

Plant Structure and Function, Biodiversity and Conservation revision notes

Plant Structure and Function, Biodiversity and Conservation is topic 4 of Pearson Edexcel International AS/A Level Biology (XBI11/YBI11), examined in Unit 2 (WBI12/01). It covers plant cell ultrastructure, cellulose and plant tissues, bacterial growth and plant-derived medicines, then classification, biodiversity, allele frequency and the conservation of endangered species. It includes Core practicals 7, 8 and 9.

For Pearson Edexcel International AS Biology XBI11 and International A Level Biology YBI11. **A Level only** is IA2 content (Units 4 to 6), not needed for the International AS.

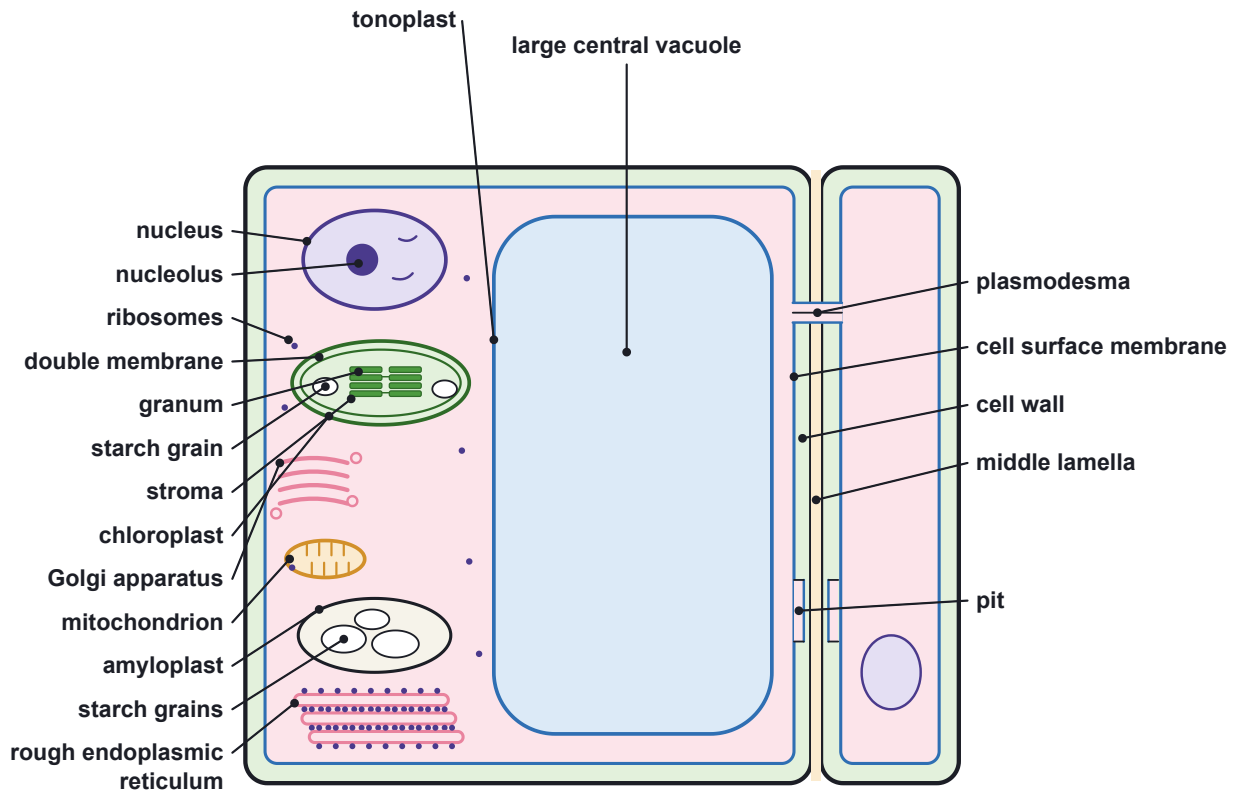
1 Plant cells and cell walls Spec 4.1–4.4

Plant cell ultrastructure and its functions Spec 4.1, 4.2

A plant cell has all the organelles of a eukaryotic cell (nucleus, mitochondria, rough endoplasmic reticulum, Golgi apparatus, ribosomes), plus several structures that animal cells lack. Learn the **structure** and the **function** of each one in the table, because both are examined.

Structures found in plant cells

Structure	Ultrastructure	Function
Cell wall	Outside the cell surface membrane. Cellulose microfibrils embedded in a matrix of other polysaccharides (such as hemicelluloses and pectins). Fully permeable.	Gives the cell strength and shape. Resists the pressure of water entering by osmosis, so the cell does not burst and becomes turgid.
Middle lamella	A thin layer between the walls of neighbouring cells, rich in pectins (calcium pectate).	Cements (sticks) adjacent cells together.
Chloroplast	Typically 3-10 µm long. Surrounded by a double membrane (envelope). Inside: stacks of thylakoid membranes (grana) joined by lamellae, containing chlorophyll; a fluid stroma containing enzymes, 70S ribosomes, circular DNA, lipid droplets and starch grains.	Site of photosynthesis. Light-dependent reactions occur on the thylakoid membranes; light-independent reactions occur in the stroma.
Amyloplast	A colourless organelle with a double membrane, packed with starch grains. No chlorophyll or thylakoids.	Stores starch, for example in potato tubers, roots and seeds.
Vacuole	A large, permanent, fluid-filled sac. The fluid is cell sap: a solution of water, sugars, amino acids, mineral ions, pigments and waste.	Stores solutes and water. Its water content helps keep the cell turgid, which supports non-woody plants.
Tonoplast	The single, partially permeable membrane surrounding the vacuole. Contains transport proteins.	Controls what enters and leaves the vacuole, for example by active transport of ions into the sap.
Plasmodesmata	Narrow channels through the cell walls, lined by the cell surface membrane and containing a thin strand of cytoplasm that links the cytoplasm of neighbouring cells.	Allow substances and chemical signals to pass directly from cell to cell.
Pits	Regions where the wall is thin because secondary thickening was not laid down there. Pits in neighbouring cells line up as pairs.	Allow water and dissolved substances to move between adjacent cells, for example from one xylem vessel to the next.



Ultrastructure of a plant cell and its junction with a neighbouring cell. The **middle lamella** lies between the two cell walls, and **plasmodesmata** pass through the walls.

Comparing plant and animal cells

Plant cells compared with animal cells		
Feature	Plant cell	Animal cell
Cell wall	Present, made of cellulose	Absent
Chloroplasts	Present in photosynthetic cells	Absent
Vacuole	One large permanent vacuole with a tonoplast	None, or small temporary vacuoles
Carbohydrate store	Starch (in amyloplasts and chloroplasts)	Glycogen (in the cytoplasm)
Shape	Fixed, often regular, because of the wall	Variable; no fixed shape
Centrioles	Absent in flowering plants	Present
Both have	Nucleus, cell surface membrane, mitochondria, ribosomes, rough endoplasmic reticulum, Golgi apparatus	Same

Exam tip: For compare questions give a similarity and a difference, and write about both cell types in the same sentence. "Plant cells have a cell wall" scores less than "plant cells have a cellulose cell wall but animal cells do not".

Recognising organelles in electron micrographs

- Chloroplast: oval, double membrane, internal stacks of discs (grana), often with pale starch grains.
- Amyloplast: rounded, double membrane, filled with large pale or dense starch grains, no internal stacks.

- Vacuole: very large, pale and featureless, bounded by a single thin line (tonoplast) and pushing the cytoplasm and nucleus to the edge.
- Cell wall and middle lamella: a thick layer outside the cell membrane; the middle lamella is the thin line between the walls of two neighbouring cells.
- Plasmodesmata: narrow gaps crossing the wall, joining the cytoplasm of two cells.

Common mistake: The tonoplast is the membrane around the vacuole. It is not the cell wall and it is not the cell surface membrane.

Starch and cellulose Spec 4.3

Both starch and cellulose are polysaccharides made by condensation reactions that join glucose monomers with glycosidic bonds. They differ in the type of glucose, the shape of the molecule and therefore the function.

Starch

Starch is the main energy store in plants and is made of α-glucose units. It is a mixture of two polysaccharides.

- Amylose: unbranched chain joined by α-1,4 glycosidic bonds. The chain coils into a helix, which makes it very compact.
- Amylopectin: α-1,4 chains with α-1,6 glycosidic bonds that make branches. Many free ends allow enzymes to hydrolyse it quickly, releasing glucose for respiration.

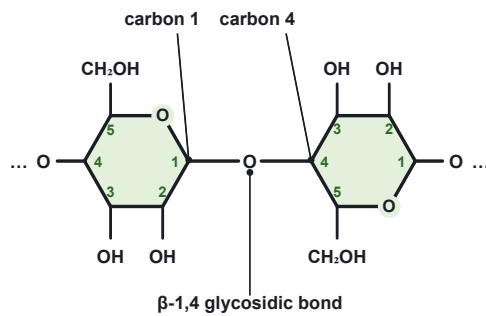
Starch is a good storage molecule because it is insoluble, so it does not change the water potential of the cell or leave the cell. It is also compact, so a lot of glucose is stored in a small space, and it is easily hydrolysed to α-glucose when energy is needed.

Cellulose

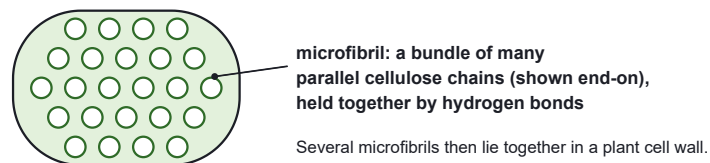
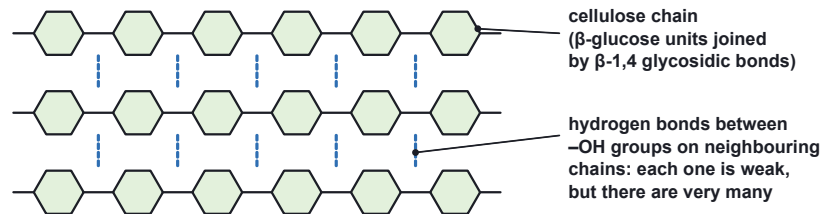
Cellulose is the main component of plant cell walls and is made of β-glucose units. In β-glucose the -OH on carbon 1 is above the ring. To form a β-1,4 glycosidic bond, every other glucose unit must be rotated through 180°. This gives a long, straight, unbranched chain.

1. Straight cellulose chains lie side by side.
2. Many hydrogen bonds form between the -OH groups on adjacent chains. Each bond is weak, but there are very many of them, so together they are strong.
3. Many chains held side by side in a bundle form a microfibril.
4. Microfibrils group into larger fibres. These have very high tensile strength and resist being pulled apart.

Starch compared with cellulose		
Feature	Starch	Cellulose
Monomer	α-glucose	β-glucose
Glycosidic bonds	α-1,4 (and α-1,6 in amylopectin)	β-1,4 only
Shape	Helical (amylose) or branched (amylopectin)	Straight, unbranched chains
Hydrogen bonds between chains	Few: the amylose helix is held by hydrogen bonds within the chain, not between chains	Yes, forming microfibrils
Function	Energy store	Structural: strengthens the cell wall



Every second β-glucose is rotated through 180°, so the chain is straight.



Cellulose: alternate **β-glucose** units are inverted and joined by β-1,4 glycosidic bonds. Many **hydrogen bonds** between parallel chains hold them together in a microfibril.

Common mistake: Do not say "cellulose is strong because it has strong bonds". Each hydrogen bond is weak. Strength comes from the very large number of hydrogen bonds between parallel chains.

Cell wall structure, secondary thickening and plant fibres Spec 4.4

The primary cell wall

In a growing cell, cellulose microfibrils are laid down in the **primary cell wall** in layers, with the microfibrils running at different angles. They are held in a matrix of other polysaccharides. This arrangement resists forces from every direction, including the outward pressure of the turgid cell, while the wall remains flexible enough to allow growth.

Secondary thickening

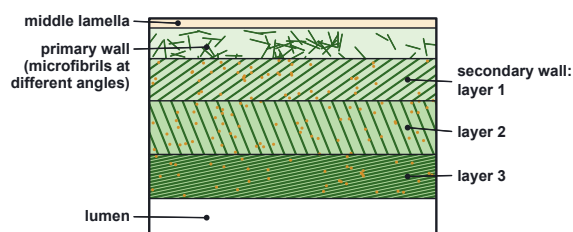
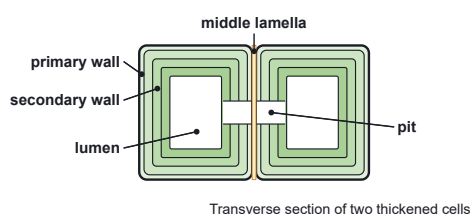
In some cells, extra layers are added on the inside of the primary wall after the cell has stopped growing. This is **secondary thickening**. The new layers contain cellulose microfibrils, and in xylem vessels and sclerenchyma fibres they are impregnated with **lignin**, a rigid, waterproof polymer. The cell contents then die, leaving a dead, hollow cell with a strong wall.

How wall structure gives xylem vessels and sclerenchyma fibres their properties

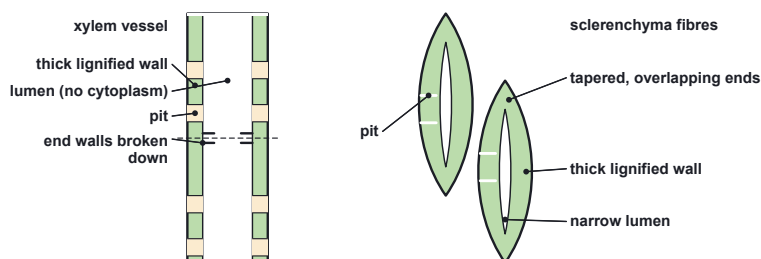
Tissue	Wall features	Physical properties
Xylem vessels	Thick secondary walls with cellulose and lignin, in rings, spirals or almost continuous layers with pits left unthickened. No end walls between vessel elements.	Lignin makes the wall waterproof, so water does not leak out. The thick, rigid wall resists collapse under the negative pressure (tension) caused by transpiration pull and gives the stem support. Pits allow sideways movement of water.
Sclerenchyma fibres	Very long, narrow cells with tapered ends, overlapping their neighbours. Thick, heavily lignified secondary walls built of layers of parallel cellulose microfibrils; in the thickest layer the microfibrils run almost along the length of the cell. Dead at maturity with a narrow lumen.	Very high tensile strength and some flexibility, so they support the plant and resist bending and stretching.

Properties exploited by humans

The long cells and tightly bound microfibrils of sclerenchyma fibres make plant fibres strong, flexible and light. Humans extract them from stems and leaves, for example flax, hemp, jute and sisal, to make rope, string, textiles, paper, packaging and fibre-reinforced composite materials. Xylem tissue in wood is used for timber because its thick lignified walls are strong in compression and bending.



Within a layer the microfibrils are parallel; the angle changes from layer to layer. Amber dots: lignin impregnating the secondary wall.



Longitudinal sections: xylem vessel (left) and sclerenchyma fibres (right)

Walls of xylem vessels and sclerenchyma fibres: the **secondary wall** is built of layers of microfibrils, each at a different angle, and is impregnated with **lignin**.

Exam tip: Link structure to property to use: "microfibrils in layers and lignin give high tensile strength, so fibres can be used for rope".

Quick check

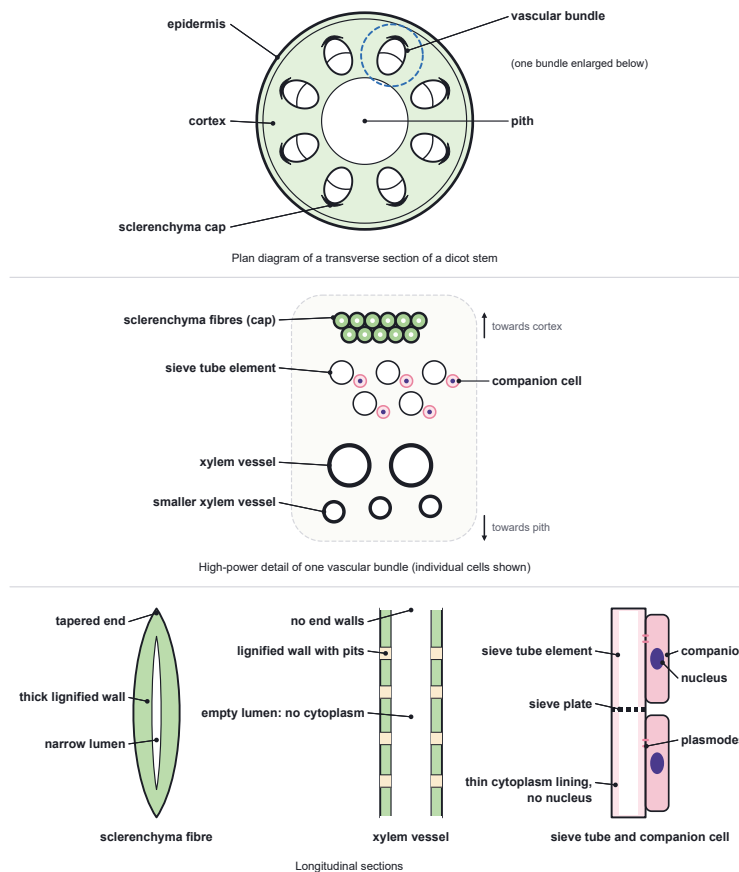
- 1 . Name the membrane that surrounds the plant vacuole.
- 2 . State the function of plasmodesmata.
- 3 . Which type of glucose is cellulose made from?
- 4 . Why does amylopectin release glucose faster than amylose?
- 5 . Name the polymer in secondary walls that makes them waterproof and rigid.

2 Plant tissues, fibres and mineral nutrition Spec 4.5-4.9

Sclerenchyma fibres, xylem vessels and phloem Spec 4.5

These three tissues are all elongated cells arranged in strands that run along the length of roots and stems. Know where they are in the stem, what their cells look like and what they do.

Sclerenchyma fibres, xylem vessels and phloem			
Feature	Sclerenchyma fibres	Xylem vessels	Phloem (sieve tubes and companion cells)
Function	Support (mechanical strength)	Support and transport of water and mineral ions	Translocation of organic solutes (mainly sucrose and amino acids)
Living or dead at maturity	Dead	Dead	Sieve tube elements living; companion cells living
Cell wall	Thick, lignified secondary wall; narrow lumen	Thick lignified secondary wall in rings, spirals or nearly continuous layers; pits	Thin cellulose walls, not lignified
Cell contents	None	None; hollow lumen, no end walls between vessel elements, forming a continuous tube	Sieve tube: little cytoplasm, no nucleus, few organelles; sieve plates with pores at the ends. Companion cell: dense cytoplasm, nucleus, many mitochondria
Cell shape	Very long, narrow, tapered ends overlapping	Long, wide tubes joined end to end	Sieve tube elements joined end to end; companion cell alongside each
Direction of flow	Not applicable	One direction only: upwards from roots	Both directions, from sources to sinks
Position in a dicot stem	In a cap on the outer side of each vascular bundle, and as a ring or patches towards the outside of the stem	Inner (central) side of each vascular bundle, towards the pith	Outer side of each vascular bundle, between the xylem and the sclerenchyma



Dicot stem: plan diagram, one vascular bundle in detail, and the three cell types seen in longitudinal section.

Similarities

- All three are elongated cells arranged end to end or side by side.
- Fibres and xylem vessels both have thick lignified walls and are dead when mature.
- All three are found in the vascular bundles (fibres also outside them).
- Fibres and xylem vessels both support the plant; phloem does not have a support role.

Roots and leaves

In a root the xylem forms a central star or cross shape, with phloem between its arms, which helps the root resist pulling forces. In a leaf, the vascular bundles in the midrib and veins have xylem on the upper side and phloem on the lower side.

Exam tip: In stems, remember the order from the centre outwards: pith, xylem, phloem, sclerenchyma cap. Questions often ask you to name a tissue from its position and description.

Core practical 7: plant tissues under the light microscope Spec 4.6

Core practical: Core practical 7: use a light microscope to make observations, draw and label plan diagrams of transverse sections of roots, stems and leaves; draw and label cells of plant tissues; and identify sclerenchyma fibres, phloem, sieve tubes and xylem vessels and their location.

Preparing a section

1. Use a sharp razor blade or scalpel to cut a very thin transverse section of the stem, root or leaf. Cut away from your fingers, and place the blade in a sharps container or as directed after use.
2. Float the sections in water in a dish. Choose the thinnest, most complete section with a fine paintbrush.

3. Stain: toluidine blue stains lignified walls blue-green; phloroglucinol with hydrochloric acid stains lignin red. Treat hydrochloric acid as corrosive and wear eye protection.
4. Mount on a slide in a drop of water or stain. Lower a coverslip at an angle, using a mounted needle, to avoid trapping air bubbles.
5. Start with the lowest power objective, focus with the coarse focus and then the fine focus, then move to a higher power for detail.

Plan diagrams

- Draw with a sharp pencil, using clean single lines, with no shading and no colouring.
- Draw the outline of the whole section and the boundaries between tissues. Do not draw individual cells.
- Make the drawing large (using at least half the page) and in proportion.
- Label with straight lines ruled from the structure, not crossing each other, with a title, and record the magnification.

What to show on plan diagrams of transverse sections

Section	Tissues to outline and label
Root	Epidermis (with root hairs), cortex, endodermis, and a central vascular cylinder with xylem in a star or cross shape and phloem between its arms
Stem	Epidermis, cortex, a ring of vascular bundles (xylem on the inner side, phloem outside it, sclerenchyma cap outermost) and central pith
Leaf	Upper and lower epidermis, palisade mesophyll, spongy mesophyll, and the midrib vascular bundle with xylem on the upper side and phloem on the lower side

Drawing cells of a tissue

Use a higher power objective and draw a small number of cells in one tissue with a continuous line, showing the cell walls, lumen and contents. Show thick walls as double lines. Identify the tissues from their appearance.

- **Xylem vessels**: large circular lumens, no contents, thick walls (stained blue-green or red by lignin stains).
- **Sclerenchyma fibres**: small lumens, very thick walls, in groups outside the phloem.
- **Phloem**: thin-walled; **sieve tubes** are larger and empty-looking, with companion cells smaller and with dense cytoplasm beside them. In a longitudinal section a sieve plate may be visible.

magnification = size of image ÷ size of real object

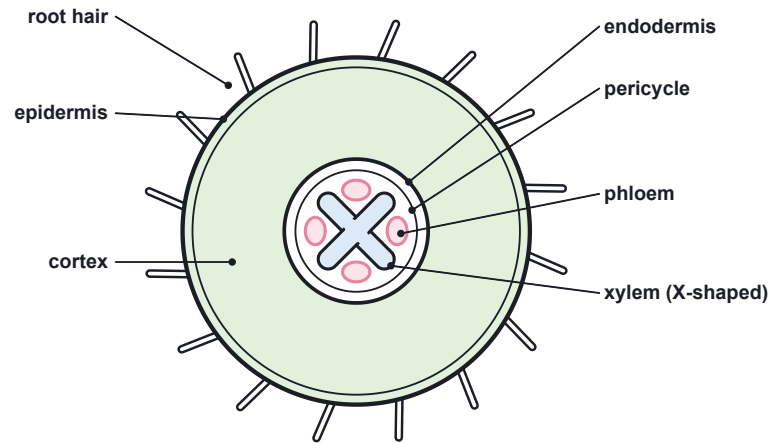
Calculating the magnification of a plan diagram

A student's plan diagram of a stem section is 96 mm wide. The real section, measured using an eyepiece graticule that was calibrated with a stage micrometer, is 3.2 mm wide. Calculate the magnification.

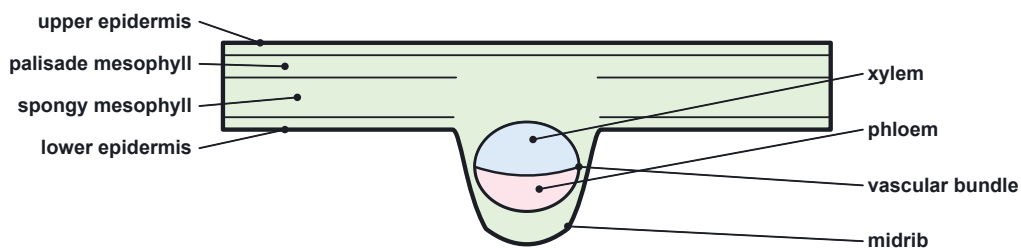
1. Both lengths must be in the same units: 96 mm and 3.2 mm.
2. magnification = $96 \div 3.2$

Answer: $\times 30$

Maths skill: To find the real size from a drawing, rearrange: $\text{real size} = \text{image size} \div \text{magnification}$.
Convert units first: $1 \text{ mm} = 1000 \mu\text{m}$.



Plan diagram of a transverse section of a young dicot root



Plan diagram of a transverse section through a dicot leaf midrib

Plan diagrams: a young root and a leaf midrib. Plan diagrams show tissues as outlines only, with no individual cells.

Common mistake: A plan diagram must not show individual cells, and labels must not cross. Both are common ways to lose marks.

Plant fibres, starch and sustainability Spec 4.7

Oil-based plastics are made from crude oil, a finite resource, and most do not break down quickly, so they build up as waste. Plant-based materials offer alternatives that can contribute to sustainability, which means meeting the needs of the present without harming the ability of future generations to meet their own needs.

Plant-based products that can replace oil-based plastics		
Material	Source	Example uses
Plant fibres (hemp, flax, jute, sisal)	Sclerenchyma fibres from stems and leaves	Rope, string, textiles, bags, and fibre-reinforced materials used in place of plastic or glass fibre composites
Starch-based plastics	Starch from crops such as maize, potato or cassava	Packaging, carrier bags, cutlery, food containers, loose-fill packaging

How they contribute to sustainability

- They are renewable: new crops can be grown each year.
- Plants take in carbon dioxide during photosynthesis, so the carbon released when products are used or break down was recently removed from the atmosphere. This can reduce net carbon dioxide emissions compared with using oil.
- They are often biodegradable, so microorganisms can break them down and less waste accumulates.
- Less crude oil is needed, which reduces the use of a finite resource.

Limitations

- Growing crops for materials needs land, water and fertiliser, which can compete with food production and reduce biodiversity if natural habitat is cleared.
- Some starch-based plastics break down only under particular conditions, such as in industrial composting, and may be less strong or less waterproof than oil-based plastics.
- Processing and transport still use energy.

Exam tip: A balanced answer scores best: give one or two points on renewability or biodegradability and a limitation such as land use competing with food crops.

Water and inorganic ions in plants Spec 4.8

Water

- Keeps cells **turgid**, which supports the stems and leaves of non-woody plants and opens stomata.
- A solvent and transport medium: ions are carried in the xylem and sucrose in the phloem.
- A reactant in photosynthesis (light-dependent reactions) and in hydrolysis reactions.
- Evaporation (transpiration) cools the leaf and pulls water and ions up the plant.
- Needed for cell expansion during growth.

Inorganic ions

Ions needed by plants		
Ion	Used for	Effect of deficiency
Nitrate (NO_3^-)	Source of nitrogen to make amino acids, proteins, nucleotides and nucleic acids (DNA and RNA bases), and chlorophyll	Stunted growth; older leaves turn yellow (chlorosis) because nitrogen is moved to young leaves
Calcium (Ca^{2+})	Forms calcium pectate in the middle lamella and helps build cell walls; also helps maintain membrane structure	Poor growth, especially of young tissues and roots; distorted young leaves
Magnesium (Mg^{2+})	Central atom of the chlorophyll molecule, needed to make chlorophyll	Chlorosis: leaves, especially older ones, turn yellow between the veins, so photosynthesis is reduced

Plants absorb these ions as dissolved ions from the soil water, mainly through the root hair cells by active transport.

The specification recommends (as an additional practical, not a core practical) that you **investigate plant mineral deficiencies**. Seedlings of the same species and age are grown in culture solutions that are identical except that one lacks nitrate, calcium or magnesium ions, with a complete solution as the control. After a few weeks, compare growth (for example height, number of leaves or dry mass) and leaf colour.

Common mistake: Say that magnesium ions are the central atom in the chlorophyll molecule, and that plants absorb nitrogen as nitrate ions, not as nitrogen gas.

Core practical 8: tensile strength of plant fibres Spec 4.9

Core practical: Core practical 8: determine the tensile strength of plant fibres.

Tensile strength

The maximum force per unit cross-sectional area that a material can withstand when it is pulled before it breaks. Units: N mm^{-2} or Pa ($1 \text{ N mm}^{-2} = 1 \text{ MPa}$).

$$\text{tensile strength} = \text{breaking force} \div \text{cross-sectional area}$$

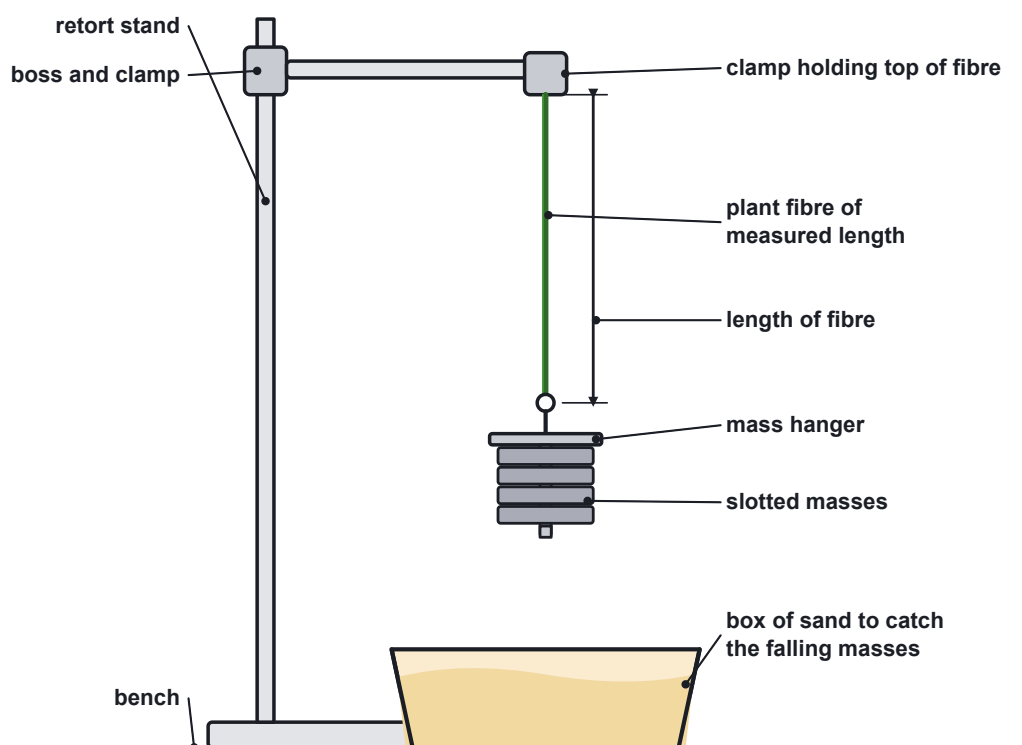
$$\text{force (N)} = \text{mass (kg)} \times 9.81 \text{ N kg}^{-1} \text{ and area} = \pi r^2$$

Method

1. Cut fibres of the same length from each plant being compared (for example 20 cm). Measure the diameter at several points with a micrometer or under a microscope with a graticule, and calculate the mean radius.
2. Clamp one end of the fibre firmly to a retort stand at the top. Tie a mass hanger securely to the other end.
3. Place a box of soft material, such as sand or cushioning, below the hanger, and wear eye protection and closed shoes.
4. Add masses one at a time (for example 100 g), waiting a few seconds each time, until the fibre breaks.
5. Record the total mass, including the hanger, at which the fibre broke. Repeat with at least five fibres of each type and calculate a mean.
6. Calculate the breaking force, the cross-sectional area from the radius, and then the tensile strength.

Variables

Type	Variable	How
Independent	Type of plant fibre (or treatment)	Choose fibres from different plants
Dependent	Mass or force at which the fibre breaks	Add masses until the fibre snaps
Control	Length of fibre; diameter (or calculate per unit area); moisture content; temperature; how the fibre is attached; size of mass increments	Same length, same age and storage, same increments and time between additions



Apparatus for measuring the tensile strength of a plant fibre. Add masses until the fibre breaks.

Risks and reliability

- Falling masses can injure feet: use a box of soft material and closed shoes.
- Fibres may snap near the knot or clamp. Discard these as anomalies and repeat.
- Fibres vary in thickness along their length, so measure the diameter in several places and calculate area per fibre.
- Repeating with many fibres and calculating a mean improves reliability. Use the same increments of mass for all fibres.

Calculating tensile strength

A plant fibre of diameter 0.40 mm breaks when the total mass hanging from it is 4.5 kg. Calculate the tensile strength in N mm^{-2} . Use $g = 9.81 \text{ N kg}^{-1}$.

1. $\text{force} = 4.5 \times 9.81 = 44.1 \text{ N}$

2. $\text{radius} = 0.40 \div 2 = 0.20 \text{ mm}$, so $\text{area} = \pi \times 0.20^2 = 0.126 \text{ mm}^2$

3. $\text{tensile strength} = 44.1 \div 0.126$

Answer: 350 N mm^{-2} (to 2 significant figures)

Maths skill: Show every step: convert mass to force, convert diameter to radius, then area, then divide. Check that the units match (N and mm^2).

Quick check

- 1 . Name the tissue that carries sucrose around the plant.
- 2 . Why can xylem vessels carry water in one continuous column?
- 3 . State the formula for magnification.
- 4 . Why do plants need magnesium ions?
- 5 . Give the unit of tensile strength.

3 Bacterial growth, plant medicines and drug testing Spec 4.10–4.13

Conditions required for bacterial growth Spec 4.10

Bacteria are prokaryotes that reproduce asexually by **binary fission**. In ideal conditions some species can divide every 20 to 30 minutes. For growth to happen, several conditions must be met.

Conditions required for bacterial growth		
Condition	Why it is needed	In the laboratory
Nutrients	A carbon source (for example glucose) for respiration and making organic molecules; a nitrogen source for amino acids and nucleotides; mineral ions and sometimes vitamins	Nutrient agar or nutrient broth
Temperature	Enzymes have an optimum temperature. Too cold: slow growth. Too hot: enzymes denature	Incubate at a suitable temperature; in school laboratories usually 25 °C and never above 30 °C, to reduce the risk of growing human pathogens (which grow best near 37 °C)
Oxygen	Aerobic bacteria need oxygen for aerobic respiration. Anaerobic bacteria are inhibited or killed by oxygen	Plates are incubated with lids on, taped in only a few places, so that oxygen can enter
pH	Enzymes work best in a narrow pH range; most bacteria grow best near neutral pH	Buffers or media at a suitable pH
Water	Metabolic reactions take place in solution	Agar gel or broth is mostly water

Providing the conditions safely

- Culture media and equipment are sterilised before use (for example in an autoclave, at 121 °C), so that unwanted microorganisms do not compete with the bacteria being grown.
- Cultures are grown at temperatures that make human pathogens unlikely to grow, and plates are not opened after incubation.
- At the end, cultures are sterilised before disposal.

Exam tip: Do not just list "warmth". Say the bacteria need a suitable temperature because enzymes have an optimum, and name nutrients (carbon and nitrogen sources).

Antimicrobial and therapeutic substances from plants Spec 4.11

Plants cannot move away from pests and pathogens, so many make chemicals that kill or deter microorganisms, fungi and herbivores. Some of these chemicals have been used in traditional medicine, and some have been developed into modern drugs.

Examples of therapeutic substances from plants		
Plant	Substance	Use
Garlic	Allicin	Antimicrobial properties (antibacterial and antifungal)
Willow bark	Salicin, from which aspirin was developed	Pain relief and reducing fever and inflammation
Foxglove (<i>Digitalis purpurea</i>)	Digitalis (digoxin)	Treating some heart conditions
Cinchona bark	Quinine	Treating malaria
Sweet wormwood (<i>Artemisia annua</i>)	Artemisinin	Treating malaria
Madagascan periwinkle	Vincristine	Treating some cancers

Using plant substances

- Extracts may contain many compounds, so the active compound must be identified and tested.
- The dose in a plant varies with species, age, growing conditions and part of the plant, so a purified, standardised dose is safer.
- Many plants have not been tested, so loss of plant species may lose possible medicines.
- Plant substances can be toxic, as with digitalis, and must be tested before use.

Exam tip: Name at least one plant, the substance and its use. "Antimicrobial" means kills or inhibits the growth of microorganisms such as bacteria.

Core practical 9: antimicrobial properties of plants and aseptic technique Spec 4.12

Core practical: Core practical 9: investigate the antimicrobial properties of plants, including aseptic techniques for the safe handling of bacteria.

Aseptic technique

Aseptic technique prevents unwanted microorganisms contaminating the culture and prevents the bacteria escaping into the environment or infecting the worker.

- Disinfect the bench before and after work, and wash hands.
- Work near a lit Bunsen burner, where rising hot air carries microorganisms away from the work area.
- Sterilise inoculating loops by heating in a flame until red hot, then let them cool before touching the culture.
- Flame the neck of bottles after opening and before closing, hold lids rather than put them down, and lift the lid of a Petri dish only as far as needed.
- Use sterile Petri dishes, agar and spreaders, and keep dishes closed when not in use.
- Seal the lid with only two or four small pieces of tape, never fully around, so oxygen can enter and anaerobic pathogens are not encouraged. Incubate upside down, so condensation does not drip on the agar.
- Do not open dishes after incubation. Sterilise all equipment and cultures in an autoclave afterwards.

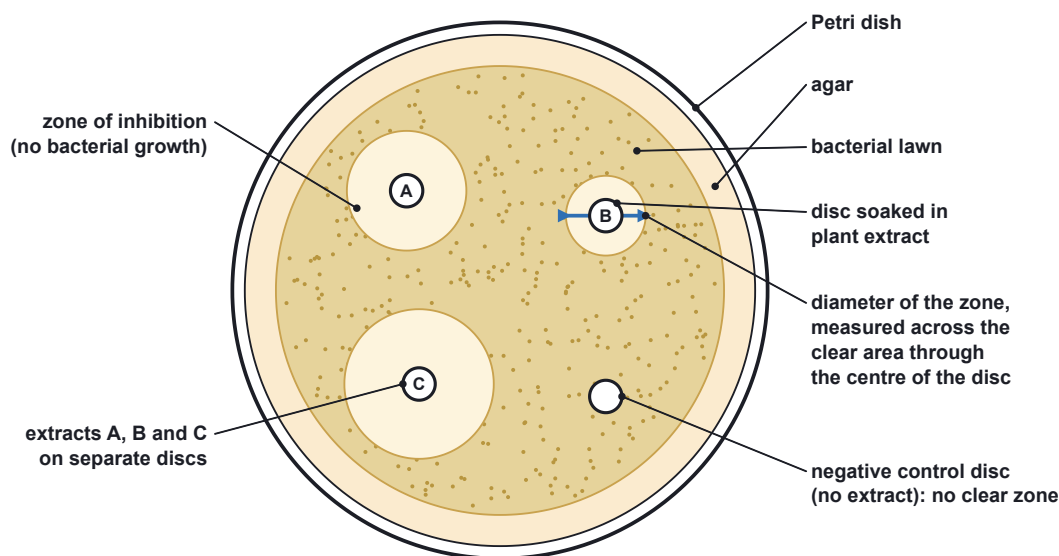
Method

1. Prepare plant extracts, for example by crushing fresh leaves or cloves in a mortar with a measured volume of solvent and filtering. Keep the same mass of plant material and volume of solvent for each plant, and make a dilution series if concentration is being tested.

- Using aseptic technique, spread a standard volume of a non-pathogenic bacterial culture evenly over the surface of nutrient agar in a Petri dish to make a bacterial **lawn**.
- Place sterile filter paper discs, soaked for the same time in each plant extract, onto the lawn with sterile forceps. Include a negative control disc soaked only in the solvent and, if available, a positive control disc with a known antibiotic.
- Tape, invert and incubate the dishes at 25 °C for 24 to 48 hours.
- Measure the diameter of the clear zone around each disc (zone of inhibition) in two directions at right angles and calculate a mean diameter. Calculate the area of the zone (area = πr^2).

Variables and controls

Type	Detail
Independent	Type of plant extract (or concentration of the extract)
Dependent	Diameter or area of the clear zone of inhibition
Control (kept constant)	Volume of bacteria spread; type of bacteria; agar depth and type; size of disc; volume and concentration of extract; incubation temperature and time
Control experiment	Disc soaked in solvent only (negative control) shows that the solvent does not prevent growth; a disc with antibiotic (positive control) shows that bacteria can be inhibited



Plan view of an agar plate. The **zone of inhibition** is the clear area where bacteria have not grown; measure its diameter through the centre of the disc.

Comparing zones of inhibition

A clear zone of diameter 14 mm formed around a disc soaked in garlic extract. Calculate the area of the zone of inhibition.

- radius = $14 \div 2 = 7$ mm
- area = $\pi r^2 = \pi \times 7^2$

Answer: 154 mm² (to 3 significant figures)

Maths skill: The area of the clear zone (πr^2) is often used because it describes the whole region where growth was inhibited; ranking extracts by diameter gives the same order. If asked for the area of inhibition only, subtract the area of the disc.

Common mistake: A zone of inhibition does not show that bacteria are killed, only that growth is stopped. A negative control is needed to show that the effect is due to the extract and not the solvent.

The development of drug testing Spec 4.13

Historic testing: William Withering and digitalis soup

In the 18th century, William Withering investigated a traditional herbal mixture ('digitalis soup') used to treat dropsy (swelling due to fluid build-up, often caused by heart failure). He found that foxglove was the active ingredient. He recorded the effects of different doses on his patients, and found the dose that worked without causing toxic effects. His work showed that plants contain active chemicals, but there was no control group, no placebo, patients and doctor knew the treatment and the results were open to bias.

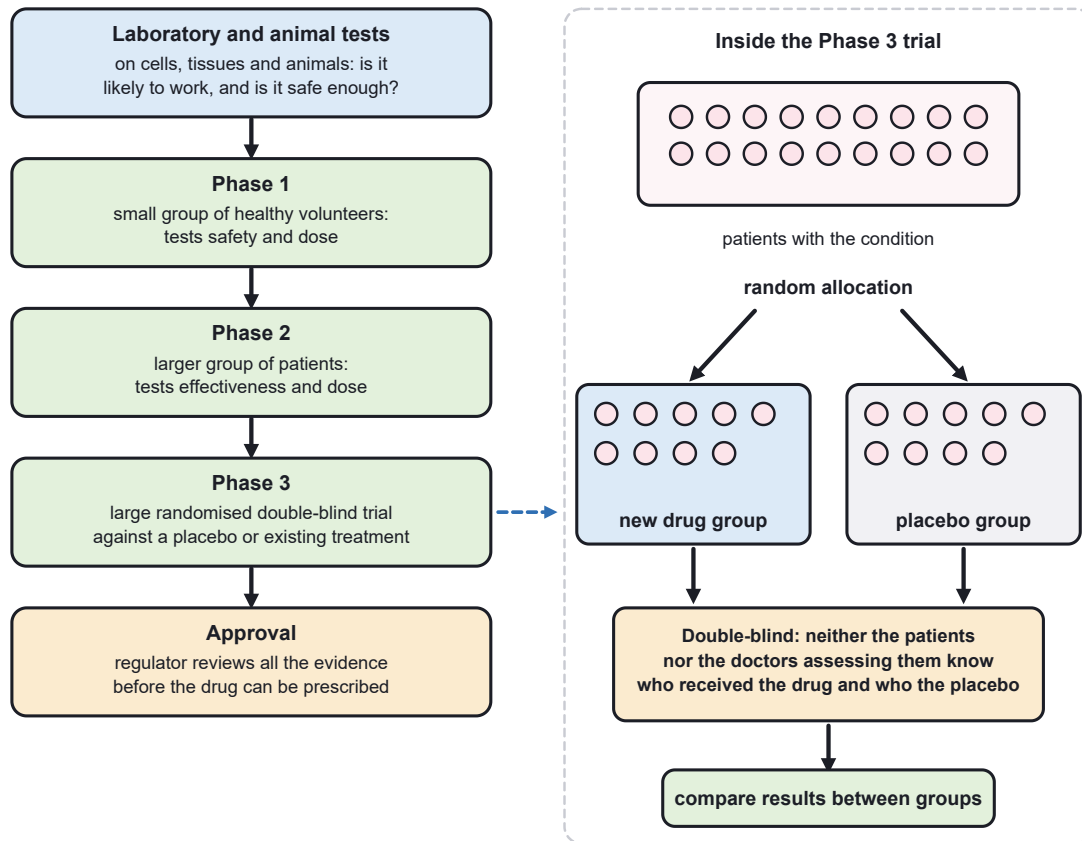
Modern drug testing

1. Laboratory tests: computer models, then tests on cells and tissues, and on animals, to check effects, toxicity and dose.
2. **Phase 1**: a small number of healthy volunteers receive the drug to test safety, side effects and how the body handles it.
3. **Phase 2**: a larger group of patients with the condition is given the drug to find out whether it works and what dose is best.
4. **Phase 3**: a very large group of patients, in a randomised, controlled trial, compares the new drug with a placebo or the best existing treatment to confirm effectiveness and side effects.

Placebo	An inactive substance that looks identical to the drug, given to the control group so the effect of the drug can be separated from the effect of believing one is being treated.
Double-blind trial	A trial in which neither the patients nor the doctors and researchers assessing them know who is receiving the drug and who is receiving the placebo, to avoid bias.

Why these features matter

- A control group and placebo allow for the placebo effect, where patients improve because they expect to.
- Random allocation makes the groups similar in age, health and other factors.
- Double-blind design stops patients and doctors influencing the results, consciously or not.
- Large samples reduce the effect of chance and allow rare side effects to be detected.
- Patients must give informed consent, and trials can be stopped if the drug causes harm or is clearly beneficial.



Stages of drug development, and how a randomised **double-blind** trial is organised.

Exam tip: If asked why a trial is double-blind, say who is not told (patients and the researchers assessing them) and what this prevents (bias). If asked why a placebo is used, mention the placebo effect.

Quick check

1. Name two nutrients that bacteria need to grow.
2. Why is a Bunsen burner lit during aseptic work?
3. What does a zone of inhibition show?
4. What is a placebo?
5. What is the purpose of Phase 1 of a clinical trial?

4 Classification and biodiversity Spec 4.14–4.18

Classification and the three-domain system Spec 4.14

Classification is a way of organising the variety of life into groups, based on the relationships between organisms. Organisms that are more closely related share more similarities in their **phenotypes** (observable features such as anatomy, physiology and behaviour) and in their **genotypes** (DNA base sequences, which can also be compared indirectly through the amino acid sequences of the proteins they code for).

The species concept and hierarchy

The basic unit of classification is the **species**: a group of organisms with similar characteristics that can interbreed to produce fertile offspring. Species are placed in a hierarchy of groups, each contained in the next: domain, kingdom, phylum, class, order, family, genus, species. Each species has a two-part name, the binomial (for example *Homo sapiens*), written with the genus first (capital letter) and the species second.

Exam tip: The species concept has limits: it does not apply to organisms that reproduce only asexually (including bacteria), or to fossils, and some different species can interbreed to produce hybrids.

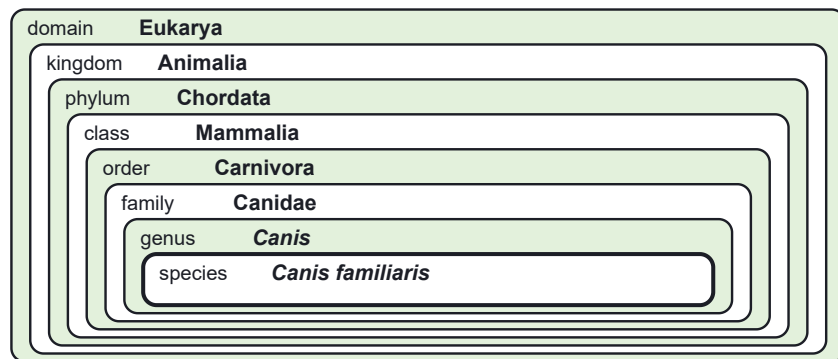
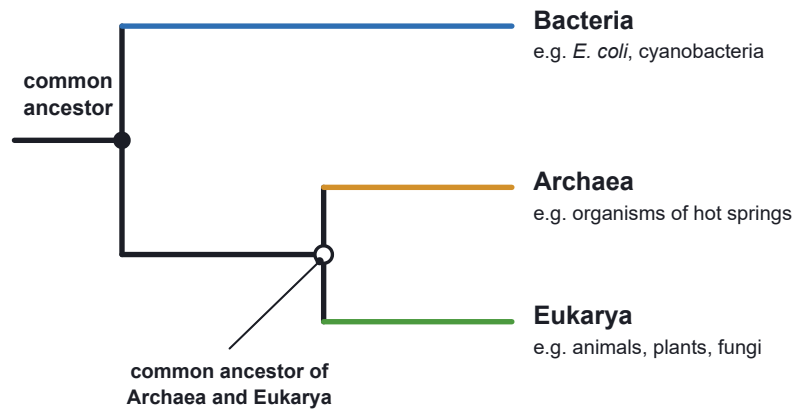
Molecular evidence

Early classification used mainly physical features, which can mislead because unrelated organisms may look similar. Comparing DNA base sequences, the amino acid sequences of proteins and RNA sequences gives more objective evidence of how closely organisms are related. The more similar the sequences, the more recently the species shared a common ancestor.

Critical evaluation and the three domains

New data are critically evaluated by the scientific community. Before publication in a scientific journal, other experts check the methods, data and conclusions (peer review). After publication and presentation at conferences, other scientists discuss the findings and try to repeat the work, often with new data. Only if the evidence is supported do the taxonomic groupings change, which keeps classification reliable. Molecular evidence showed that the prokaryotes used to be considered a single kingdom contain two very different groups, and led to the **three-domain system** in which all organisms are placed into Archaea, Bacteria or Eukarya.

Features of the three domains			
Feature	Bacteria	Archaea	Eukarya
Nucleus	Absent	Absent	Present
Cell wall	Peptidoglycan	No peptidoglycan	Cellulose (plants), chitin (fungi), none in animals
Membrane lipids	Ester-linked	Ether-linked	Ester-linked
Ribosomes	70S	70S	80S
DNA	Circular, no histones	Circular; in many species associated with histones	Linear, associated with histones
Examples	<i>Escherichia coli</i>	Methane-producing and many extreme-environment species	Plants, animals, fungi, protists



The three domains of life, and the taxonomic hierarchy using the domestic dog as an example.

Biodiversity, endemism and human threats Spec 4.15, 4.16

Biodiversity	The variety of living organisms in a given area or on Earth, measured as the number and abundance of species (species diversity), the genetic variation within species (genetic diversity) and the range of habitats (habitat diversity).
Endemism	A species being found in only one geographical region, and nowhere else in the world. Such a species is called endemic. Examples: lemurs in Madagascar; many species on isolated islands such as the Galápagos or Hawaii.

The variety of life over time

Over millions of years evolution by natural selection and speciation has produced an extensive variety of life, with millions of species, most of them not yet described. Biodiversity has been reduced in the past by mass extinctions.

The threat from human activity

The variety of life is now being reduced faster than before, and human activity is the main cause. Endemic species are especially vulnerable because they live in small areas and cannot move elsewhere if their habitat is changed.

Human threats to biodiversity

Threat	How it reduces biodiversity
Habitat loss and fragmentation	Clearing land for agriculture, housing and roads destroys habitats or divides them into small patches
Climate change	Warming changes rainfall and temperature, so species may no longer be adapted to their habitat
Pollution	Fertilisers, pesticides and plastics harm organisms or alter habitats
Overexploitation	Hunting, fishing and harvesting faster than populations can recover
Introduced (invasive) species	Compete with, prey on or bring disease to native species

Exam tip: Always say how the threat reduces biodiversity (for example "habitat destruction reduces the number of species that can live there"), not only name it.

Measuring biodiversity: species richness and heterozygosity index Spec 4.17

Species richness

Species richness is the number of different species in a habitat. It is found by sampling (for example with quadrats, transects or pitfall traps), identifying each species and counting how many species there are. It is simple, but it takes no account of how many individuals there are in each species. A habitat with 10 species of which 99% of individuals are one species has the same richness as one where the 10 species are equally common.

Genetic diversity: heterozygosity index

Genetic diversity is the variety of alleles within a species. One measure is the **heterozygosity index**, which uses the proportion of individuals in a population that are heterozygous for a gene.

heterozygosity index = number of heterozygotes ÷ number of individuals in the population

The value is between 0 and 1. A value near 1 means many individuals are heterozygous and the population has high genetic diversity. A small isolated population, in which alleles are lost and relatives interbreed, has a low index.

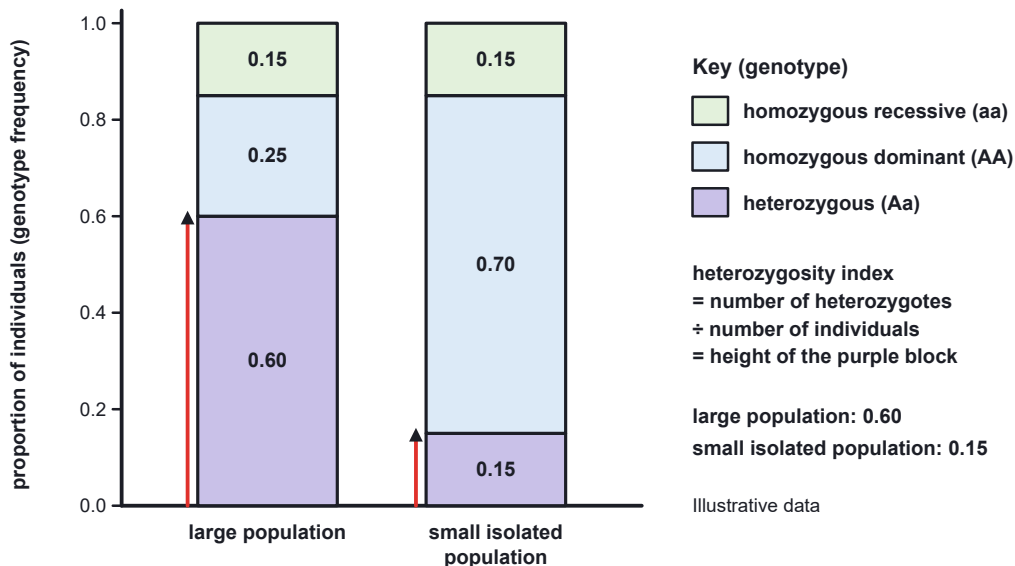
Calculating a heterozygosity index

A sample of 80 beetles was genotyped for one gene. 30 beetles were AA, 36 were Aa and 14 were aa. Calculate the heterozygosity index.

1. Heterozygotes (Aa) = 36
2. Individuals in the population sample = 80
3. $36 \div 80$

Answer: 0.45

Maths skill: Heterozygotes are individuals with two different alleles (Aa). Do not include homozygous individuals (AA or aa) in the top line.



Illustrative data: the small isolated population has a much lower heterozygosity index, so less genetic diversity.

Comparing habitats: index of diversity (D) Spec 4.18

Species richness does not tell us how evenly individuals are spread among species. The index of diversity (D) uses both the number of species and their relative abundance.

$$D = N(N - 1) \div \sum n(n - 1)$$

- N = total number of organisms of all species
- n = total number of organisms of each species
- Σ = sum of

A habitat with many species with similar numbers of individuals has a high D. A habitat dominated by one species has a low D, as does one with few species. D has a minimum of 1 for a single species.

Calculating D for a habitat

A quadrat survey of woodland A recorded five species: 12, 8, 5, 3 and 2 individuals. Calculate the index of diversity.

1. $N = 12 + 8 + 5 + 3 + 2 = 30$
2. $N(N - 1) = 30 \times 29 = 870$
3. $\sum n(n - 1) = (12 \times 11) + (8 \times 7) + (5 \times 4) + (3 \times 2) + (2 \times 1) = 132 + 56 + 20 + 6 + 2 = 216$
4. $D = 870 \div 216$

Answer: 4.0 (to 2 significant figures)

If a second woodland, B, has 30 individuals in three species (25, 3 and 2), $\sum n(n - 1) = 600 + 6 + 2 = 608$ and $D = 870 \div 608 = 1.4$. Woodland A is more diverse than woodland B, even though B also has several species, because B is dominated by one species.

Interpreting and evaluating D

- A high D means the habitat is more diverse, which often indicates a stable habitat with many niches.
- A low D suggests a habitat dominated by a few species, which may indicate pollution or disturbance.

- Results depend on sampling: use random sampling, with enough samples, from habitats of comparable area, at the same time of year.
- D uses numbers only, so it does not show how different the species are from one another, and it gives only a snapshot at one time.

Exam tip: Show N , $N(N - 1)$, the sum $\sum n(n - 1)$ and the division. A final answer with no working often loses marks.

Maths skill: $n(n - 1)$ for a species with 1 individual is 0, so single individuals do not add to the bottom line but are still counted in N .

Quick check

1. Name the three domains of life.
2. What type of evidence led to the three-domain system?
3. Define endemism.
4. What is species richness?
5. In $D = N(N - 1) \div \sum n(n - 1)$, what does n stand for?

5 Niche, allele frequency and conservation Spec 4.19–4.21

Niche and adaptations to the environment Spec 4.19

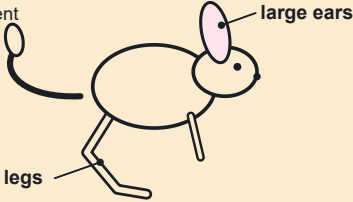
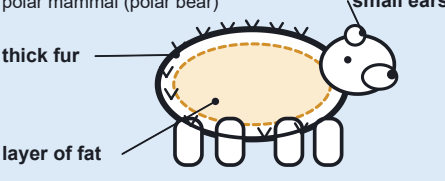
Niche

The role of a species within its habitat: how it obtains food and other resources, the abiotic conditions it tolerates and the biotic interactions it has with other species (such as feeding relationships, competition and predation).

Each species in a habitat has its own niche. If two species have very similar niches they compete for the same resources, and one is likely to decline, so competition tends to separate the niches of species living together. Organisms are adapted to their niche: a feature that helps survival and reproduction in a particular environment is an **adaptation**. Adaptations are the result of natural selection.

Types of adaptation

Type	Meaning	Examples
Behavioural	The way an organism acts	Hibernation in winter; migration; nocturnal activity in desert animals to avoid the heat of the day; courtship displays; basking to warm up
Anatomical	Structural features of the body that can be seen in a dissection or section	Marram grass has rolled leaves, hairs and sunken stomata that reduce transpiration; thick fur and a layer of fat in polar bears; large ears in desert foxes that lose heat
Physiological	Processes inside the body, including biochemical ones	Kangaroo rats produce very concentrated urine, conserving water; antifreeze proteins in polar fish; production of venom or toxins; slowed metabolism during hibernation

	Behavioural	Anatomical	Physiological
Hot dry habitat desert rodent 	Nocturnal: active at night and shelters in a cool burrow by day, avoiding the heat	Large ears lose heat; long hind legs let it hop fast, keeping the body off the hot ground	Very concentrated urine, so little water is lost (as in the kangaroo rat)
Cold habitat polar mammal (polar bear) 	Shelters from wind and cold in a snow den (mothers den to give birth to cubs)	Thick fur and a layer of fat insulate the body; small ears reduce heat loss	Stored fat is broken down for energy and water when food is scarce

Behavioural, anatomical and physiological adaptations in a hot dry habitat and a cold habitat.

Exam tip: When asked to discuss adaptations, name the type (behavioural, anatomical or physiological), give the feature and link it to survival in that environment, for example by reducing water loss.

The Hardy-Weinberg equation, mutation and natural selection Spec 4.20(i), 4.20(ii)

The **allele frequency** is the proportion of a particular allele among all the alleles of that gene in a population. For a gene with two alleles, the dominant allele has frequency p and the recessive allele has

frequency q .

$$p + q = 1$$

$$p^2 + 2pq + q^2 = 1$$

- p = frequency of the dominant allele; q = frequency of the recessive allele
- p^2 = frequency of the homozygous dominant genotype; q^2 = frequency of the homozygous recessive genotype
- $2pq$ = frequency of the heterozygous genotype

Conditions for the equation to hold

The equation predicts that allele frequencies stay the same from one generation to the next only if the population is large, mating is random, there is no mutation, no natural selection and no migration into or out of the population (gene flow).

Using it to detect change

If the population does not meet these conditions, allele frequencies may change over time. Allele frequencies are calculated from a sample of one generation and used to predict the genotype frequencies (p^2 , $2pq$, q^2) expected if nothing changes. These are compared with the frequencies observed in later generations: if they differ significantly, a change in allele frequency is occurring, that is, the population is evolving.

Using the Hardy-Weinberg equation

In a population of 500 plants, 45 plants have white flowers. White is caused by a recessive allele and the population is in Hardy-Weinberg equilibrium. Calculate the number of heterozygous plants.

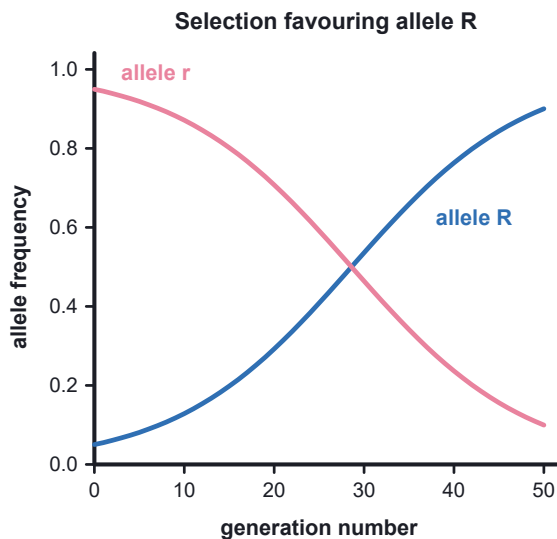
1. $q^2 = 45 \div 500 = 0.09$
2. $q = \sqrt{0.09} = 0.3$
3. $p = 1 - 0.3 = 0.7$
4. $2pq = 2 \times 0.7 \times 0.3 = 0.42$
5. number = 0.42×500

Answer: 210 heterozygous plants

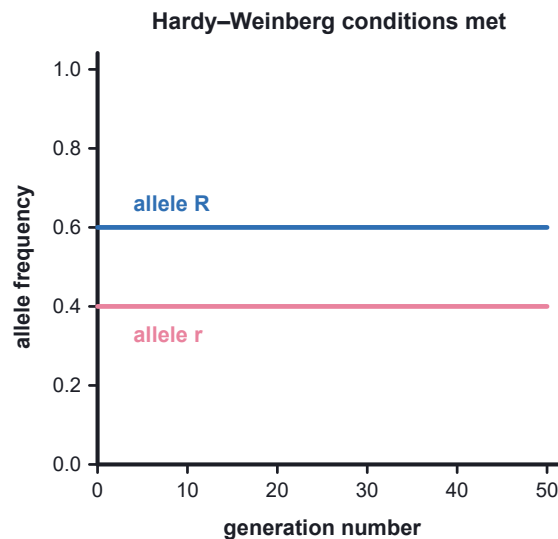
Maths skill: Always start from the one genotype you can see: the homozygous recessive (q^2). Take the square root to get q , then $p = 1 - q$, then $2pq$.

How allele frequencies change

- **Mutation**: a change in the DNA base sequence can create a new allele. Mutation is random and rare, but it is the source of new alleles.
- **Natural selection**: individuals with advantageous alleles are more likely to survive and reproduce and to pass those alleles on, so the frequency of advantageous alleles increases from generation to generation. For example, a mutation giving bacteria resistance to an antibiotic is selected for when the antibiotic is present, and the frequency of the resistance allele increases.



Illustrative data



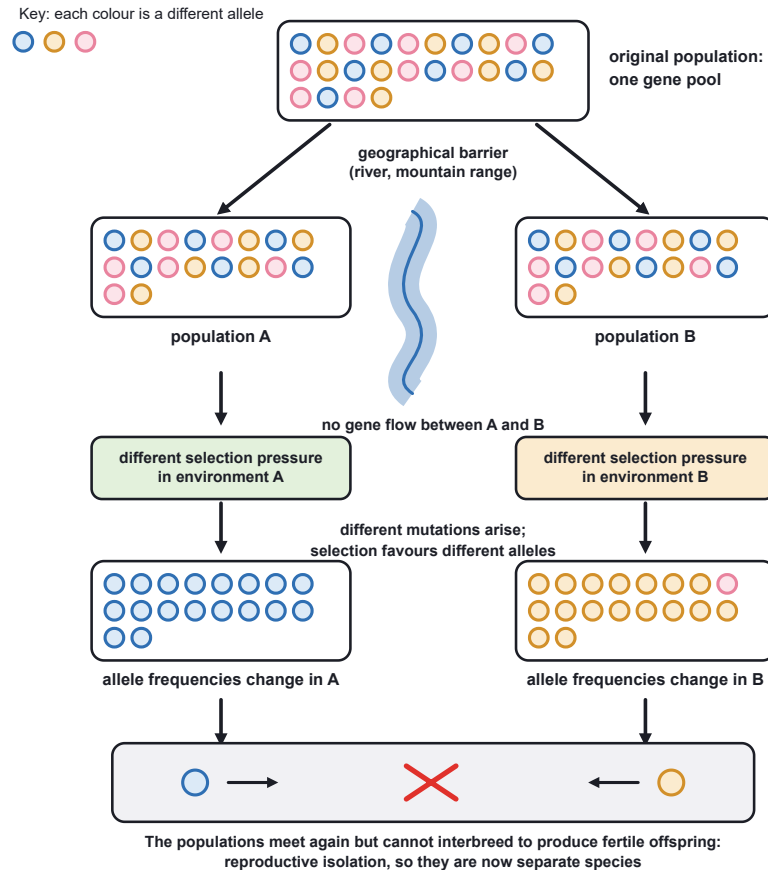
no selection: allele frequencies stay constant

Allele frequencies change under selection (left) but stay constant when the Hardy-Weinberg conditions are met (right).
Illustrative data.

Reproductive isolation and the formation of new species Spec 4.20(iii)

Reproductive isolation means that two populations can no longer exchange genes, so their gene pools become separate. It can be caused by:

- geographical barriers, such as a mountain range, river or sea (a population is divided into two)
 - ecological or seasonal barriers, such as flowering or breeding at different times
 - behavioural barriers, such as different courtship songs or displays that stop mating
1. A population is split into two (or two populations stop interbreeding).
 2. There is no gene flow between them.
 3. Mutations occur independently in each population, creating different alleles.
 4. If the environments differ, natural selection favours different alleles in each population.
 5. Allele frequencies in the two gene pools become increasingly different.
 6. Eventually members of the two populations cannot interbreed to produce fertile offspring. A new species has formed (**speciation**).



Allopatric speciation: **geographical isolation** is followed by different selection pressures and, eventually, **reproductive isolation**.

Exam tip: Include all of: isolation, no gene flow, different mutations, different selection pressures, change in allele frequency, and finally the inability to interbreed to produce fertile offspring.

Evaluating zoos and seed banks in conservation Spec 4.21

Zoos

- **Captive breeding programmes**: animals are bred under controlled conditions. Studbooks record pedigrees and animals are exchanged between zoos so mating partners are unrelated, to maintain genetic diversity and avoid inbreeding.
- **Reintroduction programmes**: animals bred in captivity are released into protected habitat, increasing wild populations.
- **Scientific research**: study of the biology, behaviour and breeding of species, and techniques such as artificial insemination and embryo storage, help conservation in the wild.
- **Education**: visitors learn about threatened species, which increases support and funding.

Seed banks

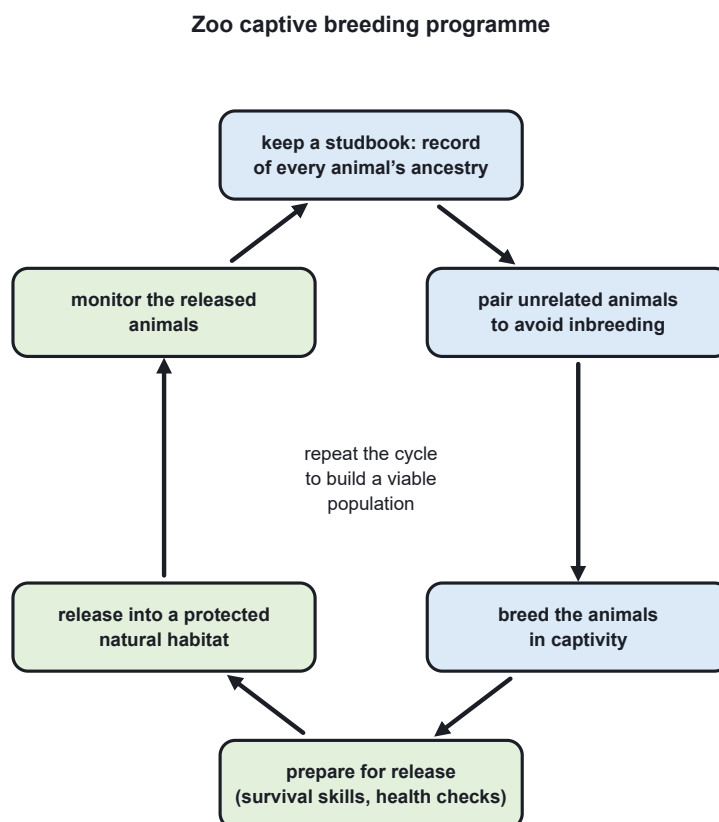
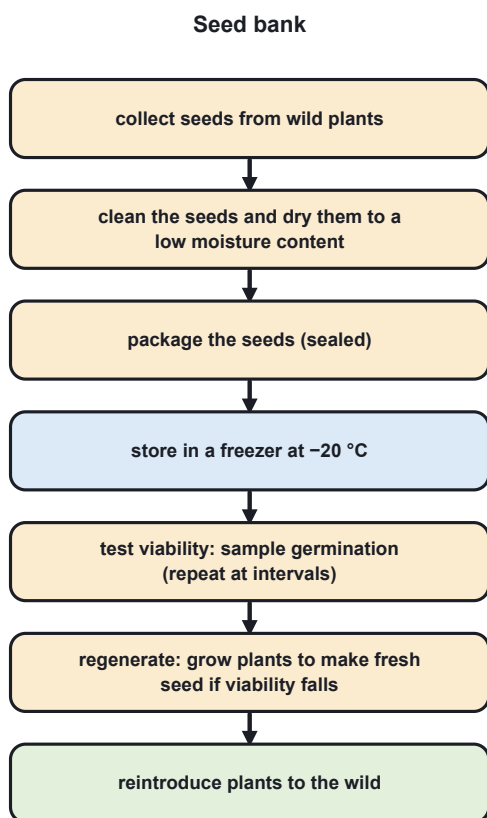
Seed banks store seeds from many species, dried to a low water content and kept at low temperature (for example -20°C), which slows metabolism and keeps seeds viable for many years. Seeds can later be germinated to grow new plants or to reintroduce a species.

- Seeds are collected from many plants across a species' range, to capture as much of its genetic diversity as possible.
- **Scientific research**: seed banks study the best conditions for drying, storing and germinating each species, and regularly test samples for viability by germinating them.

- **Reintroduction**: plants grown from stored seeds can be used to restore populations in protected or restored habitats.
- **Education**: seed banks and botanic gardens explain the value of plant diversity, for example as a source of food crops and medicines.

Evaluating zoos and seed banks		
	Strengths	Limitations
Zoos	Protect animals from threats such as hunting and habitat loss; managed breeding maintains genetic diversity; research and education; reintroduction can restore wild populations	Expensive and space-limited, so only a few individuals of a few species; small populations may lose genetic diversity; animals may lose natural behaviours and not survive reintroduction; habitat must still be protected for release; risk of disease
Seed banks	Cheap, take little space and store many individuals of many species; protect against loss in the wild; store a wide range of alleles for future use; seeds can be tested for viability and regrown	Some seeds cannot be dried or frozen; seeds may lose viability, so need testing and regrowing; plants in storage do not adapt to changing conditions; seeds from small samples may carry limited genetic diversity; a reintroduction needs suitable habitat

Exam tip: In evaluate questions give a balanced judgement: weigh strengths against limitations and end with a reasoned conclusion, for example that both are useful but only if natural habitats are also protected.



Two ex situ conservation methods: a **seed bank** (left) and a **captive breeding** programme in a zoo (right).

Quick check

1. Define niche.
2. Name the three types of adaptation.
3. In the Hardy-Weinberg equation, what does $2pq$ represent?
4. Name two causes of changes in allele frequency.
5. Why are seeds dried before storage in a seed bank?

Quick check answers

Plant cells and cell walls

1. The tonoplast.
2. They are channels through cell walls, lined by the cell surface membrane, that link the cytoplasm of adjacent cells and allow substances and signals to pass between them.
3. β -glucose, with alternate units rotated through 180° , joined by β -1,4 glycosidic bonds.
4. It is branched, so it has many free ends where enzymes can hydrolyse glycosidic bonds at the same time.
5. Lignin.

Plant tissues, fibres and mineral nutrition

1. Phloem (sieve tubes).
2. They are dead, have no cell contents and no end walls, so form a hollow tube.
3. Magnification = size of image $\div$ size of real object.
4. Magnesium is the central atom in chlorophyll, so it is needed to make chlorophyll for photosynthesis.
5. N mm^{-2} (equivalent to MPa) or Pa.

Bacterial growth, plant medicines and drug testing

1. A carbon source (for example glucose) and a nitrogen source (for amino acids), plus mineral ions.
2. Rising hot air carries microorganisms away from the work area, reducing contamination.
3. That the substance inhibits bacterial growth.
4. An inactive substance that looks the same as the drug, given to the control group.
5. To test the safety, side effects and dose of a drug on a small number of healthy volunteers.

Classification and biodiversity

1. Archaea, Bacteria and Eukarya.
2. Molecular evidence, such as differences in rRNA and other nucleotide or protein sequences.
3. A species being found in only one geographical region and nowhere else.
4. The number of different species in a habitat.
5. The total number of organisms of each species.

Niche, allele frequency and conservation

1. The role of a species within its habitat, including how it obtains resources and its interactions with other species.
2. Behavioural, anatomical and physiological.
3. The frequency of the heterozygous genotype.
4. Mutation and natural selection.
5. To reduce water content, which slows metabolism and prevents germination and damage from ice crystals.