells, and damaged cells.

T helper cell
A type of T lymphocyte that recognises foreign antigens and releases cytokines that activate T and B cells.

taxon
A taxonomic category or group, such as a phylum, order, family, genus, or species.

template strand
The non-coding strand of a DNA molecule, used as a template for RNA synthesis.

terminator sequence
A nucleic acid sequence that marks the end of a gene during DNA transcription.

tertiary structure
The three-dimensional structure of a protein, created by the folding of the helices or sheets.

thylakoid discs
Membrane-bound sacs containing chlorophyll; the site of the light-dependent reactions of photosynthesis.

thymine
One of the nucleobases in DNA. Pairs with adenine, and is not a component of RNA.

thymus
A primary lymphoid organ of the immune system where T cells mature.

transcription
The process of copying a segment of DNA into a strand of mRNA.

transgenic organism
An organism whose genome has been altered by the insertion of a novel gene.

transitional fossil
A fossil that exhibits traits common to both an ancestral group and its derived descendants.

translation
The process of decoding a strand of mRNA to produce a sequence of amino acids.

transmission *(of disease)*
The transfer of an infectious agent from an infected host individual to another individual.

triose phosphate
A 3-C molecule produced in photosynthesis.

UVW

uracil
One of the nucleobases in RNA. Pairs with adenine, and is not a component of DNA.

vaccination
Inoculation with a vaccine to help the immune system develop immunity to a particular disease.

validity *(of data)*
How accurately a method measures what it is intended to measure.

variable
A measurable property that changes over time or can take on different values.

vector *(disease)*
An agent that carries an infectious pathogen into another living organism.

Wernicke's area
A region of the brain associated with understanding written and spoken language.

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A-2 Appendix 2: Equipment List

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

1: Nucleic Acids and Proteins

INVESTIGATION 1.1
Extracting DNA

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

INVESTIGATION 1.2
Creating a model of a DNA molecule

Per pair
- Scissors
- Tape or paste

INVESTIGATION 1.3
Modelling protein structure

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

3: Regulation of Biochemical pathways

INVESTIGATION 3.1
Investigating peroxidase activity

Per pair/group
- 13 x boiling tubes
- 42 mL distilled water
- 1.8 mL 0.1% H_2O_2 solution
- 1.2 mL prepared guaiacol solution
- Parafilm
- 6 mL of each pH buffered solution (pH 3, 5, 6, 7, 8, 10)
- 9 mL turnip peroxidase solution
- Test tube rack
- Timer

4: Photosynthesis

INVESTIGATION 4.1
Investigating photosynthetic rate

Per pair/group
- 1.0 g *Cabomba aquatica*
- Balance
- Scissors
- Water
- 1 x large beaker (large enough to hold the glass funnel)
- 1 x glass funnel
- 0.2 mol/L sodium hydrogen carbonate solution (enough to cover the plant)
- 1 x test tube
- 1 x lamp with a 60W bulb
- Lux meter
- Timer
- 1 x ruler or tape measure

5: Cellular Respiration

INVESTIGATION 5.1
Measuring respiration in germinating seeds

Per group
- 3 x boiling tubes
- Marker pen
- 6 x cotton balls
- 15% KOH solution
- 2 x eye dropper or plastic pipette
- 3 x gauze pieces
- Germinated bean seeds (enough to fill one quarter of the boiling tube)
- Ungerminated bean seeds (enough to fill one quarter of the boiling tube)
- Glass beads (enough to fill one quarter of the boiling tube)
- 3 x 2-hole tube stoppers
- 3 x bent glass tubes or pipettes
- 3 x tubes (must be able to be clamped shut)
- 3 x screw clips
- A few drops of colored liquid
- 3 x syringes (must fit tube with screw clamp attached)
- 3 x clamp stands or rack
- Water bath (25°C)
- Ruler
- Timer

INVESTIGATION 5.2
Measuring fermentation in yeast

Per pair
- 1 x 100 mL beaker
- 10 g of active yeast
- 50 mL tap water at 24°C
- 25 g of substrate (glucose, maltose, sucrose, or lactose)
- 1 x glass stirring rod
- 1 x conical flask (to hold 275 mL)
- Parafilm
- Single hole stopper
- Tubing
- 1 x 100 mL measuring cylinder
- 1 x small basin to hold inverted cylinder
- Stopwatch

9: Disease Challenges and Strategies

INVESTIGATION 9.1
Investigating the effectiveness of handwashing

Per class
- Warm water
- Soap
- Hand sanitiser

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

INVESTIGATION 9.2
Modelling disease outbreak and spread

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

10: Genetic Changes in a Population

INVESTIGATION 10.1
Investigating natural selection

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

INVESTIGATION 10.2
Modelling genetic drift

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

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Credits

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