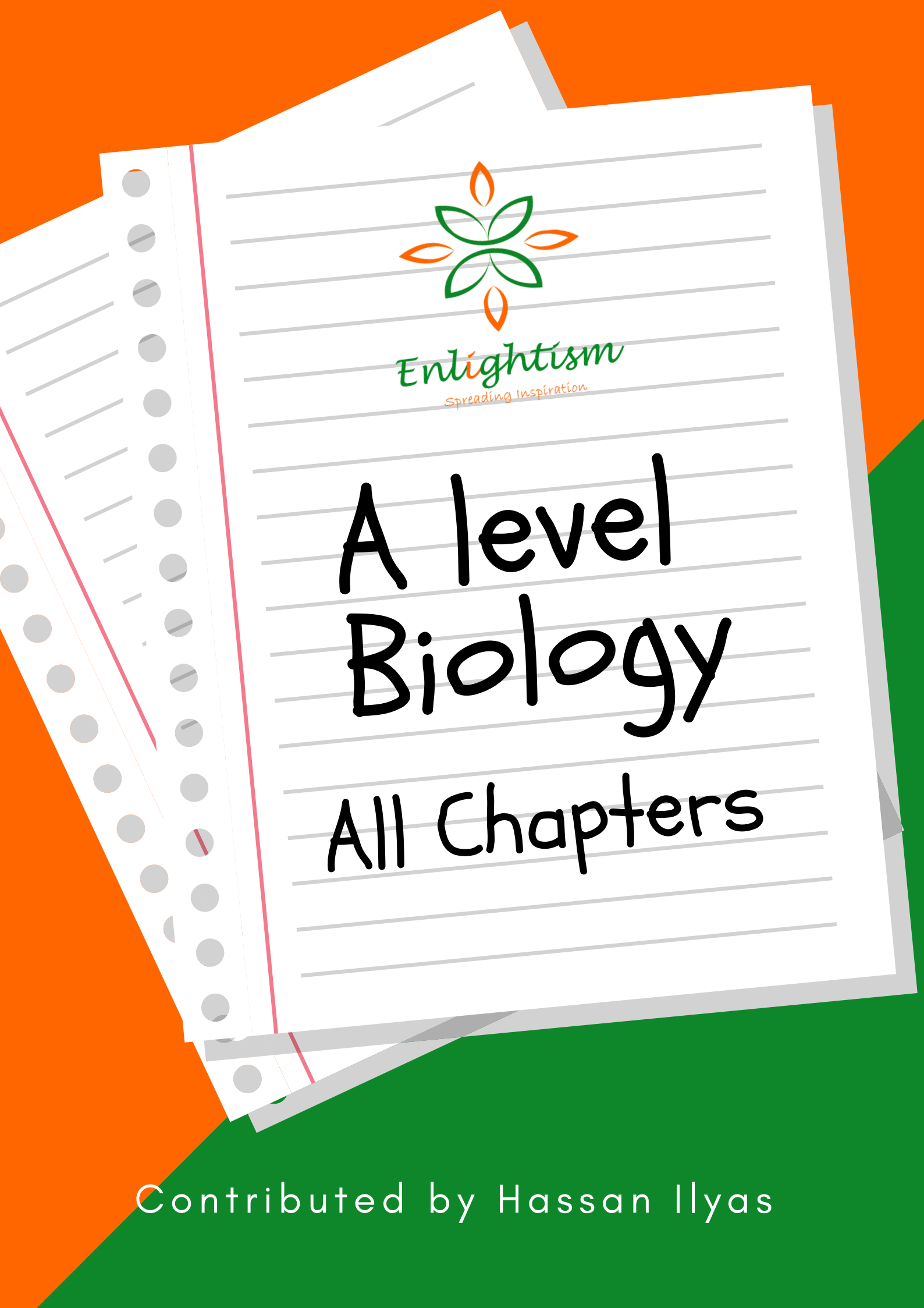
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12. Energy & Respiration

The Need for Energy

- Living organisms are composed of cells, and within each cell, many **activities** and **processes** are constantly being carried out to maintain life
- Work in a living organism requires **energy** and usable carbon compounds

Type of work	Examples
Transporting substances across membranes	◦ Active transport using the sodium–potassium pump in cell membranes ◦ Exocytosis of digested bacteria from white blood cells
Anabolic reactions	◦ Synthesis of DNA from nucleotides ◦ Synthesis of protein from amino acids
Movement	◦ Cellular movement of chromosomes via the spindle ◦ Mechanical contraction of muscles
Maintaining body temperature	◦ Only occurs in mammals and birds

The source of energy & materials

- For nearly all organisms the sun is the primary source of energy
- The reactions of **photosynthesis** store energy in organic molecules
    - Light energy from the sun is transformed into chemical potential energy in the synthesis of carbohydrates
    - The carbohydrates formed are then used in the synthesis of ATP (from their breakdown) or are combined and modified to form all the usable organic molecules that are essential for all metabolic processes within the plant
    - Photosynthesis is carried out by the first organism in a food chain, such as plants and some other small organisms
- **Respiration** in all living cells releases energy from the breakdown of organic molecules
- Respiration involves the transfer of **chemical potential energy** from nutrient molecules (such as carbohydrates, fats and proteins) into a usable energy form (through the synthesis of **ATP**) that can be used for work within an organism

Glucose equations

glucose + oxygen → carbon dioxide + water + energy

$$C_6H_{12}0_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_20 + 2870kJ$$

- Autotrophs are organisms that are able to **synthesise their own usable carbon compounds** from carbon dioxide in the atmosphere through photosynthesis
- Heterotrophs don't have this ability. They require a supply of pre-made usable carbon compounds which they get from their food

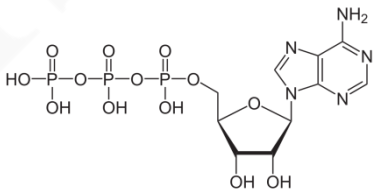
❖ According to the laws of thermodynamics, energy cannot be created or destroyed; it is **transformed** from one form into another. Be careful not to say that energy is "created" when talking about photosynthesis and respiration.

ATP: Universal Energy Currency

- Energy released during the reactions of respiration is transferred to the molecule **adenosine triphosphate** (ATP)
- ATP is a small and soluble molecule that provides a **short-term store** of chemical energy that cells can use to do work
- It is vital in linking energy-requiring and energy-yielding reactions
- ATP is described as a universal energy currency because it is used in all organisms and like money, it can be used for different purposes (reactions) and is reused countless times
- Be careful not to use the terms energy and ATP interchangeably. Energy is the capacity or power to do work. ATP is a molecule which stores (chemical potential) energy and carries it to places in the cell that need energy to do work.
- The use of ATP as an 'energy-currency' is beneficial for many reasons:
    - The hydrolysis of ATP can be carried out quickly and easily wherever energy is required within the cell by the action of just one enzyme, ATPase
    - A useful (not too small, not too large) quantity of energy is released from the hydrolysis of one ATP molecule – this is beneficial as it reduces waste but also gives the cell control over what processes occur
    - ATP is relatively stable at cellular pH levels

Structure of ATP

- ATP is a phosphorylated **nucleotide**
- It is made up of: Ribose sugar, Adenine base and Three phosphate groups



Hydrolysis of ATP

- When ATP is hydrolysed (broken down), ADP and phosphate (H_3PO_4) are produced
- As ADP forms **free energy** is released that can be used for processes within a cell eg. DNA synthesis
    - Removal of one phosphate group from ATP releases 30.8 kJ mol^{-1} of energy, forming ADP
    - Removal of a second phosphate group from ADP also releases 30.8 kJ mol^{-1} of energy, forming AMP
    - Removal of the third and final phosphate group from AMP releases 14.2 kJ mol^{-1} of energy, forming adenosine

Feature	Benefit
Releases a small but sufficient amount of energy (75.8 kJ mol^{-1} from the complete hydrolysis of ATP)	This is enough energy to drive important metabolic reactions while keeping energy wastage low
Exists as a stable molecule	It doesn't break down unless a catalyst (ATPase) is present so energy won't be wasted
Can be recycled	The breakdown of ATP is a reversible reaction, ATP can be reformed from ADP and Pi. This means the same molecule can be reused elsewhere in the cell for different reactions
Hydrolysis is quick and easy	Allows cells to respond to a sudden increase in energy demand
Soluble and moves easily within cells	Can transport energy to different areas of the cell
Forms phosphorylated intermediates	This can make metabolites more reactive and lower the activation energy required for a reaction

ATP Synthesis

- On average humans use more than 50 kg of ATP in a day but only have a maximum of ~ 200g of ATP in their body at any given time
- Organisms **cannot build up large stores of ATP** and it rarely passes through the cell surface membrane
- This means the cells must make ATP as and when they need it
- ATP is formed when ADP is combined with an inorganic phosphate (Pi) group
    - This is an **energy-requiring reaction**
    - **Water is released** as a waste product (therefore ATP synthesis is a condensation reaction)

Types of ATP synthesis

- ATP is made during the reactions of respiration and photosynthesis
    - All of an animal's ATP comes from respiration
- ATP can be made in two different ways:
    - **Substrate-linked phosphorylation**
    - **Chemiosmosis**

Substrate-linked phosphorylation

- ATP is formed by **transferring a phosphate directly from a substrate molecule to ADP**

$$\mathbf{ADP + P_i \text{ —> } ATP}$$

- The energy required for the reaction is **provided directly by another chemical reaction**
- This type of ATP synthesis occurs in the cell cytoplasm and in the matrix of the mitochondria
- It only accounts for a small amount of the ATP synthesised during aerobic respiration
    - ~ 4 / 6 ATP per glucose molecule
- This type of ATP synthesis takes place in glycolysis

Chemiosmosis

- This specific type of ATP synthesis involves a proton (hydrogen ion) gradient across a membrane
- It takes place across the inner membrane of the mitochondria and the **thylakoid membrane of chloroplasts**
- An electron transport chain helps to establish the **proton concentration gradient**
    - High energy electrons move from carrier to carrier releasing energy that is used to pump protons (up a concentration gradient) across the inner membrane into the intermembrane space
    - Protons are pumped from a low concentration in the mitochondrial matrix to a high concentration in the intermembrane space
- The protons then move down the concentration gradient into the matrix which **releases energy**
- The protons move through the **ATP synthase complex** which uses the released energy to **drive the phosphorylation of ATP**
- Oxygen acts as the **final electron and proton acceptor** to form water
- Most of the ATP made during respiration is synthesised via chemiosmosis
    - ~ 32 / 34 ATP per glucose molecule

	Substrate–linked phosphorylation	Chemiosmosis
Process	The phosphate of a substrate molecule is **directly transferred** to ADP to form ATP It uses energy directly provided by another chemical reaction	The energy released by the **movement of hydrogen ions** down a concentration gradient is used to synthesise ATP via the enzyme **ATP synthase** **Oxygen** acts as the **final hydrogen/electron acceptor.**
Location	Cytoplasm of cells/ Matrix of mitochondria	Inner mitochondrial membrane/ Thylakoid membrane of chloroplasts
Quantity of ATP produced during respiration	Small (4/6 per glucose molecule)	Large (34/32 per glucose molecule)

The Role of NAD & FAD

- Coenzymes NAD and FAD play a critical role in aerobic respiration
- When hydrogen atoms become available at different points during respiration NAD and FAD accept these hydrogen atoms
    - A hydrogen atom consists of a hydrogen ion and an electron
- When the coenzymes gain a hydrogen they are '**reduced'** (**O**xidation **I**s **L**oss, **R**eduction **I**s **G**ain)
- They **transfer the hydrogen atoms** (**hydrogen ions** and **electrons**) from the different stages of respiration to the **electron transport chain** on the inner mitochondrial membrane, the site where hydrogens are removed from the coenzymes
- When the hydrogen atoms are removed the coenzymes are '**oxidised'**
- Hydrogen ions and electrons are important in the electron transport chain at the end of respiration as they play a role in the **synthesis of ATP**
    - Electrons from reduced NAD (NADH) and reduced FAD ($FADH_2$) are given to the electron transport chain
    - Hydrogen ions from reduced NAD (NADH) and reduced FAD ($FADH_2$) are released when the electrons are lost
    - The electron transport chain drives the movement of these hydrogen ions (protons) across the inner mitochondrial membrane into the mitochondrial matrix, creating a proton gradient (more hydrogen ions in the matrix)
    - Movement of hydrogen ions down proton gradient, back into the intermembrane space, gives the energy required for ATP synthesis

Sources of reduced NAD & FAD

- A certain amount of reduced NAD and FAD is produced during the aerobic respiration of a single glucose molecule
- Reduced NAD:
    - 2 x 1 = 2 from Glycolysis
    - 2 x 1 = 2 from the Link Reaction
    - 2 x 3 = 6 from the Krebs cycle
- Reduced FAD:
    - 2 x 1 = 2 from the Krebs cycle

❖ Note at all stages there is a doubling (2x) of reduced NAD and FAD. This is because one glucose molecule is split in two in glycolysis and so these **reactions occur twice per single molecule of glucose.**

The Electron Transport Chain

- Synthesis of ATP is associated with the electron transport chain on the membranes of mitochondria and chloroplasts
- The electron transport chain is made up of a **series of membrane proteins**/ electron carriers
- They are positioned close together which allows the electrons to pass from carrier to carrier
- The inner membrane of the mitochondria is impermeable to hydrogen ions so these electron carriers are required to **pump the protons across the membrane** to establish the concentration gradient

❖ Oxygen acts as the **final electron acceptor.** Without oxygen, the electron transport chain cannot continue as the electrons have nowhere to go. Without oxygen accepting the electrons (and hydrogens), the reduced coenzymes NADH and $FADH_2$ cannot be oxidised to regenerate NAD and FAD, so they can't be used in further hydrogen transport.

Energy Values of Respiratory Substrates

- **Glucose** is the main respiratory substrate for aerobic respiration in most cells
- When the supply of glucose in a cell has been used up a cell may continue respiration using other substrates
- These may be: Other carbohydrates, Lipids and Proteins
- Amino acids from proteins are only respired aerobically when all other substrates have been used up
    - This is because they often have **essential functions elsewhere** in the cell
    - Amino acids are required to make proteins which have structural (eg. in the cytoskeleton) and functional (eg. enzymatic) roles
- When these different substrates are broken down in respiration, they **release different amounts of energy**

Explaining the differences in energy values

- Lipids have the highest energy value (39.4 kJ g^{-1}) followed by proteins (17.0 kJ g^{-1}) and then carbohydrates (15.8 kJ g^{-1})
- The differences in the energy values of substrates can be explained by their **molecular composition**
    - Specifically how many **hydrogen atoms** become available when the substrate molecules are broken down
- During respiration hydrogen atoms play a vital role:
    - The substrate molecules are broken down and the hydrogen atoms become available
    - **Hydrogen carrier molecules** called **NAD and FAD** pick them up (become reduced) and transfer them to the inner mitochondrial membrane
    - Reduced NAD and FAD release the hydrogen atoms which split into protons and electrons
    - The protons are pumped across the inner mitochondrial membrane into the intermembrane space – forming a **proton / chemiosmotic gradient**
    - This proton gradient is used in **chemiosmosis** to **produce ATP**
    - After the protons have flowed back into the matrix of the mitochondria via ATP synthase they are **oxidised to form water**
- This means that a molecule with a **higher hydrogen content** will result in a **greater proton gradient** across the mitochondrial membrane which allows for the formation of **more ATP** via chemiosmosis
- Fatty acids in lipids are made up of **long hydrocarbon chains** with lots of hydrogen atoms. These hydrogen atoms are released when the lipid is broken down

Respiratory substrate	Energy value / kJ g^{-1}
Carbohydrate	15.8
Lipid	39.4
Protein	17.0

Respiratory substrate	RQ
Carbohydrate	1.0
Lipid	0.7
Protein	0.9

Respiratory Quotient (RQ)

- The respiratory quotient (RQ) is: the ratio of carbon dioxide molecules produced to oxygen molecules taken in during respiration

$$\mathbf{RQ = CO_2 / O_2}$$

RQ values of different respiratory substrates

- Carbohydrates, lipids and proteins have different typical RQ values
- This is because of the number of carbon-hydrogen bonds differs in each type of biological molecule
    - More carbon-hydrogen bonds means that more hydrogen atoms can be used to create a proton gradient
    - More hydrogens means that more ATP molecules can be produced
    - More oxygen is therefore required to breakdown the molecule (in the last step of oxidative phosphorylation to form water)
- When glucose is aerobically respired equal amounts of carbon dioxide are produced to oxygen taken in, meaning it has an RQ value of 1

$$C_6H_{12}0_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

<u>Calculating RQs</u>

- The respiratory quotient is calculated from respiration equations
- It involves comparing the ratios of carbon dioxide given out to oxygen taken in
- The formula for this is:

$$RQ = \frac{moles\ or\ molecules\ of\ carbon\ dioxide\ given\ out}{moles\ or\ molecules\ of\ oxygen\ taken\ in}$$

- If you know the **molecular formula** of the substrate being aerobically respired then you can create a balanced equation to calculate the RQ value
- In a **balanced** equation the **number before the chemical formula** can be taken as the **number of molecules/moles** of that compound
    - This is because the same number of molecules of any gas take up the same volume eg. 12 molecules of carbon dioxide take up the same volume as 12 molecules of oxygen
- **Glucose has a simple 1:1 ratio** and RQ value of 1 but other substrates have more complex ratios leading to different RQ values

Calculating the RQ for anaerobic respiration

- Anaerobic respiration is respiration that takes place **without oxygen** but does produce a small amount of ATP
- Depending on the organism anaerobic respiration in cells can be done via lactate or ethanol fermentation
    - Mammalian muscle cells use **lactate fermentation**
    - Plant tissue cells and yeast use **ethanol fermentation**
- The RQ cannot be calculated for anaerobic respiration in muscle cells because **no oxygen is used and no carbon dioxide is produced** during lactate fermentation
- For yeast cells the RQ tends towards infinity as **no oxygen is used while carbon dioxide is still being produced**

Investigating RQs

- Respirometers are used to measure and investigate the **rate of oxygen consumption** during respiration in organisms
- They can also be used to calculate respiratory quotients
- The experiments usually involve organisms such as seeds or invertebrates

Equation for calculating change in gas volume

- The volume of oxygen consumed ($cm^3\ min^{-1}$) can be worked out using the diameter of the capillary tube *r* (cm) and the distance moved by the manometer fluid *h* (cm) in a minute using the formula:

$$\pi r^2 h$$

Using a respirometer to determine the RQ

Method

- Measure oxygen consumption: set up the respirometer and run the experiment with soda-lime present in both tubes. Use the manometer reading to calculate the change in gas volume within a given time, *x* $cm^3\ min^{-1}$
- Reset the apparatus: allow air to re-enter the tubes via the screw cap and reset the manometer fluid using the syringe
- Run the experiment again: **remove the soda-lime** from both tubes and use the manometer reading to calculate the change in gas volume in a given time, *y* $cm^3\ min^{-1}$

Calculations

- *x* tells us the volume of oxygen consumed by respiration within a given time
- *y* tells us the volume of oxygen consumed by respiration within a given time minus the volume of carbon dioxide produced within a given time
    - ***y* may be a positive or negative** value depending on the direction that the manometer fluid moves (up = positive value, down = negative value)
- The two measurements *x* and *y* can be used to calculate the RQ

$$\mathbf{RQ = CO_2 / O_2}$$

$$\mathbf{RQ = (\mathit{x} + \mathit{y})\ /\ \mathit{x}}$$

❖ When equal volumes of oxygen are consumed and carbon dioxide produced (as seen with glucose) the manometer fluid will not move and *y* will be 0, making the RQ 1.

Analysis

- Respirometers can be used in experiments to investigate how different factors affect the RQ of organisms over time
- When an **RQ value changes** it means **the substrate being respired has changed**
- Some cells may also be **using a mixture of substrates** in respiration an RQ value of 0.85 suggests **both** carbohydrates and lipids are being used
    - This is because the RQ of glucose is 1 and the RQ of lipids is 0.7
- Under normal cell conditions the order substrates are used in respiration: carbohydrates, lipids then proteins
- The RQ can also give an indication of under or overfeeding:
    - An RQ value of more than 1 suggests excessive carbohydrate/calorie intake
    - An RQ value of less than 0.7 suggests underfeeding
- There are several ways you can manage variables and increase the reliability of results in respirometer experiments:
    - Use a controlled water bath to keep the **temperature** constant
    - Have a control tube with an equal volume of inert material to the volume of the organisms to compensate for changes in atmospheric **pressure**
    - Repeat the experiment multiple times and use an **average**

<u>Structure & Function of the Mitochondria</u>

- Mitochondria are rod-shaped organelles 0.5 – 1.0 μm in diameter
- They are the site of aerobic respiration in eukaryotic cells
- The function of mitochondria is to **synthesize ATP**
- Synthesis of ATP in mitochondria occurs during last stage of respiration called oxidative phosphorylation
    - This relies on membrane proteins that make up the 'electron transport chain' and the ATP synthase enzyme – the details of this are covered later in the notes

Structure

- Mitochondria have two phospholipid membranes
- The outer membrane is:
    - Smooth
    - Permeable to several small molecules
- The inner membrane is:
    - Folded (cristae)
    - **Less permeable**
    - The site of the **electron transport chain** (used in oxidative phosphorylation)
    - Location of **ATP synthase** (used in oxidative phosphorylation)
- The intermembrane space:
    - Has a low pH due to the **high concentration of protons**
    - The concentration gradient across the inner membrane is formed during oxidative phosphorylation and is **essential for ATP synthesis**
- The matrix:
    - Is an aqueous solution within the inner membranes of the mitochondrion
    - Contains ribosomes, enzymes and circular mitochondrial DNA necessary for mitochondria to function

Relationship between structure & function

- The structure of mitochondria makes them well adapted to their function
- They have a **large surface area** due to the presence of **cristae** (inner folds) which enables the membrane to hold many electron transport chain proteins and ATP synthase enzymes
- More active cell types can have larger mitochondria with longer and more tightly packed cristae to enable the **synthesis of more ATP** because they have a **larger surface area**
- The **number** of mitochondria in each cell can vary depending on cell activity
    - Muscle cells are more active and have more mitochondria per cell than fat cells

<u>The Four Stages in Aerobic Respiration</u>

- Glucose is the main respiratory substrate used by cells
- Aerobic respiration is the process of breaking down a respiratory substrate in order to **produce ATP using oxygen**
- The process of aerobic respiration using glucose can be split into four stages
- Each stage occurs at a particular location in a eukaryotic cell:
    - **Glycolysis** takes place in the cell cytoplasm
    - The **Link reaction** takes place in the matrix of the mitochondria
    - The **Krebs cycle** takes place in the matrix of the mitochondria
    - **Oxidative phosphorylation** occurs at the inner membrane of the mitochondria

Stage	Description	Location
1. Glycolysis	Phosphorylation and splitting of glucose	Cell cytoplasm
2. Link reaction	Decarboxylation and dehydrogenation of pyruvate	Matrix of mitochondria
3. Krebs cycle	Cyclical pathway with enzyme-controlled reactions	Matrix of mitochondria
4. Oxidative phosphorylation	Production of ATP through oxidation of hydrogen atoms	Inner membrane of mitochondria

<u>Aerobic Respiration: Glycolysis</u>

- Glycolysis is the first stage of respiration
- It takes place in the cytoplasm of the cell and involves:
    - **Trapping glucose** in the cell by phosphorylating the molecule
    - **Splitting the glucose molecule in two**
- It results in the production of
    - 2 Pyruvate (3C) molecules
    - Net gain 2 ATP
    - 2 reduced NAD

Steps of glycolysis

- **Phosphorylation**: glucose (6C) is phosphorylated by 2 ATP to form fructose bisphosphate (6C)

Glucose + 2ATP → Fructose bisphosphate

- **Lysis**: fructose bisphosphate (6C) splits into two molecules of triose phosphate (3C)

Fructose bisphosphate → 2 Triose phosphate

- **Oxidation**: hydrogen is removed from each molecule of triose phosphate and transferred to coenzyme NAD to form 2 reduced NAD

$4H + 2NAD \rightarrow 2NADH + 2H^+$

- **Dephosphorylation:** phosphates are transferred from the intermediate substrate molecules to form 4 ATP through **substrate-linked phosphorylation**

$4P_i + 4ADP \rightarrow 4ATP$

- **Pyruvate is produced**: the end product of glycolysis which can be used in the next stage of respiration

2 Triose phosphate → 2 Pyruvate

❖ It may seem strange that ATP is used and also produced during glycolysis. At the start ATP is used to **make glucose more reactive** (it is usually very stable) and to lower the activation energy of the reaction. Since 2 ATP are used and 4 are produced during the process, there is a **net gain of 2 ATP per glucose molecule.**

<u>Entering the Link Reaction</u>

- The end product of glycolysis is **pyruvate**
- Pyruvate contains a substantial amount of chemical energy that can be further utilized in respiration to produce more ATP
- When **oxygen is available** pyruvate will **enter the mitochondrial matrix** and **aerobic** respiration will continue
- It moves across the double membrane of the mitochondria via **active transport**
    - It requires a transport protein and a small amount of ATP
- Once in the mitochondrial matrix **pyruvate** takes part in the **link reaction**

The Link Reaction

- The link reaction takes place in the matrix of the mitochondria
- It is referred to as the link reaction because it **links glycolysis** to the **Krebs cycle**
- The steps are:
    1. Decarboxylation and dehydrogenation of pyruvate by enzymes to produce an **acetyl group, $CH_3C(O)$-**
    2. **Combination with coenzyme A** to form **acetyl coA**
- It produces:
    - Acetyl coA
    - Carbon dioxide (CO_2)
    - Reduced NAD (NADH)

pyruvate + NAD + CoA → acetyl CoA + carbon dioxide + reduced NAD

Role of coenzyme A

- A coenzyme is a molecule that **helps an enzyme carry out its function** but is **not used in the reaction itself**
- Coenzyme A consists of a nucleoside (ribose and adenine) and a vitamin
- In the link reaction, CoA binds to the remainder of the pyruvate molecule (acetyl group 2C) to form acetyl CoA
- It then **supplies the acetyl group to the Krebs cycle** where it is used to continue aerobic respiration
- This is the stage that brings part of the carbohydrate (or lipid/amino acid) into the further stages of respiration and **links** the initial stage of respiration in the cytoplasm to the later stages in the mitochondria

❖ There are two pyruvate molecules produced per glucose molecule so you need to **multiply everything by 2** when thinking about what happens to a single glucose molecule in aerobic respiration.

<u>Outline of the Krebs Cycle</u>

- The Krebs cycle (sometimes called the citric acid cycle) consists of a **series of enzyme-controlled reactions**
- **Acetyl CoA (2C) enters** the circular pathway via the **link reaction**
- 4 carbon (4C) oxaloacetate accepts the 2C acetyl fragment from acetyl CoA to form citrate (6C)
- **Citrate is then converted back to oxaloacetate** through a series of small reactions

❖ The Krebs cycle is often referred to as cyclical or circular. This is because the acceptor molecule **oxaloacetate** is **regenerated** throughout the reaction so that it can start all over again by adding another acetyl CoA.

The Krebs Cycle

- **Oxaloacetate is regenerated** in the Krebs cycle through a series of reactions
- Decarboxylation of citrate
    - Releasing **2 CO_2** as waste gas
- Dehydrogenation of citrate
    - Releasing H atoms that reduce coenzymes NAD and FAD
    - 3 NAD and 1 FAD → **3NADH + H^+ and 1 $FADH_2$**
- Substrate-linked phosphorylation
    - A phosphate is transferred from one of the intermediates to ADP, forming **1 ATP**

<u>Oxidative Phosphorylation</u>

- **Oxidative phosphorylation** is the last stage of aerobic respiration
- It takes place at the inner membrane of the mitochondria
- Several steps occur: Hydrogen atoms are donated by reduced NAD and FAD
- Hydrogen atoms **split into protons and electrons**
- The high energy electrons release energy as they move through the **electron transport chain**
- The released energy is used to **transport protons** across the inner mitochondrial membrane from the intermembrane space **into the matrix**
- A **concentration gradient** of protons is established between the intermembrane space and the matrix
- The protons return to the matrix via facilitated diffusion through the channel protein ATP synthase
- The movement of protons down their concentration gradient provides energy for **ATP synthesis**
- **Oxygen** combines with protons and electrons at the end of the electron transport chain to form water

The electron transport chain

- The electron transport chain is made up of a **series of membrane proteins**/ electron carriers
- They are positioned close together which allows the electrons to pass from carrier to carrier
- The inner membrane of the mitochondria is impermeable to hydrogen ions so these electron carriers are required to **pump the protons across the membrane** to establish the concentration gradient

❖ Oxygen acts as the **final electron acceptor.** Without oxygen the electron transport chain cannot continue as the electrons have nowhere to go. Without oxygen accepting the electrons (and hydrogens) the reduced coenzymes NADH and $FADH_2$ cannot be oxidised to regenerate NAD and FAD, so they can't be used in further hydrogen transport.

<u>Anaerobic Respiration</u>

- Sometimes cells experience conditions with **little or no oxygen**
- There are several consequences when there is not enough oxygen available for respiration:
    - There is **no final acceptor of electrons** from the electron transport chain
    - The electron transport chain stops functioning
    - No more ATP is produced via oxidative phosphorylation
    - Reduced NAD and FAD aren't oxidised by an electron carrier
    - No oxidised NAD and FAD are available for dehydrogenation in the Krebs cycle
    - The Krebs cycle stops
- However, there is still a way for cells to produce some ATP in low oxygen conditions through anaerobic respiration

Anaerobic pathways

- Some cells are able to **oxidise the reduced NAD** produced during glycolysis so it can be used for further hydrogen transport
- This means that **glycolysis can continue** and **small amounts of ATP** are still produced
- Different cells use different pathways to achieve this
    - Yeast and microorganisms use **ethanol fermentation**
    - Other microorganisms and mammalian muscle cells use **lactate fermentation**

Ethanol fermentation

- In this pathway reduced NAD transfers its hydrogens to ethanal to form ethanol
- In the first step of the pathway **pyruvate is decarboxylated** to ethanal
    - Producing **CO_2**
- Then **ethanal is reduced** to ethanol by the enzyme alcohol dehydrogenase
- Ethanal is the hydrogen acceptor
- Ethanol cannot be further metabolised; it is a waste product

Lactate fermentation

- In this pathway reduced NAD transfers its hydrogens to pyruvate to form lactate
- **Pyruvate is reduced** to lactate by enzyme lactate dehydrogenase
- Pyruvate is the hydrogen acceptor
- The final product lactate can be further metabolised

Metabolization of lactate

- After lactate is produced two things can happen:
  1. It can be **oxidised back to pyruvate** which is then channeled into the Krebs cycle for ATP production
  2. It can be **converted into glycogen** for storage in the liver
- The oxidation of lactate back to pyruvate needs extra oxygen
  - This extra oxygen is referred to as an **oxygen debt**
  - It explains why animals **breathe deeper and faster after exercise**

❖ Note that ethanol fermentation is a two-step process (lactate fermentation is a one-step process). Carbon dioxide is also produced alongside the waste ethanol.

<u>Aerobic & Anaerobic Respiration</u>

- In cells there is a much greater energy yield from respiration in aerobic conditions than in anaerobic conditions
- In anaerobic respiration glucose is only partially oxidised meaning only some of its chemical potential energy is released and transferred to ATP
- The only ATP producing reaction that continues is glycolysis (~2 ATP)
- As there is no oxygen to act as the final electron acceptor none of the reactions within the mitochondria can take place
- The stages that take place inside the mitochondria produce much more ATP than glycolysis alone (~36 ATP)

	Aerobic respiration	Anaerobic respiration
Stages	Glycolysis Link reaction The Krebs cycle Oxidative phosphorylation	Glycolysis Fermentation
Oxidation of glucose	Complete	Incomplete
Total ATP produced	High (~36)	Low (2)
Location	Cytoplasm and mitochondria	Cytoplasm
Products	CO_2, H_2O	Yeast: CO_2, ethanol Mammals: Lactate

<u>Anaerobic Adaptations of Rice</u>

- Flooding is a major problem when growing crops
- As water rises and it covers the different parts of a plant it can create problems:
  - Plant roots don't get the **oxygen** they need for aerobic respiration
  - Plant leaves don't get the **carbon dioxide** they need for photosynthesis
- These gases are less readily available in water as they diffuse more slowly in liquid compared to air
- Rice plants possess several adaptations that enable them to survive and grow in waterlogged conditions

Adaptations for aerobic respiration

- Some types of rice show an **increased rate of upward growth** away from the waterline
    - Leaves are above water so there is **access to oxygen and carbon dioxide through the stomata**
- Rice plants possess **aerenchyma** tissue in the stems and roots
    - This specialised plant tissue contains useful air spaces that allow **gases that enter the stomata to diffuse** to other parts of the plant that are above and under the water
    - Oxygen and carbon dioxide can therefore be held in this tissue even when underwater and can be transferred from parts of the plant that has access to air

Adaptations for anaerobic respiration

- When there isn't enough energy being supplied to the cells by aerobic respiration plants resort to anaerobic respiration as a source of ATP
- Plants use **ethanol fermentation** during anaerobic respiration
    - Toxic ethanol is produced which can build up in the plant tissue causing damage
- Rice plants can **tolerate higher levels of toxic ethanol** compared to other plants
- They also **produce more ethanol dehydrogenase**
    - This is the enzyme that breaks down ethanol
- The resilience that rice plants have towards ethanol allows them to carry out anaerobic respiration for longer so enough ATP is produced for the plant to survive and actively grow
- You might be wondering why farmers would grow rice in paddies (intentionally flooded fields)?
    - Growing rice in these conditions actually increases the yield. The plants or weeds that would usually be competitors for nutrients and light are unable to survive in these conditions and so the rice has more resources for its growth.

<u>Effect of Temperature & Substrate Concentration</u>

- A **redox indicator** is a substance that changes colour when it is reduced or oxidised
- **DCPIP** and **methylene** blue are redox indicators
    - They are used to investigate the effects of **temperature and substrate concentration** on the **rate of aerobic respiration** in yeast
- These dyes can be added to a suspension of living **yeast cells** as they don't damage cells
- Yeast can respire both aerobically and anaerobically, in this experiment it is their rate of anaerobic respiration that is being investigated

Mechanism

- **Dehydrogenation** happens regularly throughout the different stages of aerobic respiration
- The hydrogens that are removed from substrate molecules are transferred to the final stage of aerobic respiration, oxidative phosphorylation, via the hydrogen carriers NAD and FAD
- When DCPIP and methylene blue are present they can also take up hydrogens and get reduced
- Both redox indicators undergo the same colour change when they are reduced (**Blue → colourless**)
- The faster the rate of respiration, the faster the rate of hydrogen release and the faster the dyes get reduced and change colour
    - This means that the **rate of colour change** can correspond to the **rate of respiration** in yeast
- The rate of respiration is inversely proportional to the time taken

Rate of respiration (sec^{-1}) = 1 / time (sec)

Investigating the effect of temperature & substrate concentration on the rate of respiration in yeast

- The effect of temperature can be investigated by adding the test tubes containing the yeast suspension to a **temperature-controlled water bath** and recording the time taken for a colour change to occur once the dye is added
    - Repeat across a range of temperatures. For example, 30°C, 35°C, 40°C, 45°C
- The effect of substrate concentration can be investigated by adding **different concentrations of a substrate** to the suspension of yeast cells and recording the time taken for a colour change to occur once the dye is added
    - For example, 0.1% glucose, 0.5% glucose, 1.0% glucose

Controlling other variables

- It is important when investigating one variable to ensure that the other variables in the experiment are being controlled
    - **Volume of dye added**: if there is more dye molecules present then the time taken for the colour change to occur will be longer
    - **Volume of yeast suspension**: when more yeast cells are present the rate of respiration will be inflated
    - **Type of substrate**: yeast cells will respire different substrates at different rates
    - **Concentration of substrate**: if there is limited substrate in one tube then the respiration of those yeast cells will be limited
    - **Temperature**: an increase or decrease in temperature can affect the rate of respiration due to energy demands and kinetic energy changes. The temperature of the dye being added also needs to be considered

❖ Although the DCPIP and methylene blue undergo a colour change from blue to colourless it is important to remember that the **yeast suspension in the test tube may have a slight colour** (usually yellow). That means when the dye changes to colourless there may still be an overall yellow colour in the test tube. If this is the case it can be useful to have a control tube containing the same yeast suspension but with no dye added, then you can tell when the dye has completely changed colour.

Effect of Temperature: Respirometer

- Respirometers are used to measure and investigate the **rate of oxygen consumption** during aerobic respiration in organisms
- By adding the apparatus to a thermostatically controlled **water bath** the **effect of temperature on the rate of respiration** can be investigated
- The experiments usually involve organisms such as seeds or invertebrates

Method

- **Measure oxygen consumption**: set up the respirometer and run the experiment with both tubes in a controlled temperature water bath. Use the manometer reading to calculate the change in gas volume within a given time, *x* cm^3 min^{-1}
- **Reset the apparatus**: Allow air to reenter the tubes via the screw cap and reset the manometer fluid using the syringe. **Change the temperature of the water bath** and allow the tubes to acclimate, then close the screw clip to begin the experiment
- **Run the experiment again**: use the manometer reading to calculate the change in gas volume in a given time, *y* cm^3 min^{-1}
- **Repeat** experiment several times at different temperatures

Calculations

- The volume of oxygen consumed (cm^3 min^{-1}) can be worked out using the diameter of the capillary tube *r* (cm) and the distance moved by the manometer fluid *h* (cm) in a minute using the formula:

$$\pi r^2 h$$

Analysis

- The **rate of oxygen consumption** (cm^3 min^{-1}) is often taken as the rate of respiration for organisms
- The different volumes of oxygen consumed obtained for the different temperatures can be presented in table or graph form to show the effects of temperature

❖ If you think back to learning about proteins and enzymes you will remember that at extreme high temperatures, proteins become **denatured** and are unable to carry out their function. At low temperatures, molecules and enzymes don't collide very frequently as they don't have a lot of energy. This same trend can often be seen in the rate of respiration as the reactions rely on enzymes.

❖ The respirometer set up above is for measuring the rate of **aerobic** respiration. It cannot be used to measure the rate of aerobic respiration as no oxygen is consumed during aerobic respiration, as shown by the different equations for aerobic and anaerobic respiration.

❖ Aerobic respiration:

Glucose + Oxygen → Energy + Carbon Dioxide

❖ Anaerobic respiration (in mammals)

Glucose → Energy + Lactic acid

13. Stages of Photosynthesis

The Two Stages of Photosynthesis

- Photosynthesis occurs in two stages: the **light-dependent stage**, which takes place in the **thylakoids**, and the **light-independent stage**, which takes place in the **stroma**
- During the **light-dependent** stage of photosynthesis:
    - **Reduced NADP** is produced when hydrogen ions combine with the **carrier molecule NADP** using electrons from the **photolysis** of water
    - **ATP** is produced (from ADP and P_i by **ATP synthase** in a process called **photophosphorylation** (ADP + $P_i \rightarrow$ ATP)
    - Photophosphorylation uses the **proton (H^+) gradient** generated by the photolysis of water
    - **Energy from ATP** and **hydrogen from reduced NADP** are passed from the light-dependent stage to the light-independent stage of photosynthesis
- The energy and hydrogen are used during the **light-independent** reactions (known collectively as the **Calvin cycle**) to produce complex organic molecules, including (but not limited to) **carbohydrates**, such as:
    - **Starch** (for storage)
    - **Sucrose** (for translocation around the plant)
    - **Cellulose** (for making cell walls)

Thylakoids & the Stroma

- Plant cells contain **chloroplasts** which is the site of photosynthesis
- Chloroplasts are filled with a fluid known as the **stroma**
- The **system of membranes** found in the stroma of the chloroplast consists of a series of flattened fluid-filled sacs known as **thylakoids**
- In places, these thylakoids stack up to form structures known as **grana** (singular – granum)
- The **light-dependent stage** of photosynthesis occurs in the **thylakoid membranes** and the **thylakoid spaces** (the spaces inside the thylakoids)
- The thylakoid membranes contain the **pigments**, **enzymes** and **electron carriers** required for the light-dependent reactions
- The membranes of the grana create a **large surface area** to **increase the number of light-dependent reactions** that can occur
- This membrane system provides a large number of pigment molecules in an arrangement that ensures **as much light as necessary is absorbed**
- The pigment molecules are arranged in light-harvesting clusters known as **photosystems**
- In a photosystem, the different pigment molecules are arranged in **funnel-like structures** the thylakoid membrane (each pigment molecule passes energy down to the next pigment molecule in the cluster until it reaches the primary pigment reaction centre)
- The **stroma** is the fluid that fills the chloroplasts and surrounds thylakoids
- CO_2, sugars, enzymes and other molecules are dissolved in the stroma
- The stroma is the site of the **light-independent stage** of photosynthesis

Chloroplast Pigments

- Chloroplasts contain several different **photosynthetic pigments** within the **thylakoids**, which **absorb different wavelengths of light**
    - In places, these thylakoids stack up to form structures known as **grana** (singular – granum)
    - The thylakoid membrane system provides a large number of pigment molecules in an arrangement that ensures **as much light as necessary is absorbed**
    - The pigment molecules are arranged in light-harvesting clusters known as **photosystems**
    - In a photosystem, the different pigment molecules are arranged in **funnel-like structures** the thylakoid membrane (each pigment molecule passes energy down to the next pigment molecule in the cluster until it reaches the primary pigment reaction centre)
- The **light-dependent stage** of photosynthesis occurs in the **thylakoid membranes** and the **thylakoid spaces** (the spaces inside the thylakoids)
- This is why the thylakoid membranes contain the **pigments**, **enzymes** and **electron carriers** required for the light-dependent reactions
- There are **two groups** of pigments: primary pigments known as **chlorophylls** and accessory pigments known as **carotenoids**
- **Chlorophylls** absorb wavelengths in the **blue-violet and red regions** of the light spectrum
    - They reflect green light, causing plants to appear green
- **Carotenoids** absorb wavelengths of light mainly in the **blue-violet region** of the spectrum

Pigment group	Name of pigment	Colour of pigment
Chlorophylls	Chlorophyll a Chlorophyll b	Yellow–green Blue–green
Carotenoids	β carotene Xanthophyll	Orange Yellow

Absorption Spectra & Action Spectra

- An **absorption spectrum** is a graph that shows the **absorbance** of different wavelengths of light by a particular pigment
- **Chlorophylls** absorb wavelengths in the **blue-violet and red regions** of the light spectrum
- **Carotenoids** absorb wavelengths of light mainly in the **blue-violet region** of the spectrum
- An **action spectrum** is a graph that shows the **rate of photosynthesis** at different wavelengths of light
- The rate of photosynthesis is **highest** at the **blue-violet** and **red** regions of the light spectrum, as these are the wavelengths of light that plants can **absorb** (i.e. the wavelengths of light that chlorophylls and carotenoids can absorb)
- There is a strong **correlation** between the cumulative absorption spectra of all pigments and the action spectrum:
    - Both graphs have **two main peaks** – at the **blue-violet** region and the **red** region of the light spectrum
    - Both graphs have a **trough** in the **green-yellow** region of the light spectrum

Chromatography of Chloroplast Pigments

- **Chromatography** is an experimental technique that is used to **separate mixtures**:
    - The mixture is **dissolved** in a fluid/**solvent** (called the mobile phase) and the dissolved mixture then passes through a static material (called the stationary phase)
    - **Different components** within the mixture travel through the material at **different speeds**
    - This causes the different components to **separate**
    - A retardation factor (**R_f**) can be calculated for each component of the mixture

        R_f value = distance travelled by component ÷ distance travelled by solvent
- Two of the most common techniques for separating these photosynthetic pigments are:
    - **Paper chromatography** – the mixture of pigments is passed through paper (cellulose)
    - **Thin-layer chromatography** – the mixture of pigments is passed through a thin layer of adsorbent (eg. silica gel), through which the mixture travels faster and separates more distinctly
- Chromatography can be used to separate and identify chloroplast pigments that have been extracted from a leaf as each pigment will have a unique R_f value
- The R_f value demonstrates how far a dissolved pigment travels through the stationary phase
    - A **smaller R_f value** indicates the pigment is **less soluble** and **larger** in size
- Although specific R_f values depend on the solvent that is being used, in general:
    - **Carotenoids** have the **highest R_f values** (usually close to 1)
    - **Chlorophyll *b*** has a **much lower R_f value**
    - **Chlorophyll *a*** has an R_f value somewhere **between** those of carotenoids and chlorophyll *b*
    - The R_f value demonstrates how far a
    - **Small R_f values** indicate the pigment is l**ess soluble** and **larger** in size

Types of Photophosphorylation

- The **thylakoid membrane** is the site of the **light-dependent stage** of photosynthesis
- During the **light-dependent** stage of photosynthesis:
    - Light energy is used to breakdown water (photolysis) to produce hydrogen ions, electrons and oxygen in the **thylakoid lumen**
    - A **proton gradient** is formed due to the photolysis of water resulting in a high concentration of hydrogen ions in the thylakoid lumen
    - Electrons travel through an **electron transport chain** of proteins within the membrane
    - **Reduced NADP** (NADPH) is produced when hydrogen ions in the stroma and electrons from the electron transport chain combine with the **carrier molecule NADP**
    - **ATP** is produced during a process known as **photophosphorylation** (ADP + P_i → ATP) using the proton gradient between the thylakoid lumen and stroma to drive the enzyme ATP synthase
- The photophosphorylation of ADP to ATP can be **cyclic** or **non-cyclic**, depending on the pattern of electron flow in photosystem I or photosystem II or both
    - In **cyclic** photophosphorylation, **only photosystem I** is involved
    - In **non-cyclic** photophosphorylation, **both photosystem I and photosystem II** are involved

- **Photosystems** are collections of photosynthetic pigments that absorb light energy and transfer the energy onto electrons, each photosystem contains a **primary pigment**
    - **Photosystem II** has a primary pigment that absorbs light at a wavelength of 680 nm and is therefore called **P680**
    - Photosystem II is at the beginning of the electron transport chain and is where the photolysis of water takes place
    - **Photosystem I** has a primary pigment that absorbs light at a wavelength of 700 nm and is therefore called **P700**
    - Photosystem I is in the middle of the electron transport chain
- The energy carried by the ATP is then used during the **light-independent** reactions of photosynthesis

Stages of the Calvin Cycle

- **Energy from ATP** and **hydrogen from reduced NADP** are passed from the light-dependent stage to the light-independent stage of photosynthesis
- The energy and hydrogen are used during the **light-independent** reactions (known collectively as the **Calvin cycle**) to produce complex organic molecules, including (but not limited to) **carbohydrates**, such as:
    - **Starch** (for storage)
    - **Sucrose** (for translocation around the plant)
    - **Cellulose** (for making cell walls)
- This stage of photosynthesis does not, in itself, require energy from light (hence **light-independent**) and can therefore take place in light or darkness. However, as it requires inputs of ATP and reduced NADP from the light-dependent stage, the Calvin cycle cannot continue indefinitely in darkness, as these inputs will run out
- There are **three main steps** within the **Calvin cycle**:
    - **Rubisco** catalyses the **fixation of carbon dioxide** by combination with a molecule of **ribulose bisphosphate** (RuBP), a 5C compound, to yield two molecules of **glycerate 3-phosphate** (GP), a 3C compound
    - **GP is reduced** to **triose phosphate** (TP) in a reaction involving reduced NADP and ATP
    - **RuBP is regenerated** from TP in reactions that use ATP

Carbon fixation

- Carbon dioxide combines with a five-carbon (5C) sugar known as **ribulose bisphosphate** (RuBP)
- An **enzyme** called **rubisco** (ribulose bisphosphate carboxylase) catalyses this reaction
- The resulting six-carbon (6C) compound is **unstable** and splits in two
- This gives two molecules of a three-carbon (3C) compound known as **glycerate 3-phosphate** (GP)
- The carbon dioxide has been **'fixed'** (it has been removed from the external environment and has become part of the plant cell)
- Glycerate 3-phosphate (GP) is not a carbohydrate but the next step in the Calvin cycle convert it into one

Reduction of glycerate 3-phosphate

- **Energy from ATP** and **hydrogen from reduced NADP** – both produced during the light-dependent stage of photosynthesis – are used to **reduce glycerate 3-phosphate** (GP) to a phosphorylated three-carbon (3C) sugar known as **triose phosphate** (TP)
- **One-sixth** of the triose phosphate (TP) molecules are used to produce useful organic molecules needed by the plant:
    - Triose phosphates can condense to become **hexose phosphates** (6C), which can be used to produce **starch, sucrose or cellulose**
    - Triose phosphates can be converted to **glycerol** and glycerate 3-phosphates to **fatty acids**, which join to form **lipids for cell membranes**
    - Triose phosphates can be used in the **production of amino acids for protein synthesis**

Regeneration of ribulose bisphosphate

- **Five-sixths** of the triose phosphate (TP) molecules are used to **regenerate ribulose bisphosphate** (RuBP)
- This process **requires ATP**

Calvin Cycle Intermediates

- Intermediate molecules of the Calvin cycle (such as **glycerate 3-phosphate** and **triose phosphate**) are used to produce other molecules
- **Glycerate 3-phosphate (GP)** is used to produce some amino acids
- **Triose phosphate (TP)** is used to produce:
    - **Hexose phosphates** (6C), which can be used to produce **starch, sucrose or cellulose**
    - **Lipids** for **cell membranes**
    - **Amino acids** for **protein synthesis**

<u>Limiting Factors of Photosynthesis</u>

- Plants need several factors for **photosynthesis** to occur:
    - the presence of **photosynthetic pigments**
    - a supply of **carbon dioxide**
    - a supply of **water**
    - **light** energy
    - a **suitable temperature**
- If there is a **shortage** of any of these factors, photosynthesis cannot occur at its **maximum possible rate**
- The main external factors that affect the rate of photosynthesis are:
    - **light intensity**
    - **carbon dioxide concentration**
    - **temperature**
- These are known as **limiting factors** of photosynthesis
- If any one of these factors is **below the optimum level** for the plant, its rate of photosynthesis will be **reduced**, even if the other two factors are at the optimum level
- Light intensity, CO_2 concentration and temperature are the three limiting factors of photosynthesis that you need to learn. Although a lack of water can reduce the rate of photosynthesis, water shortages usually affect other processes in the plant before affecting photosynthesis.

Limiting Factors of Photosynthesis: Effects

- **Changes** in **light intensity**, **carbon dioxide concentration** and **temperature** are all limiting factors that affect the **rate of photosynthesis**:

Light intensity

- When temperature and carbon dioxide concentration remain constant, changes in light intensity affect the rate of photosynthesis
- **The rate of photosynthesis increases as light intensity increases**:
    - The greater the light intensity, the more energy supplied to the plant and therefore **the faster the light-dependent stage** of photosynthesis can occur
    - This **produces more ATP and reduced NADP for the Calvin cycle** (light-independent stage), which can then also occur at a greater rate
    - During this stage, light intensity is said to be a limiting factor of photosynthesis
- At some point, if light intensity continues to increase, the relationship above will no longer apply and the rate of photosynthesis will reach a **plateau**
- At this point, **light intensity is no longer a limiting factor** – another factor is limiting the rate
- The factors which could be limiting the rate when the line on the graph is horizontal include **temperature** being too low or too high, or not enough **carbon dioxide**

Carbon dioxide concentration

- **The rate of photosynthesis increases as carbon dioxide concentration increases**:
    - Carbon dioxide is one of the raw materials required for photosynthesis
    - It is required for the **light-independent stage of photosynthesis, when CO_2 is combined with the five-carbon compound ribulose bisphosphate (RuBP)**
    - This means the more carbon dioxide that is present, the faster this step of the **Calvin cycle** can occur and **the faster the overall rate of photosynthesis**
- This trend will continue until some other factor required for photosynthesis prevents the rate from increasing further because it is in short supply
- The factors which could be limiting the rate when the line on the graph is horizontal include **temperature** being too low or too high, or not enough **light**

Temperature

- As temperature increases the rate of photosynthesis increases as the reaction is controlled by enzymes
- However, as the reaction is controlled by enzymes, **this trend only continues up to a certain temperature** beyond which the enzymes begin to **denature** and the rate of reaction **decreases**
- For most metabolic reactions, temperature has a large effect on reaction rate
- For photosynthesis, **temperature has no significant effect on the light-dependent reactions**, as these are driven by energy from light rather than the kinetic energy of the reacting molecules
- However, **the Calvin cycle is affected by temperature, as the light-independent reactions are enzyme-controlled reactions** (eg. rubisco catalyses the reaction between CO_2 and the five-carbon compound ribulose bisphosphate)
- In the section of the graph where the rate is increasing (the line is going up), the limiting factor is whatever the label on the x-axis (the bottom axis) of the graph is. In the section of the graph where the rate is not increasing (the line is horizontal), the limiting factor will be something other than what is on the x-axis – choose from temperature, light intensity or carbon dioxide concentration.

Limiting Factors of Photosynthesis: Increasing Crop Yields

- An understanding of limiting factors on the rate of photosynthesis can be used to **increase crop yields** in **protected environments**, such as **glasshouses**
- In the most sophisticated glasshouses, for example, **sensors** can be used to monitor the **light intensity**, the **humidity** of the atmosphere and the **carbon dioxide concentration** around the crops
- All these factors can be **managed** by a computer and their levels **adjusted** to ensure the crop can **photosynthesis at the highest rate possible**
- This **maximizes the yield** of the crop

<u>Investigating the Rate of Photosynthesis: Redox Indicators</u>

- The **light-dependent reactions** of photosynthesis take place in the thylakoid membrane and involve the **release of high-energy electrons** from chlorophyll *a* molecules
- These electrons are **picked up by electron acceptors** and then passed down the electron transport chain
- However, if a **redox indicator** (such as **DCPIP** or **methylene blue**) is present, the indicator **takes up the electrons instead**
- This causes the indicator to **change colour**
    - DCPIP: oxidised (**blue**) → accepts electrons → reduced (**colourless**)
    - Methylene blue: oxidised (**blue**) → accepts electrons → reduced (**colourless**)
    - *The colour of the reduced solution may appear green because the chlorophyll have a green colour*
- The **rate** at which the redox indicator changes colour from its **oxidised** state to its **reduced** state can be used as a measure of the **rate of photosynthesis**
    - When light is at a higher intensity, or at more preferable light wavelengths, the rate of photoactivation of electrons is faster, therefore the rate of reduction of the indicator is faster

Method

Step 1: Leaves are crushed in a liquid known as an **isolation medium**

- This produces a concentrated leaf extract that contains a **suspension of intact and functional chloroplasts**
- The medium must have the **same water potential** as the leaf cells (so the chloroplasts don't shrivel or burst) and contain a **buffer** (to keep the pH constant). It should also be **ice-cold** (to avoid damaging the chloroplasts and to maintain membrane structure)

Step 2: Small tubes are set up with **different intensities**, or **different colours (wavelengths)** of light shining of them

- If different intensities of light are used, they must all be of the same wavelength (same colour of light)
- If different wavelengths of light are used, they must all be of the same light intensity

Step 3: DCPIP of **methylene blue** indicator is added to each tube, as well as a small volume of the leaf extract

Step 4: The **time taken** for the redox indicator to go **colourless** is recorded

- This is a measure of the **rate of photosynthesis**

Investigating the Rate of Photosynthesis: Aquatic Plants

- Investigations to determine the effects of light intensity, carbon dioxide concentration and temperature on the **rate of photosynthesis** can be carried out using **aquatic plants**, such as *Elodea* or *Cabomba* (types of **pondweed**)
- The effect of these limiting factors on the rate of photosynthesis can be investigated in the following ways:
    - **Light intensity** – change the distance (*d*) of a light source from the plant (light intensity is proportional to $1/d^2$)
    - **Carbon dioxide concentration** – add different quantities of sodium hydrogencarbonate ($NaHCO_3$) to the water surrounding the plant, this dissolves to produce CO_2
    - **Temperature (of the solution surrounding the plant)** – place the boiling tube containing the submerged plant in water baths of different temperatures
- Whilst changing one of these factors during the investigation (as described below), **ensure the other two remain constant**
    - For example, when investigating the effect of light intensity on the rate of photosynthesis, a glass tank should be placed in between the lamp and the boiling tube containing the pondweed to absorb heat from the lamp – this prevents the solution surrounding the plant from changing temperature

Method

Step 1: Ensure the water is **well aerated** before use by **bubbling air through it**
- This will ensure oxygen gas given off by the plant during the investigation form **bubbles** and **do not dissolve in the water**

Step 2: Ensure the plant has been **well illuminated** before use
- This will ensure that the plant contains all the enzymes required for photosynthesis and that any changes of rate are due to the independent variable

Step 3: Set up the apparatus in a **darkened room**
- Ensure the pondweed is submerged in **sodium hydrogencarbonate solution (1%)** – this ensures the pondweed has a controlled **supply of carbon dioxide** (a reactant in photosynthesis)

Step 4: Cut the stem of the pondweed **cleanly** just before placing into the boiling tube

Step 5: Measure the volume of gas collected in the gas-syringe in a **set period of time** (eg. 5 minutes)

Step 6: Change the independent variable (i.e. change the light intensity, carbon dioxide concentration or temperature depending on which limiting factor you are investigating) and repeat step 5

Step 7: Record the results in a table and plot a graph of volume of oxygen produced per minute against the distance from the lamp (if investigating light intensity), carbon dioxide concentration, or temperature

- The 3 limiting factors and how each one can be altered in a laboratory environment:
    - Light intensity – the distance of the light source from the plant (intensity $\propto 1/d^2$)
    - Temperature – changing the temperature of the water bath the test tube sits in
    - Carbon dioxide – the amount of $NaHCO_3$ dissolved in the water the pondweed is in
    - Also remember that the variables not being tested (the control variables) must be kept constant.

Chloroplast Structures & their Functions

- Chloroplasts are the **organelles** in plant cells where **photosynthesis** occurs
- Each chloroplast is surrounded by a **double-membrane envelope**
    - Each of the envelope membranes is a phospholipid bilayer
- Chloroplasts are filled with a fluid known as the **stroma**
    - The stroma is the site of the **light-independent stage** of photosynthesis
- A separate **system of membranes** is found in the stroma
    - This membrane system is the site of the **light-dependent stage** of photosynthesis
    - The membrane contains the **pigments**, **enzymes** and **electron carriers** required for the light-dependent reactions
    - This membrane system consists of a series of flattened fluid-filled sacs known as **thylakoids**
    - These thylakoids stack up to form structures known as **grana** (singular – granum)
    - Grana are connected by membranous channels called stroma lamellae, which ensure the stacks of sacs are connected but distanced from each other
    - The membranes of the grana create a **large surface area** to **increase the number of light-dependent reactions** that can occur
    - This membrane system provides a large number of pigment molecules in an arrangement that ensures **as much light as necessary is absorbed**
- The stroma also contains small (70S) **ribosomes**, a loop of **DNA** and **starch grains**:
    - The loop of DNA codes for some of the **chloroplast proteins** (other chloroplast proteins are coded for by the DNA in the plant cell nucleus)
    - The proteins coded for by this loop of chloroplast DNA are **produced at the 70S ribosomes**
    - Sugars formed during photosynthesis are **stored** as **starch** inside starch grains

C4 Plants

- In the **light-independent stage** of photosynthesis, **carbon dioxide** combines with **RuBP** to form a **six-carbon compound**, which immediately splits to form **two three-carbon molecules**
- Plants that do this are known as **C3 plants**
- In some plants, such as **maize** and **sorghum** (tropical grasses), the first compound that is produced in the light-independent stage is a **four-carbon molecule**
- Plants that do this are known as **C4 plants**

Adaptations of C4 plants for high rates of carbon fixation at high temperatures

- The enzyme **rubisco** catalyses the reaction of **carbon dioxide** with **RuBP**
- However, under certain conditions, it can **also** catalyse the reaction of **oxygen** with **RuBP**
- This process (known as **photorespiration**) results in **less photosynthesis** taking place, as **less RuBP** is available to combine with carbon dioxide
- This mainly occurs at **high temperatures** and **high light intensities** (conditions often found at low altitudes in tropical parts of the world
- Tropical grasses, such as maize and sorghum, have evolved an adaptation to **minimise photorespiration** – they keep RuBP and rubisco **separated** from high oxygen concentrations:
    - The cells that contain **RuBP** and **rubisco** are arranged around the vascular bundles and are known as **bundle sheath cells**. They have **no direct contact with air** inside the leaf
    - Carbon dioxide is absorbed by a different group of cells, known as **mesophyll cells**, that **are** in contact with air
    - Inside the mesophyll cells, carbon dioxide is combined with a three-carbon compound known as phosphoenolpyruvate (PEP) to form a **four-carbon compound** known as oxaloacetate, which is then converted to another **four-carbon compound** known as malate
    - Malate then enters the **bundle sheath cells**, where **carbon dioxide is removed** from the malate molecules and delivered to RuBP by rubisco in the normal way
    - The light-independent reaction then continues in the same way as in a C3 plant
- C4 plants have also evolved an adaptation to growing in **hot climates** – their **enzymes** generally have **higher optimum temperatures** than those of C3 plants

14. Homeostasis

Homeostasis

- In order to **function** properly and **efficiently**, organisms have different **control systems** that ensure their **internal conditions** are kept relatively **constant**
- The process of maintaining constant internal body conditions is known as **homeostasis**
- Homeostasis is **critically important** for organisms as it ensures the maintenance of **optimal conditions** for **enzyme action** and **cell function**
- **Sensory cells** can **detect** information about the **conditions** inside and outside of the body
- Examples of physiological factors that are **controlled** by **homeostasis** in **mammals** include:
    - Core body temperature
    - Metabolic waste (eg. carbon dioxide and urea)
    - Blood pH
    - Concentration of glucose in the blood
    - Water potential of the blood
    - Concentration of the respiratory gases (carbon dioxide and oxygen) in the blood

❖ **Homeostasis** is the regulation of the internal conditions of a cell or organism to maintain optimum conditions for function, in response to internal and external changes.

Principles of Homeostasis

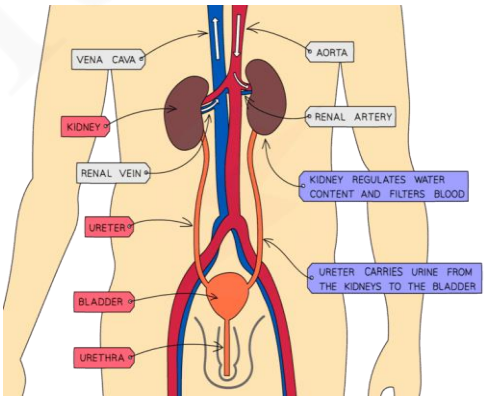
- The majority of homeostatic control mechanisms in organisms use **negative feedback** to maintain **homeostatic balance** (i.e. to keep certain physiological factors, such as blood glucose concentration, **within certain limits**)
- Negative feedback control loops involve:
    - A **receptor** (or sensor) – to **detect** a **stimulus** that is involved with a condition / physiological factor
    - A **coordination system** (nervous system and endocrine system) – to **transfer information** between different parts of the body
    - An **effector** (muscles and glands) – to **carry out a response**
- Outcome of a negative feedback loop:
    - The factor / stimulus is **continuously monitored**
    - If there is an increase in the factor, the body responds to make the factor decrease
    - If there is a decrease in the factor, the body responds to make the factor increase
- Homeostasis in **mammals** relies on **two different coordination systems** to transfer information between different parts of the body:
    - **Nervous system** – information is transmitted as **electrical impulses** that travel along **neurones**
    - **Endocrine system** – information is transmitted as **chemical messengers** called **hormones** that travel in the **blood**
- Although the nervous and endocrine systems are both important in homeostasis and the regulation of certain physiological factors, there are some fundamental differences between them. Information is transmitted through these two systems in different ways (electrical impulses vs. hormones). Also, the nervous system is usually required for fast, but short-lived responses, whereas the endocrine system is involved in slower, but longer-lasting responses.

Production of Urea

- Many **metabolic reactions** within the body produce **waste products**
- The removal of these waste products is known as **excretion**
- Many excretory products are formed in humans, with two in particular (**carbon dioxide** and **urea**) being formed in **much greater quantities** than others

Urea

- **Urea** is produced in the **liver**
- It is produced from **excess amino acids**
- If more protein is eaten than is required, the excess **cannot be stored** in the body
- However, the amino acids within the protein can still provide **useful energy**
- To make this energy accessible, the **amino group** is **removed** from each amino acid
- This process is known as **deamination**:
    - The amino group (**-NH_2**) of an amino acid is removed, together with an extra hydrogen atom
    - These combine to form **ammonia** (NH_3)
    - The remaining keto acid may enter the Krebs cycle to be **respired**, be converted to **glucose**, or converted to **glycogen / fat for storage**
- **Ammonia** is a **very soluble** and **highly toxic** compound that is produced during deamination. It can be very damaging if allowed to build up in the blood
- This is avoided by **converting ammonia** to **urea**
    - Urea is **less soluble** and **less toxic** than ammonia
- Ammonia is **combined with carbon dioxide** to form urea



Structure of the Human Kidney

- Humans have **two** kidneys
- The kidneys are responsible for carrying out two very important functions:
    - As an **osmoregulatory organ** – **they regulate the water content of the blood** (vital for maintaining blood pressure)
    - As an **excretory organ** – **they excrete the toxic waste products of metabolism** (such as **urea**) and substances in excess of requirements (such as **salts**)
- The kidney itself is surrounded by a fairly tough outer layer known as the **fibrous capsule**
- Beneath the fibrous capsule, the kidney has **three main areas**:
    - The **cortex** (contains the glomerulus, as well as the Bowman's capsule, proximal convoluted tubule, and distal convoluted tubule of the nephrons)
    - The **medulla** (contains the loop of Henle and collecting duct of the nephrons)
    - The **renal pelvis** (where ureter joins kidney)

Structure	Function
Renal artery	Carries oxygenated blood (containing urea and salts) to kidneys
Renal vein	Carries deoxygenated blood (that has had urea and excess salts removed) away from kidneys
Kidney	Regulates water content of blood and filters blood
Ureter	Carries urine from kidneys to bladder
Bladder	Stores urine (temporarily)
Urethra	Releases urine outside of the body

Nephron Structure

- Each kidney contains **thousands** of **tiny tubes**, known as **nephrons**
- The nephron is the **functional unit** of the kidney – the nephrons are responsible for the **formation of urine**
- There is also a network of **blood vessels** associated with each nephron:
    - **Within the Bowman's capsule** of each nephron is a structure known as the **glomerulus**
    - Each glomerulus is supplied with blood by an **afferent arteriole** (which carries blood from the **renal artery**)
    - The capillaries of the glomerulus rejoin to form an **efferent arteriole**
    - Blood then flows from the efferent arteriole into a network of capillaries that run closely alongside the rest of the nephron
    - Blood from these capillaries eventually flows into the **renal vein**

Formation of Urine in the Nephron

- The nephron is the **functional unit** of the kidney – the nephrons are responsible for the **formation of urine**
- The process of urine formation in the kidneys occurs in **two stages**:
    1. **Ultrafiltration**
    2. **Selective reabsorption**

Stage	Name of process	Where process occurs	Explanation of process
1	Ultrafiltration	Bowman's capsule	Small molecules (including **amino acids, water, glucose, urea and inorganic ions**) are filtered out of the blood capillaries of the glomerulus and **into the Bowman's capsule** to form filtrate known as glomerular filtrate.
2	Selective reabsorption	Proximal convoluted tubule	**Useful molecules** are taken back (**reabsorbed**) from the filtrate and returned to the blood as the filtrate flows along the nephron.

- After the necessary reabsorption of amino acids, water, glucose and inorganic ions is complete (even some urea is reabsorbed), **the filtrate eventually leaves the nephron** and is now referred to as **urine**
- This urine flows out of the kidneys, along the ureters and into the **bladder**, where it is temporarily stored

Ultrafiltration

- The blood in the **glomerular capillaries** is **separated** from the **lumen** of the **Bowman's capsule** by **two cell layers** with a **basement membrane** in between them:
    - The first cell layer is the **endothelium of the capillary** – each capillary endothelial cell is perforated by thousands of tiny membrane-lined circular holes
    - The next layer is the **basement membrane** – this is made up of a network of collagen and glycoproteins
    - The second cell layer is the **epithelium of the Bowman's capsule** – these epithelial cells have many tiny finger-like projections with gaps in between them and are known as **podocytes**
- As blood passes through the glomerular capillaries, the holes in the capillary endothelial cells and **the gaps between the podocytes** allows substances dissolved in the blood plasma to pass into the Bowman's capsule
    - The fluid that filters through from the blood into the Bowman's capsule is known as the **glomerular filtrate**
    - The main substances that pass out of the capillaries and form the glomerular filtrate are: **amino acids, water, glucose, urea and inorganic ions** (mainly Na^+, K^+ and Cl^-)
- **Red and white blood cells** and **platelets** remain in the blood as they are **too large** to pass through the holes in the capillary endothelial cells
- The **basement membrane** acts as a **filter** as it **stops large protein molecules** from getting through

How ultrafiltration occurs

- Ultrafiltration occurs due to the **differences in water potential** between the **plasma** in the **glomerular capillaries** and the **filtrate** in the **Bowman's capsule**
    - Remember – water moves down a water potential gradient, from a region of higher water potential to a region of lower water potential. Water potential is increased by high pressure and decreased by the presence of solutes
- Overall, the effect of the pressure gradient **outweighs** the effect of solute gradient
- Therefore, the water potential of the blood plasma in the glomerulus is **higher** than the water potential 8of the filtrate in the Bowman's capsule
- This means that as blood flows through the glomerulus, there is an **overall movement of water down the water potential gradient from the blood into the Bowman's capsule**

Factor affecting water potential	How factor affects water potential in the glomerulus and Bowman's capsule	Resulting movement of water
Pressure	○ As the afferent arteriole is **wider** than the efferent arteriole, the **blood pressure** is relatively **high** in the **glomerular capillaries.** ○ This **raises** the water potential of the blood plasma in the glomerular capillaries above the water potential of the filtrate in the Bowman's capsule.	Water moves down the water potential gradient, from the blood plasma in the glomerular capillaries into the Bowman's capsule.
Solute concentration	○ Whilst the basement membrane allows most solutes within the blood plasma to filter into the Bowman's capsule, **plasma protein molecules are too big** to get through and stay in the blood. ○ As a result, the solute concentration in the blood plasma in the glomerular capillaries is still higher than that in the filtrate in the Bowman's capsule. ○ This makes the water potential of the blood plasma **lower** than that of the filtrate in the Bowman's capsule.	Water moves down the water potential gradient from the Bowman's capsule into the blood plasma in the glomerular capillaries.

Adaptation of proximal convoluted tubule epithelial cell	How adaptation aids reabsorption
Many microvilli present on the luminal membrane (the cell surface membrane that faces the lumen).	This increases the surface area for reabsorption.
Many co–transporter proteins in the luminal membrane.	Each type of co–transporter protein transports a specific solute (eg. glucose or a particular amino acid) across the luminal membrane.
Many mitochondria.	These provide energy for sodium–potassium ($Na^+ – K^+$) pump proteins in the basal membranes of the cells.
Cells tightly packed together.	This means that no fluid can pass between the cells (all substances reabsorbed must pass through the cells).

Selective Reabsorption

- Many of the substances that end up in the **glomerular filtrate** actually need to be **kept** by the body
- These substances are **reabsorbed** into the blood as the filtrate passes along the nephron
- This process is known as **selective reabsorption** as only certain substances are reabsorbed
- Most of this reabsorption occurs in the **proximal convoluted tubule**
- The lining of the proximal convoluted tubule is composed of a single layer of epithelial cells, which are adapted to carry out reabsorption in several ways:
    - Microvilli
    - Co-transporter proteins
    - A high number of mitochondria
    - Tightly packed cells

How selective reabsorption occurs

- Blood capillaries are located very close to the outer surface of the proximal convoluted tubule
    - As the blood in these capillaries comes straight from the glomerulus, it has very little plasma and has lost much of its water, inorganic ions and other small solutes
- The basal membranes (of the proximal convoluted tubule epithelial cells) are the sections of the cell membrane that are closest to the blood capillaries
- **Sodium-potassium pumps** in these **basal membranes** move sodium ions out of the epithelial cells and into the blood, where they are carried away
- This **lowers the concentration of sodium ions inside the epithelial cells**, causing sodium ions in the filtrate to diffuse down their concentration gradient through the luminal membranes (of epithelial cells)
- These sodium ions do not diffuse freely through the luminal membranes – they must pass through **co-transporter proteins** in the membrane
- There are several types of these co-transporter proteins – each type transports **a sodium ion and another solute** from the filtrate (eg. glucose or a particular amino acid)
- Once inside the epithelial cells these solutes diffuse down their concentration gradients, passing through transport proteins in the basal membranes (of the epithelial cells) into the blood

Molecules reabsorbed from the proximal convoluted tubule during selective reabsorption

- **All glucose** in the glomerular filtrate is reabsorbed into the blood
    - This means no glucose should be present in the urine
- **Amino acids, vitamins and inorganic ions** are reabsorbed
- The movement of all these solutes from the proximal convoluted tubule into the capillaries **increases** the water potential of the **filtrate** and **decreases** the water potential of the **blood** in the capillaries
    - This creates steep water potential gradient and causes **water to move into the blood by osmosis**
- A significant amount of **urea** is reabsorbed too
    - The concentration of urea in the filtrate is higher than in the capillaries, causing urea to diffuse from the filtrate back into the blood
- Selective reabsorption in the proximal convoluted tubule uses the same method of membrane transport that moves sucrose into companion cells in phloem tissue! As sodium ions move passively down their concentration gradient into the epithelial cells of the proximal convoluted tubule, this provides the energy needed to reabsorb solute molecules (eg. glucose and amino acids) into the epithelial cells, even against their concentration gradients. This is known as indirect or secondary active transport, as the energy (ATP) is used to pump sodium ions, not the solutes themselves.

Osmoregulation

- The control of the **water potential** of body fluids is known as **osmoregulation**
- Osmoregulation is a key part of **homeostasis**
- Specialised **sensory neurones**, known as **osmoreceptors**, monitor the water potential of the blood (these osmoreceptors are found in an area of the brain known as the **hypothalamus**)
- If the osmoreceptors detect a **decrease** in the water potential of the **blood**, nerve impulses are sent along these sensory neurones to the **posterior pituitary gland** (another part of the brain just below the hypothalamus)
- These nerve impulses stimulate the posterior pituitary gland to release **antidiuretic hormone (ADH)**
- ADH molecules enter the blood and travel throughout the body
- ADH causes the **kidneys** to **reabsorb** more water
- This **reduces the loss of water in the urine**

The effect of ADH on the kidneys

- Water is reabsorbed by **osmosis** from the **filtrate** in the **nephron**
- This reabsorption occurs as the filtrate passes through structures known as **collecting ducts**
- **ADH** causes the **luminal membranes** (ie. those facing the lumen of the nephron) of the collecting duct cells to become **more permeable** to water
- ADH does this by causing an **increase** in the number of **aquaporins** (water-permeable channels) in the luminal membranes of the collecting duct cells. This occurs in the following way:
    - Collecting duct cells contain **vesicles**, the membranes of which contain many **aquaporins**
    - ADH molecules bind to receptor proteins, activating a signalling cascade that leads to the **phosphorylation** of the aquaporin molecules
    - This activates the aquaporins, causing the **vesicles** to **fuse** with the **luminal membranes** of the collecting duct cells
    - This **increases the permeability** of the membrane to water
- As the filtrate in the nephron travels along the collecting duct, water molecules move from the collecting duct (**high water potential**), through the aquaporins, and into the **tissue fluid** and **blood plasma** in the **medulla** (**low water potential**)
- As the filtrate in the collecting duct loses water it becomes more **concentrated**
- As a result, a **small volume of concentrated urine** is produced. This flows from the kidneys, through the ureters and into the bladder
- If the water potential of the blood is too high, the exact opposite happens:
    - Osmoreceptors in the hypothalamus are not stimulated
    - No nerve impulses are sent to the posterior pituitary gland
    - No ADH released
    - Aquaporins are moved out of the luminal membranes of the collecting duct cells
    - Collecting duct cells are no longer permeable to water
    - The filtrate flows along collecting duct but loses no water and is very dilute
    - A large volume of dilute urine is produced
    - This flows from the kidneys, through the ureters and into the bladder

The Control of Blood Glucose

- If the concentration of glucose in the blood **decreases** below a certain level, cells may not have enough glucose for **respiration** and may not be able to function normally
- If the concentration of glucose in the blood **increases** above a certain level, this can also **disrupt the normal function of cells**, potentially causing major problems
- The control of blood glucose concentration is a key part of **homeostasis**
- Blood glucose concentration is controlled by **two hormones** secreted by **endocrine tissue** in the **pancreas**
- This tissue is made up of groups of cells known as the **islets of Langerhans**
- The islets of Langerhans contain **two cell types**:
    - **α cells** that secrete the hormone **glucagon**
    - **β cells** that secrete the hormone **insulin**
- These α and β cells act as the **receptors** and initiate the response for controlling blood glucose concentration
- The control of blood glucose concentration by **glucagon** can be used to demonstrate the principles of **cell signalling**

Decrease in blood glucose concentration

- If a **decrease** in blood glucose concentration occurs, it is **detected** by the **α and β cells** in the pancreas:
    - The **α cells** respond by **secreting glucagon**
    - The **β cells** respond by **stopping the secretion of insulin**
- The decrease in blood **insulin** concentration **reduces** the use of **glucose** by liver and muscle cells
- **Glucagon** binds to **receptors** in the cell surface membranes of **liver cells**
- This binding causes a **conformational change** in the receptor protein that activates a **G protein**
- This activated G protein activates the enzyme **adenylyl cyclase**
- Active adenylyl cyclase catalyses the conversion of **ATP** to the second messenger, **cyclic AMP** (cAMP)
- **cAMP** binds to **protein kinase A enzymes**, activating them
- Active protein kinase A enzymes activate **phosphorylase kinase enzymes** by adding **phosphate groups** to them
- Active phosphorylase kinase enzymes activate **glycogen phosphorylase enzymes**
- Active glycogen phosphorylase enzymes **catalyse the breakdown of glycogen to glucose**
    - This process is known as **glycogenolysis**
- The enzyme cascade described above **amplifies** the original signal from glucagon and results in the **releasing of extra glucose by the liver** to increase the blood glucose concentration back to a normal level
- Make sure you know where this response to a decrease in blood glucose concentration occurs! The enzyme cascade only occurs in liver cells, there are no glucagon receptors on muscle cells.

Negative Feedback Control of Blood Glucose

- Blood glucose concentration is **regulated** by **negative feedback** control mechanisms
- In negative feedback systems:
    - **Receptors detect** whether a specific level is **too low** or **too high**
    - This information is communicated through the **hormonal or nervous system** to effectors
    - **Effectors react** to counteract the change by **bringing the level back to normal**
- In the control of blood glucose concentration:
    - **α and β cells** in the pancreas act as the **receptors**
    - They release the **hormones glucagon** (secreted by α cells) and **insulin** (secreted by β cells)
    - **Liver cells** act as the **effectors** in response to **glucagon** and **liver, muscle and fat cells** act as the **effectors** in response to **insulin**

<u>Test Strips & Biosensors</u>

Measuring urine glucose concentration

- People with **diabetes** cannot **control** their blood glucose concentration so that it remains within normal, safe limits
- The presence of **glucose** in **urine** is an **indicator** that a person may have diabetes
    - If blood glucose concentration increases above a value known as the **renal threshold**, not all of the glucose from the filtrate in the proximal convoluted tubule is **reabsorbed** and some will be left in the urine
- **Test strips** can be used to test urine for the **presence and concentration of glucose**
- **Two enzymes** are **immobilised** on a small pad at one end of the test strip. These are:
    - glucose oxidase
    - peroxidase
- The pad is immersed in the urine sample for a short time
- If glucose is present:
    - Glucose oxidase catalyses a reaction in which **glucose** is **oxidised** to form **gluconic acid** and **hydrogen peroxide**
    - Peroxidase then catalyses a reaction between the **hydrogen peroxide** and a colourless chemical in the pad to form a **brown compound** and water
- The colour of the pad is compared to a **colour chart** – different colours represent different concentrations of glucose (the higher the concentration of glucose present, the darker the colour)
- Urine tests only show whether or not the blood glucose concentration was above the renal threshold **whilst urine was collecting in the bladder** – they do not indicate the **current** blood glucose concentration

Measuring blood glucose concentration

- A **biosensor** can be used by people with **diabetes** to show their **current** blood glucose concentration
- Similar to the test strips, a biosensor uses **glucose oxidase** (but no peroxidase) immobilised on a **recognition layer**
- Covering the recognition layer is a **partially permeable membrane** that only allows small molecules from the blood to reach the immobilised enzymes
- When a small sample of blood is tested, glucose oxidase catalyses a reaction in which any **glucose** in the blood sample is **oxidised** to form **gluconic acid** and **hydrogen peroxide**
- The hydrogen peroxide produced is **oxidised** at an **electrode** that detects **electron transfers**
- The **electron flow is proportional to the glucose concentration** of the blood sample
- The biosensor amplifies the current, which is then read by a processor to produce a digital reading for blood glucose concentration
- This process is complete within a matter of seconds
- The urine test strip will only produce a positive result for glucose. Other sugars such as fructose, sucrose and lactose will give a negative result. This is due to the specificity of the glucose oxidase enzyme.

<u>Stomata</u>

- Plants carry out **homeostasis** – just like animals they need to maintain a **constant internal environment**
    - Ex: mesophyll cells in leaves require a **constant supply of carbon dioxide** for **photosynthesis**
- **Stomata** (specifically the **guard cells**) control the diffusion of gases in and out of leaves
    - This means stomata **control the entry of carbon dioxide** into leaves

Environmental stimuli causing stomata to open	Environmental stimuli causing stomata to close
Increasing light intensity	Darkness
Low carbon dioxide concentrations in the air spaces within the leaf	High carbon dioxide concentrations in the air spaces within the leaf
	Low humidity
	High temperature
	Water stress — when the supply of water from the roots is limited and/or there are high rates of transpiration

- Regulation of stomatal aperture balances the need for carbon dioxide uptake by diffusion with the need to minimise water loss by transpiration
- A stoma is actually the aperture (hole) between two guard cells, but the term is often used to refer to the whole unit (the two guard cells and the hole between them).
- Don't forget – stoma (singular) refers to one of these units, whereas stomata (plural) refers to many!

	Stomata open during the day	Stomata closed during the day
Advantage	Leaves gain carbon dioxide for photosynthesis	Water is retained inside the leaf, which is important in times of water stress
Disadvantage	Leaves lose large amounts of water by transpiration	Supply of carbon dioxide decreases so the rate of photosynthesis decreases

Opening & Closing of Stomata

- Stomata **open** and **close** in a **daily rhythm**
    - Even when the plant is kept in **constant light** or **constant darkness**, the daily rhythm of opening and closing of the stomata continues
- Opening of stomata **during the day**:
    - maintains the **inward diffusion of carbon dioxide** and the **outward diffusion of oxygen**
    - allows the **outward diffusion of water vapour** in transpiration
- Closing of stomata **at night** when photosynthesis cannot occur:
    - **reduces** the rate of **transpiration**, which conserves water

<u>Guard Cells</u>

Structure of guard cells

- Each stoma is surrounded by **two guard cells**
- Guard cells have the following features:
    - **Thick** cell walls facing the air outside the leaf and the **stoma**
    - **Thin** cell walls facing **adjacent epidermal cells**
    - **Cellulose microfibrils** arranged in bands around the cell
    - Cell walls have **no plasmodesmata**
    - Cell surface membrane is often **folded** and contains **many channel and carrier proteins**
    - Cytoplasm has a **high density of chloroplasts and mitochondria**
    - Chloroplasts have thylakoids but with **few grana** (unlike those in mesophyll cell chloroplasts)
    - Mitochondria have **many cristae**
    - **Several small vacuoles** rather than one large vacuole

Mechanism to open stomata

- Guard cells **open** when they **gain water** and become **turgid**
- Guard cells gain water by **osmosis**
- A **decrease in water potential** in the guard cells is required for water to enter the cells by osmosis
- In response to light, ATP-powered proton pumps in the guard cell surface membranes actively transport **hydrogen (H^+) ions out of the guard cell**
- This leaves the **inside** of the guard cells **negatively charged** compared to the outside
- This causes **channel proteins** in the guard cell surface membranes to **open, allowing potassium (K^+) ions** to move down the electrical gradient and **enter** the guard cells
- The potassium (K^+) ions also diffuse into the guard cells down a concentration gradient
    - The combination of the electrical gradient and concentration gradient is known as an **electrochemical gradient**
- The influx of potassium (K^+) ions **increases the solute concentration** inside the guard cells, **lowering the water potential** inside the cells
- Water now **enters** the guard cells by **osmosis** through **aquaporins** in the guard cell surface membranes
    - Most of the water enters the vacuoles, causing them to increase in size
- This increases the **turgor pressure** of the guard cells, causing the stoma to **open**
    - The bands of cellulose microfibrils only allow the guard cells to increase in length (not diameter)
    - The **thin outer walls of the guard cells bend more easily** than thick inner walls
    - This causes the guard cells to become **curved**, opening up the stoma

Mechanism to close stomata

- When certain environmental stimuli are detected (that lead to the closing of the stomata), the proton pumps in the guard cell surface membranes **stop actively transporting hydrogen (H^+) ions out of the guard cell**
- The **potassium (K^+) ions leave** the guard cells
- The **water potential gradient** is now **reversed** and water **leaves** the guard cells by **osmosis**
- This causes the guard cells to become **flaccid**, closing the stoma

<u>Abscisic Acid & Stomatal Closure</u>

- During times of **water stress,** the hormone **abscisic acid** (**ABA**) is produced by plants to **stimulate the closing of their stomata**
    - Certain environmental conditions can cause water stress, such as **very high temperatures** or **reduced water supplies**
- **Guard cells** have **ABA receptors** on their cell surface membranes
- ABA binds with these receptors, **inhibiting the proton pumps** and therefore **stopping** the active transport of hydrogen (H^+) ions out of the guard cells
- ABA also causes **calcium (Ca^{2+}) ions** to move **into the cytoplasm** of the guard cells through the cell surface membranes
- The calcium ions act as **second messengers**:
    - They cause **channel proteins** to **open** that allow negatively charged ions to leave the guard cells
    - This stimulates the **opening of further channel proteins** that allow **potassium (K^+) ions** to **leave** the guard cells
    - The calcium ions also stimulate the **closing of channel proteins** that allow potassium (K^+) ions to **enter** the guard cells
- This **loss of ions increases the water potential** of the guard cells
- Water **leaves** the guard cells by **osmosis**
- The guard cells become **flaccid**, causing the stomata to **close**

15. Control & coordination

The Endocrine System

- A **hormone** is a **chemical substance** produced by an **endocrine gland** and carried by the **blood**
    - They are chemicals which **transmit information from one part of the organism to another** and bring about a **change**
    - They **alter** the **activity** of one or more specific **target organs**
- Hormones are used to control functions that **do not need instant responses**
- The endocrine glands that produce hormones in animals are known collectively as the **endocrine system**
    - A **gland** is a **group of cells** that produces and releases one or more substances (a process known as **secretion**)
- Hormones such as **insulin, glucagon, ADH and adrenaline** are **cell-signalling molecules** that are released into the blood
- Endocrine glands have a good **blood supply** as when they make hormones they need to get them into the bloodstream (specifically the **blood plasma**) as soon as possible so they can travel around the body to the **target organs** to bring about a **response**
- Hormones only affect cells with **receptors** that the hormone can bind to
    - These are either found on the cell surface membrane, or inside cells
    - Receptors have to be **complementary** to hormones for there to be an **effect**
- Hormones such as **insulin, glucagon and ADH** are peptides or small proteins
    - They are **water-soluble** and so **cannot cross the phospholipid bilayer** of cell surface membranes
    - These hormones bind to receptors on the cell surface membranes of their target cells, which activates **second messengers** to transfer the signal throughout the cytoplasm
- Hormones such as **testosterone, oestrogen and progesterone** are steroid hormones
    - They are **lipid-soluble** and so **can cross the phospholipid bilayer**
    - These hormones bind to receptors in the cytoplasm or nucleus of their target cells

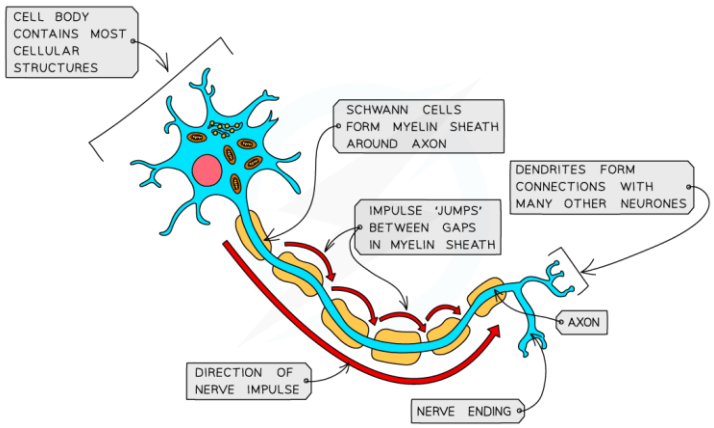
The Nervous System

- The human nervous system consists of the:
    - **Central nervous system** (CNS) – the brain and the spinal cord
    - **Peripheral nervous system** (PNS) – all of the nerves in the body
- It allows us to make sense of our surroundings and respond to them and to **coordinate and regulate body functions**
- Information is sent through the nervous system as **nerve impulses** – electrical signals that pass along nerve cells known as **neurones**
- A bundle of neurones is known as a nerve
- Neurones **coordinate** the activities of **sensory receptors** (eg. those in the eye), **decision-making centres in the central nervous system**, and **effectors** such as muscles and glands

	Nervous system	Endocrine system
Parts of the system	Brain, spinal cord, nerves/neurones	Glands
Type of message	Electrical impulse	Chemical hormone
Method of transmission	Nerves/neurones	Bloodstream
Effectors	Muscles or glands	Target cells in specific tissues
Speed of transmission	Very fast	Slower
Length of effect	Short – until electrical impulses stop	Longer – until hormone is broken down

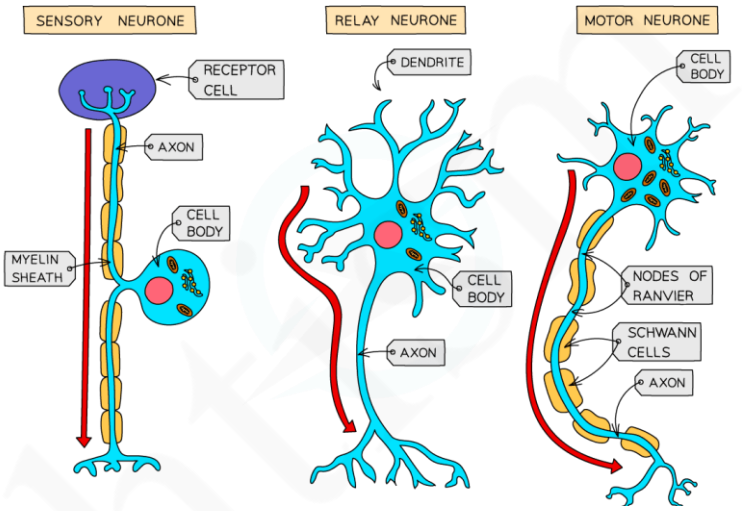
Neurones

- Neurones have a **long fibre** known as an **axon**
- The axon is **insulated** by a **fatty sheath** with small uninsulated sections along its length (called **nodes of Ranvier**)
    - The sheath is made of **myelin**, a substance made by specialised cells known as **Schwann cells**
    - Myelin is made when Schwann cells wrap themselves around the axon along its length
- This means that the electrical impulse does not travel down the whole axon, but jumps from one node to the next
- This means that less time is wasted transferring the impulse from one cell to another
- Their **cell bodies** contain many extensions called **dendrites**
- This means they can **connect** to **many other neurones** and receive **impulses** from them, forming a **network** for easy **communication**
- There are **three main types** of neurone: **sensory, relay and motor**
    - **Sensory** neurones carry impulses from **receptors** to the **CNS** (brain or spinal cord)
    - **Relay** (intermediate) neurones are found entirely within the CNS and **connect sensory** and **motor** neurones
    - **Motor** neurones carry impulses from the **CNS** to **effectors** (muscles or glands)



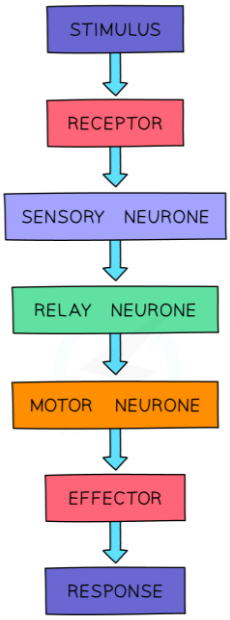
The three types of neurone – the red line shows the direction of impulses

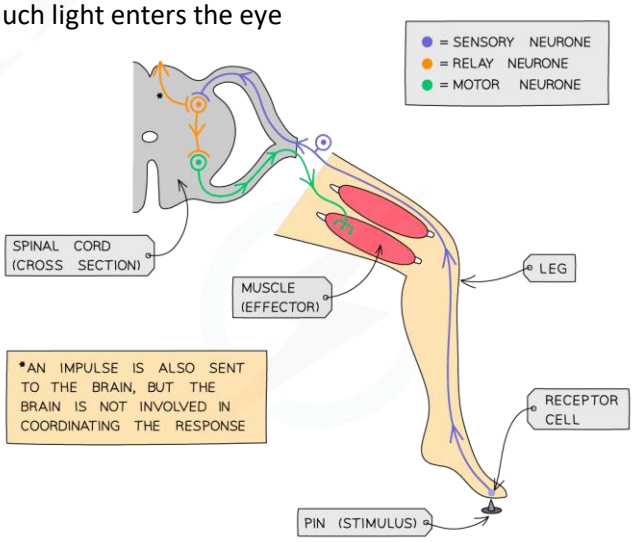
- Each type of neurone has a **slightly different structure**
- **Motor neurones** have:
    - A large cell body at one end, that lies within the spinal cord or brain
    - A nucleus that is always in its cell body
    - Many highly-branched dendrites that extend from the cell body, providing a large surface area for the axon terminals of other neurones
- **Sensory neurones** have the same basic structure as motor neurones, but have:
    - One long axon with a cell body that branches off in the middle of the axon – it may be near the source of stimuli or in a swelling of a spinal nerve known as a ganglion



Reflex arc

- **Sensory** neurones, **relay** (**intermediate**) neurones and **motor** neurones work together to bring about a **response** to a **stimulus**
- A **reflex arc** is a **pathway** along which impulses are transmitted from a receptor to an effector **without involving 'conscious' regions of the brain**
- As it does not involve the brain, a reflex response is **quicker** than any other type of nervous response
- Examples of simple reflex actions that are coordinated by these pathways are:
    - Removing the hand rapidly from a sharp or hot object
    - Blinking
    - Focusing of the eye on an object
    - Controlling how much light enters the eye





How sensory neurones, intermediate relay neurones and motor neurones work together to carry out a reflex action

- A pin (the **stimulus**) is detected by a pain **receptor** in the skin
- The **sensory neurone** sends electrical **impulses** to the spinal cord (the **coordinator**)
- Electrical impulses are passed on to **relay neurone** in the spinal cord
- The relay neurone connects to the **motor neurone** and passes the impulses on
- The motor neurone carries the impulses to the muscle in the leg (the **effector**)
- The impulses cause the muscle to contract and pull the leg up and away from the sharp object (the **response**)

Sensory Receptor Cells

- A cell that **responds** to a **stimulus** is called a **receptor cell**
- Receptor cells are **transducers** – they **convert energy** in one form (such as light, heat or sound) into energy in an **electrical impulse** within a sensory neurone
- Receptor cells are often found in **sense organs** (eg. light receptor cells are found in the eye)
    - Some receptors, such as light receptors in the eye and chemoreceptors in the taste buds, are specialised cells that detect a specific type of stimulus and influence the electrical activity of a sensory neurone
    - Other receptors, such as some kinds of touch receptors, are just the ends of the sensory neurones themselves
- When receptors cells are stimulated they are **depolarised**
    - If the **stimulus** is very **weak**, the cells are **not sufficiently depolarised** and the sensory neurone is not activated to send impulses
    - If the **stimulus** is **strong** enough, the sensory neurone is **activated** and **transmits impulses to the CNS**

Sequence of Events Resulting in an Action Potential

- The **surface** of the **tongue** is covered in many small bumps known as **papillae**
- The surface of each papilla is covered in many **taste buds**
- Each taste bud contains many receptor cells known as **chemoreceptors**
    - These chemoreceptors are sensitive to **chemicals** in food and drinks
- Each chemoreceptor is covered with **receptor proteins**; different receptor proteins detect different chemicals

Factor	How factor contributes to maintaining the resting potential
Sodium–potassium pumps in the axon membrane.	These pumps move sodium (Na^+) ions out of the axon and potassium (K^+) ions into the axon. The pump proteins use energy from the hydrolysis of ATP to continue moving these ions against their concentration gradients.
Many large, negatively charged molecules (anions) inside the axon.	This attracts potassium ions, reducing the chance of them diffusing out of the axon.
Impermeability of the axon membrane to ions.	Sodium ions cannot diffuse through the axon membrane when the neurone is at rest.
Closure of voltage–gated channel proteins (required for action potentials) in the axon membrane.	Stops sodium and potassium ions diffusing through the axon membrane.

An example of the sequence of events that results in an action potential in a sensory neurone

- Chemoreceptors in the taste buds that detect **salt** (sodium chloride) respond directly to **sodium ions**
- If salt is present in the food (dissolved in saliva) being eaten or the liquid being drunk:
    - Sodium ions **diffuse** through **highly selective channel proteins** in the cell surface membranes of the **microvilli** of the **chemoreceptor cells**
    - This leads to **depolarisation** of the chemoreceptor **cell membrane**
    - The **increase** in **positive charge** inside the cell is known as the **receptor potential**
    - If there is **sufficient stimulation** by sodium ions and sufficient depolarisation of the membrane, the receptor potential becomes large enough to stimulate **voltage-gated calcium ion channel proteins** to **open**
    - As a result, **calcium ions enter the cytoplasm** of the chemoreceptor cell and **stimulate exocytosis of vesicles containing neurotransmitter** from the basal membrane of the chemoreceptor
    - The neurotransmitter stimulates an **action potential** in the **sensory neurone**
    - The sensory neurone then transmits an **impulse** to the brain
- When receptors (such as chemoreceptors) are stimulated, they are **depolarised**
- If the stimulus is very **weak** or **below a certain threshold**, the receptor cells won't be sufficiently depolarised and the sensory neurone will not be activated to send impulses
- If the stimulus is strong enough to increase the **receptor potential above the threshold potential** then the receptor will **stimulate the sensory neurone** to send impulses
- This is an example of the **all-or-none law**
    - An impulse is only transmitted if the **initial stimulus is sufficient** to increase the membrane potential above a **threshold potential**
- Rather than staying constant, **threshold levels in receptors often increase with continued stimulation**, so that a greater stimulus is required before impulses are sent along sensory neurones
- Some receptors, like the chemoreceptors described above, are **specialised cells** that detect a **specific type of stimulus** and affect the sensory neurone's electrical activity. Other receptors are just the **ends of the sensory neurones** (for example, many types of touch receptors).

<u>Transmission of Nerve Impulses</u>

- **Neurones** transmit **electrical impulses**, which travel extremely quickly along the neurone **cell surface membrane** from one end of the neurone to the other
- Unlike a normal electric current, these impulses are **not** a flow of electrons
- These impulses, known as **action potentials**, occur via very **brief changes in the distribution of electrical charge** across the cell surface membrane
    - Action potentials are caused by the **rapid movement of sodium ions and potassium ions** across the membrane of the **axon**

Resting potential

- In a **resting axon** (one that is **not transmitting impulses**), the **inside** of the axon always has a slightly **negative electrical potential** compared to outside the axon
- This **potential difference** is usually about **-70mV** (i.e. the inside of the axon has an electrical potential about 70mV **lower** than the outside)
- This is called the **resting potential**
- Several factors contribute to **maintaining** the resting potential:

Action potentials

- There are **channel proteins** in **axon membrane** that allow **sodium ions**/**potassium ions** to pass through
- These open and close **depending on the electrical potential**/**voltage across the axon membrane** and are closed **voltage-gated channel proteins** (they are **closed** when axon membrane is at its **resting potential**)
- When an **action potential** is stimulated (eg. by a receptor cell) in a neurone, the following steps occur:
    - **Voltage-gated channel proteins** in the axon membrane **open**
    - **Sodium ions** pass **into the axon** down the **electrochemical gradient** (there is a greater concentration of sodium ions outside the axon than inside. The inside of the axon is negatively charged, attracting the positively charged sodium ions)
    - This **reduces the potential difference** across the axon membrane as the **inside** of the axon becomes **less negative** – a process known as **depolarisation**
    - This triggers **more channels** to open, allowing **more sodium ions** to enter and causing **more depolarisation**
    - This is an example of **positive feedback** (a small initial depolarisation leads to greater and greater levels of depolarisation)
    - If the potential difference reaches around **-50mV** (known as the **threshold value**), **many more channels open** and **many more sodium ions enter** causing the inside of the axon to reach a potential of around **+30mV**
    - **An action potential is generated**
    - The depolarisation of the membrane at the site of the first action potential causes **current** to flow to the **next section** of the **axon membrane**, **depolarising it** and causing sodium ion voltage-gated channel proteins to open
    - This triggers the **production of another action potential** in this section of the axon membrane and the process continues
    - In the body, this allows action potentials to begin at one end of an axon and then pass along the entire length of the axon membrane

Repolarisation and the refractory period

- Very shortly (about 1 ms) after an action potential in a section of axon membrane is generated, **all the sodium ion voltage-gated channel proteins in this section close**, stopping any further sodium ions diffusing into the axon
- **Potassium ion voltage-gated channel proteins** in this section of axon membrane now **open**, allowing the diffusion of potassium ions **out of the axon**, down their concentration gradient
- This **returns the potential difference to normal** (about -70mV) – a process known as **repolarisation**
    - There is a short period of **hyperpolarisation**. This is when the potential difference across this section of axon membrane briefly becomes **more negative than the normal resting potential**
- The potassium ion voltage-gated channel proteins then **close** and the sodium ion channel proteins in this section of membrane **become responsive to depolarisation again**
    - Until this occurs, this section of the axon membrane is in a **period of recovery** and is **unresponsive**
    - This is known as the **refractory period**
- During the refractory period, a section of the axon is unresponsive. This ensures that 'new' action potentials are generated ahead (i.e. further along the axon), rather than behind the original action potential. This makes the action potentials discrete events and means the impulse can only travel in one direction. This is essential for the successful and efficient transmission of nerve impulses along neurones.

Speed of Conduction of Impulses

- The **speed of conduction** of an impulse refers to **how quickly the impulse is transmitted** along a neurone
- It is determined by two main factors:
    - the **presence or absence of myelin** (i.e. whether or not the axon is insulated by a myelin sheath)
    - the **diameter of the axon**

Myelination

- In **unmyelinated** neurones, the speed of conduction is **very slow**
- By insulating the axon membrane, the presence of myelin **increases the speed at which action potentials can travel** along the neurone:
    - In sections of the axon that are surrounded by a myelin sheath, **depolarisation** (and the **action potentials** that this would lead to) **cannot occur**, as the myelin sheath **stops** the diffusion of sodium ions and potassium ions
    - Action potentials can only occur at the **nodes of Ranvier** (small uninsulated sections of the axon)
    - The local circuits of current that trigger depolarisation in the next section of the axon membrane exist between the nodes of Ranvier
    - This means the **action potentials 'jump' from one node to the next**
    - This is known as **saltatory conduction**
    - This allows the impulse to travel **much faster** (up to 50 times faster) than in an unmyelinated axon of the same diameter

Diameter

- The speed of conduction of an impulse along neurones with **thicker axons** is **greater** than along those with thinner ones
- Thicker axons have an **axon membrane** with a **greater surface area** over which diffusion of ions can occur
- This **increases the rate of diffusion** of sodium ions and potassium ions, which in turn **increases the rate** at which **depolarisation** and **action potentials** can occur

The Refractory Period

- Very shortly (about 1 ms) **after an action potential** has been generated in a section of the axon membrane, all the **sodium ion voltage-gated channel protein**s in this section **close**. This stops any further sodium ions diffusing into the axon
- **Potassium ion voltage-gated channel proteins** in this section of axon membrane **open**, allowing the diffusion of potassium ions out of the axon, down their concentration gradient
- This gradually **returns the potential difference to normal** (about -70mV) – a process known as **repolarisation**
- Once the resting potential is close to being reestablished, the potassium ion voltage-gated channel proteins close and the **sodium ion channel proteins** in this section of membrane **become responsive to depolarisation again**
- Until this occurs, this section of the axon membrane is in a **period of recovery** and is **unresponsive**
- This is known as the **refractory period**

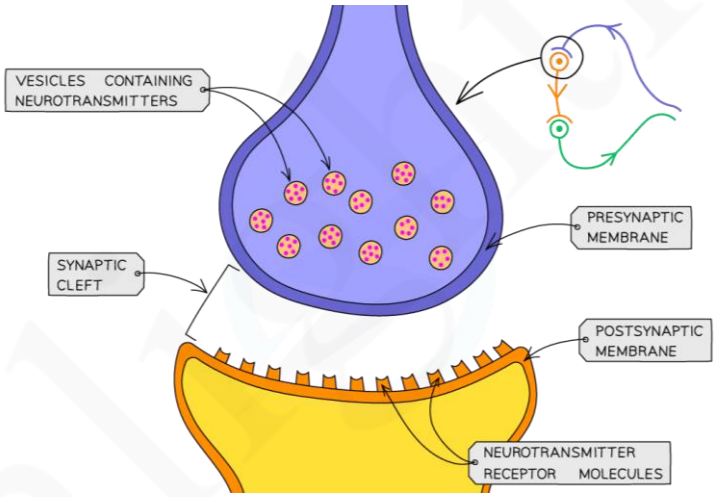
The importance of the refractory period

- The refractory period is **important** for the following reasons:
    - It ensures that action potentials are **discrete events**, stopping them from merging into one another
    - It ensures that **'new' action potentials** are **generated ahead** (i.e. further along the axon), rather than behind the original action potential, as the region behind is 'recovering' from the action potential that has just occurred
    - This means that the impulse can only travel in **one direction**, which is essential for the successful and efficient transmission of nerve impulses along neurones
    - This also means there is a **minimum time between action potentials** occurring at any one place along a neurone
    - The **length** of the refractory period is **key in determining the maximum frequency** at which impulses can be transmitted along neurones (between 500 and 1000 per second)

<u>Cholinergic Synapses</u>

- Where two neurones meet, they do not actually come into physical contact with each other – a very small gap, known as the **synaptic cleft**, separates them

- The ends of the two neurones, along with the synaptic cleft, form a **synapse**

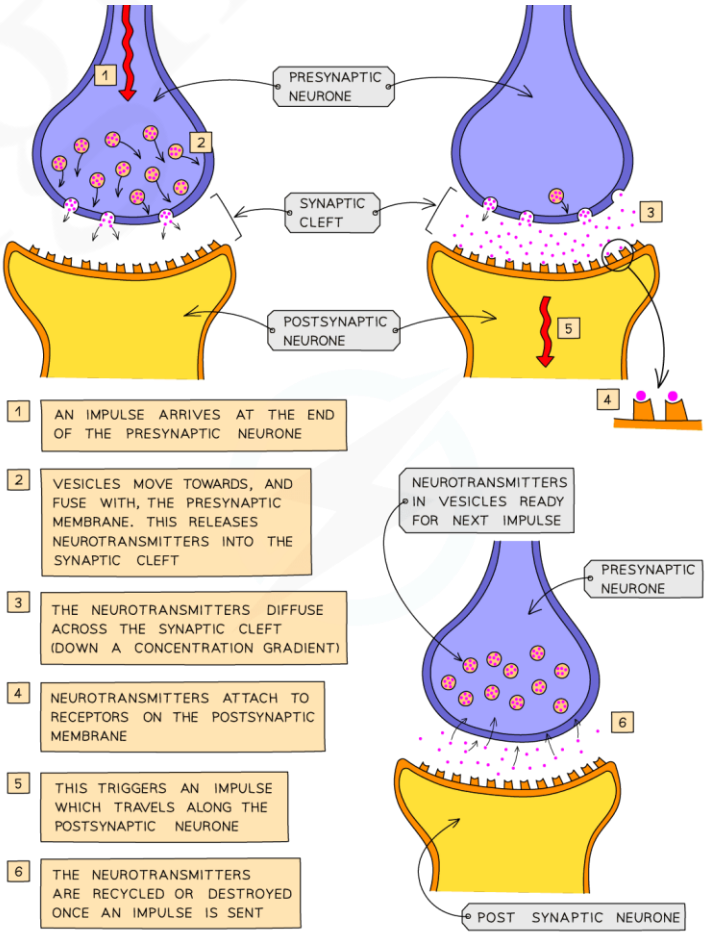


Synaptic transmission – basic mechanism

- Electrical impulses cannot 'jump' across synapses
- When an electrical impulse arrives at the end of the axon on the **presynaptic neurone**, chemical messengers called **neurotransmitters** are released from **vesicles** at the **presynaptic membrane**
- The neurotransmitters **diffuse** across the **synaptic cleft** and **temporarily bind with receptor molecules** on the **postsynaptic membrane**
- This **stimulates** the postsynaptic neurone to generate an electrical **impulse** that then travels down the **axon** of the **postsynaptic neurone**
- The neurotransmitters are then **destroyed** or **recycled** to prevent continued stimulation of the second neurone, which could cause repeated impulses to be sent

Synaptic transmission – detailed mechanism

- There are over 40 different known neurotransmitters
- One of the key neurotransmitters used throughout the nervous system is **acetylcholine (ACh)**
- Synapses that use the neurotransmitter ACh are known as **cholinergic synapses**
- The detailed process of synaptic transmission using ACh is as follows:
    1. The arrival of an **action potential** at the **presynaptic membrane** causes **depolarisation** of the membrane
    2. This **stimulates voltage-gated calcium ion channel proteins** to **open**
    3. **Calcium ions** diffuse down an **electrochemical gradient** from the tissue fluid surrounding the synapse (high concentration of calcium ions) into the **cytoplasm** of the **presynaptic neurone** (low concentration of calcium ions)
    4. This **stimulates ACh-containing vesicles to fuse with the presynaptic membrane**, releasing ACh molecules into the synaptic cleft
    5. The ACh molecules **diffuse** across the synaptic cleft and **temporarily bind to receptor proteins in the postsynaptic membrane**
    6. This causes a **conformational change** in the receptor proteins, which then **open**, **allowing sodium ions to diffuse** down an electrochemical gradient into the cytoplasm of the postsynaptic neurone
    7. The **sodium ions cause depolarisation of the postsynaptic membrane**, re-starting the electrical impulse (that can now continue down the axon of the postsynaptic neurone)
    8. To prevent the sodium ion channels staying permanently open and to stop permanent depolarisation of the postsynaptic membrane, the **ACh molecules are broken down and recycled**
    9. The enzyme **acetylcholinesterase** catalyses the **hydrolysis** of the ACh molecules into **acetate** and **choline**
    10. The **choline is absorbed back into the presynaptic membrane** and reacts with **acetyl coenzyme A** to form **ACh**, which is then packaged into presynaptic vesicles ready to be used when another action potential arrives
- This entire sequence of events takes 5 – 10 ms



Stimulating Contraction in Striated Muscle

- Striated muscle **contracts** when it receives an **impulse** from a **motor neurone** via the **neuromuscular junction**
- When an impulse travelling along the axon of a motor neurone arrives at the **presynaptic membrane**, the action potential causes **calcium ions to diffuse** into the neurone
- This stimulates **vesicles** containing the neurotransmitter **acetylcholine** (ACh) to **fuse** with the presynaptic membrane
- The ACh that is released diffuses across the neuromuscular junction and **binds to receptor proteins** on the **sarcolemma** (surface membrane of the muscle fibre cell)
- This stimulates **ion channels** in the sarcolemma to **open**, allowing **sodium ions** to **diffuse in**
- This **depolarises the sarcolemma,** generating an **action potential** that passes down the **T-tubules** towards the centre of the muscle fibre
- These action potentials cause **voltage-gated calcium ion channel proteins** in the membranes of the **sarcoplasmic reticulum** (which lie very close to the T-tubules) to **open**
- **Calcium ions** diffuse **out of the sarcoplasmic reticulum** (SR) and **into the sarcoplasm** surrounding the myofibrils
- Calcium ions **bind to troponin molecules**, stimulating them to **change shape**
- This causes the **troponin** and **tropomyosin** proteins to **change position** on the thin (actin) filaments
- The **myosin-binding sites are exposed** on the actin molecules
- The process of muscle contraction (known as the sliding filament model) can now begin
- You may have noticed that there are a lot of similarities between the events at the neuromuscular junction and those that occur at cholinergic synapses. A cholinergic synapse is between two neurones, a neuromuscular junction is between a neurone and muscle. Make sure you understand the similarities and differences and don't get confused between the two.

Part of Myofibril	Description
H band	Only thick myosin filaments present
I band	Only thin actin filaments present
A band	Contains areas where only myosin filaments are present and areas where myosin and actin filaments overlap
M line	Attachment for myosin filaments
Z line	Attachment for actin filaments
Sarcomere	The section of myofibril between two Z lines

Ultrastructure of Striated Muscle

- **Striated muscle** makes up the muscles in the body that are attached to the skeleton
- Striated muscle is made up of **muscle fibres**
- A muscle fibre is a **highly specialised** cell-like **unit**:
    - Each muscle fibre contains an organised arrangement of **contractile proteins in the cytoplasm**
    - Each muscle fibre is surrounded by a **cell surface membrane**
    - Each muscle fibre contains **many nuclei** – this is why muscle fibres are not usually referred to as cells
- The different parts of a muscle fibre have different names to the equivalent parts of a normal cell:
    - Cell surface membrane = **sarcolemma**
    - Cytoplasm = **sarcoplasm**
    - Endoplasmic reticulum = **sarcoplasmic reticulum (SR)**
- The sarcolemma has many deep tube-like projections that fold in from its outer surface:
    - These are known as transverse system tubules or **T-tubules**
    - These run **close to the SR**
- The sarcoplasm contains **mitochondria** and **myofibrils**
    - The mitochondria carry out aerobic respiration to generate the **ATP required for muscle contraction**
    - Myofibrils are **bundles of actin and myosin filaments**, which slide past each other during muscle contraction
- The membranes of the SR contain **protein pumps** that transport **calcium ions** into the lumen of the SR

Myofibrils

- Myofibrils are located in the **sarcoplasm**
- Each myofibril is made up of **two types of protein filament:**
    - **Thick** filaments made of **myosin**
    - **Thin** filaments made of **actin**
- These two types of filament are arranged in a particular order, creating different types of **bands** and **line**

Sliding Filament Model of Muscular Contraction

Structure of thick & thin filaments in a myofibril

- The **thick filaments** within a myofibril are made up of **myosin molecules**
    - These are **fibrous protein molecules** with a **globular head**
    - The **fibrous part** of the myosin molecule **anchors** the molecule into the thick filament
    - In the thick filament, many myosin molecules lie next to each other with their **globular heads all pointing away from the M line**
- The **thin filaments** within a myofibril are made up of **actin molecules**
    - These are **globular protein molecules**
    - Many actin molecules link together to form a **chain**
    - **Two** actin chains **twist** together to form one **thin filament**
    - A **fibrous** protein known as **tropomyosin** is twisted around the two actin chains
    - Another protein known as **troponin** is attached to the actin chains at regular intervals

How muscles contract – the sliding filament model

- Muscles cause movement by **contracting**
- During muscle contraction, sarcomeres within myofibrils shorten as the Z discs are pulled closer together
- This is known as the **sliding filament model of muscle contraction** and occurs via the following process:
    - An **action potential arrives** at the neuromuscular junction
    - **Calcium ions** are **released** from the **sarcoplasmic reticulum** (SR)
    - Calcium ions **bind to troponin molecules**, stimulating them to **change shape**
    - This causes **troponin** and **tropomyosin** proteins to **change position** on the actin (thin) filaments
    - **Myosin binding sites are exposed** on the actin molecules
    - The **globular heads of the myosin molecules bind** with these sites, forming cross-bridges between the two types of filament
    - The **myosin heads move and pull the actin filaments towards the centre of the sarcomere**, causing the muscle to contract a very small distance
    - **ATP hydrolysis** occurs at the myosin heads, providing the **energy** required for the myosin heads to **release** the actin filaments
    - The myosin heads move back to their **original positions** and bind to **new binding sites** on the actin filaments, **closer to the Z disc**
    - The myosin heads move again, pulling the actin filaments **even closer the centre of the sarcomere**, causing the sarcomere to **shorten** once more and pulling the Z discs closer together
    - The myosin heads hydrolyse ATP once more in order to **detach** again
    - As long as troponin and tropomyosin are not blocking the myosin-binding sites and the muscle has a supply of ATP, this process **repeats** until the muscle is **fully contracted**

<u>Control & Communication in Plants</u>

- Plants possess **communication systems** that enable them to coordinate different parts of their bodies
- The Venus flytrap is a **carnivorous plant** that gets its supply of nitrogen compounds by **trapping and digesting small animals** (mainly insects)
- The specialised leaf is divided into **two lobes** either side of a midrib
- The inside of the lobes is **red** and has **nectar-secreting glands** on the edges to attract insects
- Each lobe has three **stiff sensory hairs** that respond to being touched
- If an insect touches one of these hairs with enough force, **action potentials are stimulated**, which then travel very fast across the leaf
- These action potentials cause the two lobes to **fold together** along the midrib, capturing the insect

How the closure of the trap is achieved

- If one of the **sensory hairs** is touched with **enough force**, **calcium ion channels** in cells at the base of the hair are **activated**
- When these channels **open**, calcium ions flow in and generate a **receptor potential**
- If two of the sensory hairs are stimulated within a period of about 30 seconds, or one hair is stimulated twice within this period, **action potentials** will travel across the trap and cause it to close
- When the trap is open the lobes of the leaf are convex in shape but when the trap is triggered, the lobes become concave, bending downwards and causing the trap to shut – it is thought this occurs as a result of a release of elastic tension in the cell walls
- Sealing the trap requires **ongoing activation** of the sensory hairs – the prey trapped inside provides this ongoing stimulation, **generating further action potentials**
- Further stimulation of the sensory hairs stimulate **calcium ions** to enter **gland cells** where they stimulate the **exocytosis of vesicles containing digestive enzymes**
- The trap then stays shut for up to a week to allow the prey to be digested and the nutrients from it to be absorbed by the plant

<u>The Role of Auxin in Elongation Growth</u>

- **Plant hormones** (also known as **plant growth regulators**) are responsible for most communication within plants
- **Auxins** are a type of plant growth regulator that influence many aspects of growth, including **elongation growth** which determines the **overall length of roots and shoots**
- The principle chemical in the group of auxins made by plants is **IAA** (indole 3-acetic acid) and this chemical is often simply referred to as **'auxin'**
- Auxin (IAA) is **synthesised in the growing tips of roots and shoots** (i.e. in the **meristems**, where cells are **dividing**)
- Growth in these meristems occurs in three stages:
    - cell division by mitosis
    - cell elongation by absorption of water
    - cell differentiation
- **Auxin** (IAA) is involved in controlling **growth by elongation**

Controlling growth by elongation

- Auxin molecules bind to a **receptor protein** on the cell surface membrane
- Auxin stimulates **ATPase** proton pumps to pump **hydrogen ions** from the cytoplasm **into the cell wall** (across the cell surface membrane)
- This **acidifies** the cell wall (lowers the pH of the cell wall)
- This activates proteins known as **expansins**, which **loosen the bonds between cellulose microfibrils**
- At the same time, **potassium ion channels** are stimulated to **open**
- This leads to an **increase in potassium ion concentration in the cytoplasm**, which **decreases the water potential of the cytoplasm**
- This causes the cell to **absorb water** by **osmosis** (water enters the cell through **aquaporins**)
- This **increases** the **internal pressure** of the cell, causing the **cell wall** to **stretch** (made possible by expansin proteins)
- The cell **elongates**

The Role of Gibberellin in Germination of Barley

- **Gibberellins** are a type of plant growth regulator involved in controlling **seed germination** and **stem elongation**
- When a barley seed is shed from the parent plant, it is in a state of dormancy (contains very little water and is metabolically inactive)
- This allows the seed to survive harsh conditions until the conditions are right for successful germination (eg. the seed can survive a cold winter until temperatures rise again in spring)
- The barley seed contains:
    - An **embryo** – will grow into the new plant when the seed germinates
    - An **endosperm** – a starch-containing energy store surrounding the embryo
    - An **aleurone layer** – a protein-rich layer on the outer edge of the endosperm
- When the conditions are right, the barley seed starts to **absorb water** to begin the process of **germination**
- This stimulates the **embryo** to produce **gibberellins**
- Gibberellin molecules diffuse into the **aleurone layer** and stimulate the cells there to **synthesise amylase**
    - In barley seeds, it has been shown that gibberellin does this by **regulating genes involved in the synthesis of amylase**, causing an **increase in the transcription of mRNA coding for amylase**
- The amylase **hydrolyses starch** molecules in the **endosperm**, producing soluble **maltose** molecules
- The maltose is **converted to glucose** and transported to the **embryo**
- This glucose can be **respired** by the embryo, providing the embryo with the **energy** needed for it to **grow**

16. Inheritance

Haploid & Diploid Cells

- A diploid cell is a cell that contains **two complete sets of chromosomes (2n)**
    - These chromosomes contain the DNA necessary for protein synthesis and cell function
    - Nearly all cells in the human body are **diploid** with 23 **pairs** (46) of chromosomes in their nucleus
- Haploid cells contain **one complete set of chromosomes (n)**
    - In other words they have half the number of chromosomes compared to diploid cells
    - Humans have **haploid** cells that contain 23 chromosomes in their nucleus
    - These haploid cells are called **gametes** and they are involved in sexual reproduction
    - For humans they are the female egg and the male sperm
- Haploidy and diploidy are terms that can be applied to cells across different species
    - They describe the number of **sets** of chromosomes, not the total number of chromosomes
- Red blood cells are an exception when it comes to chromosome number as they don't have a nucleus!

The Need for Reduction Division during Meiosis

- During fertilization the **nuclei of gametes fuse together** to form the **nucleus of the zygote**
- Both gametes must contain the correct number of chromosomes in order for the zygote to be viable. If a zygote has too many or too few chromosomes it may not survive
- For a diploid zygote this means that the **gametes must be haploid**
    - n + n = 2n
- Meiosis produces haploid gametes during sexual reproduction
- The first cell division of meiosis is a **reduction division**
    - This is a **nuclear division that reduces the chromosome number** of a cell
    - In humans the chromosome number is reduced from 46 (diploid) to 23 (haploid)
- The reduction in chromosome number during meiosis ensures the gametes formed are haploid

Homologous Chromosomes

- In **diploid cells there are two complete sets of chromosomes in the nucleus**
- **Chromosomes have a characteristic shape**
    - **They have a fixed length**
    - **The position of the centromere is in a particular location**
- These characteristic features allow for each chromosome to be identified in a photomicrograph
- In photomicrographs chromosomes are often grouped into their homologous pairs
- **Homologous chromosomes**:
    - Carry the **same genes in the same positions**
    - Are the **same shape**
- During fertilization a diploid **zygote is formed**
    - **In a zygote one chromosome of each homologous pair comes from the female gamete and the other comes from the male gamete**
- Having the same genes in the same order helps homologous chromosomes line up alongside each other during meiosis
- Although homologous pairs of chromosomes contain the same genes in the same order they don't necessarily carry the same alleles (form) of each gene!

Meiosis in Animal & Plant Cells

- Meiosis is a form of **nuclear division** that results in the **production of haploid cells** from diploid cells
- It produces **gametes in plants and animals that are used in sexual reproduction**
- It has many similarities to mitosis however it has **two divisions**: meiosis I and meiosis II
- Within each division there are the following stages: prophase, metaphase, anaphase and telophase

Prophase I

- **DNA condenses** and becomes visible as chromosomes
- DNA replication has already occurred so each chromosome consists of **two sister chromatids** joined together by a centromere
- The chromosomes are arranged side by side in **homologous pairs**
    - A pair of homologous chromosomes is called a bivalent
- As the homologous chromosomes are very close together the **crossing over** of non-sister chromatids may occur. The point at which the crossing over occurs is called the **chiasma** (chiasmata; plural)
- In this stage centrioles migrate to opposite poles and the **spindle is formed**
- The **nuclear envelope breaks down** and the nucleolus disintegrates

Metaphase I

- The **bivalents line up along the equator of the spindle**, with the spindle fibres attached to the centromeres

Anaphase I

- The homologous pairs of chromosomes are separated as **microtubules pull whole chromosomes to opposite ends** of the spindle
- The centromeres do not divide

Telophase I

- The chromosomes arrive at opposite poles
- Spindle fibres start to break down
- **Nuclear envelopes form around the two groups of chromosomes** and nucleoli reform
- Some plant cells go straight into meiosis II without reformation of the nucleus in telophase I

Cytokinesis

- This is when the **division of the cytoplasm** occurs
- Cell organelles also get distributed between the two developing cells
- In animal cells: the **cell surface membrane** pinches inwards creating a **cleavage furrow** in the middle of the cell which contracts, dividing the cytoplasm in half
- In plant cells, vesicles from the Golgi apparatus gather along the equator of the spindle (the cell plate). The **vesicles** merge with each other to form the **new cell surface membrane** and also secrete a layer of calcium pectate which becomes the **middle lamella.** Layers of cellulose are laid upon the middle lamella to form the primary and secondary walls of the cell
- The end product of cytokinesis in meiosis I is **two complete haploid cells**

Second division of Meiosis : Meiosis II

- There is no interphase between meiosis I and meiosis II so the DNA is not replicated
- The second division of meiosis is almost identical to the stages of mitosis
- Prophase II
    - The **nuclear envelope breaks down** and **chromosomes condense**
    - A **spindle forms at a right angle to the old one**

Metaphase II

- **Chromosomes** line up in a **single file along the equator** of the spindle

Anaphase II

- Centromeres divide and individual **chromatids are pulled to opposite poles**
- This creates **four groups of chromosomes** that have half the number of chromosomes compared to the original parent cell

Telophase II

- **Nuclear membranes form** around each group of chromosomes

Cytokinesis

- **Cytoplasm divides** as new cell surface membranes are formed **creating four haploid cells**

❖ Understanding the difference between chromosomes and chromatids can be difficult. We count chromosomes by the **number of centromeres present**. So when the 46 chromosomes duplicate during interphase and the **amount of DNA in the cell doubles** there are still only 46 chromosomes present because there are still only 46 centromeres present. However, there are now 92 chromatids, which are strands of replicated chromosomes.

<u>Identifying the Stages of Meiosis</u>

- Cells undergoing **meiosis can be observed and photographed using specialised microscopes**
- The different stages of meiosis have distinctive characteristics meaning they can be identified from photomicrographs or diagrams

Meiosis I or Meiosis II

- **Homologous chromosomes** pair up side by side in meiosis I only
- This means if there are **pairs of chromosomes** in a diagram or photomicrograph **meiosis I** must be occurring
- The number of cells forming can help distinguish between meiosis I and II
- If there are **two new cells** forming it is **meiosis I** but if there are **four new cells** forming it is **meiosis II**

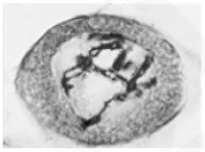
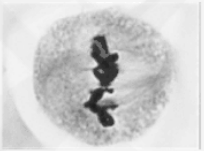
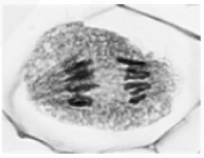
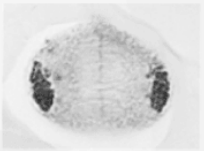
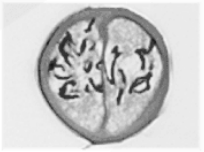
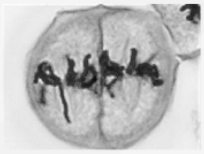
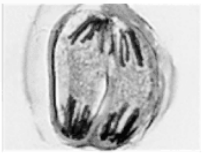
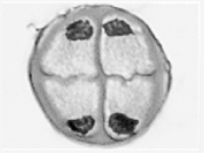
The distinguishing features at each stage of Meiosis I

- Prophase I: **Homologous pairs** of chromosomes are visible
- Metaphase I: Homologous pairs are lined up **side by side** along the **equator** of spindle
- Anaphase I: **Whole chromosomes** are being pulled to opposite **poles** with **centromeres intact**
- Telophase I: There are **2 groups** of condensed chromosomes around which nuclei membranes are forming
- Cytokinesis: Cytoplasm is dividing and **cell membrane is pinching inwards** to form **two cells**

The distinguishing features at each stage of Meiosis II

- Prophase II: **Single whole chromosomes** are visible
- Metaphase II: Single whole chromosomes are lined up along the **equator** of the spindle in **single file** (at 90 degree angle to the old spindle)
- Anaphase II: **Centromeres divide** and **chromatids** are being pulled to opposite **poles**
- Telophase II: Nuclei are forming around the **4 groups** of condensed chromosomes
- Cytokinesis: Cytoplasm is dividing and **four haploid cells** are forming

❖ For metaphase remember **M for the middle** of the spindle and cell which is where the chromosomes will be lined up. For anaphase remember **A for away** from the middle to the poles, which is where the chromosomes / chromatids are being pulled.

Stage	Micrograph
Prophase I One group of chromosomes become visible as DNA condenses	
Metaphase I Chromosome pairs are located along the middle of the spindle	
Anaphase I Whole chromosomes are being pulled away from the middle	
Telophase I There are two groups of chromosomes at each pole. Nucleus is reforming and cytoplasm is pinching in	
Prophase II Two groups of chromosomes are visible as DNA condenses	
Metaphase II Chromosomes line up along middle of the spindles in single file	
Anaphase II Chromatids are pulled away from the middle of the spindles	
Telophase II There are four groups of chromosomes and cytoplasm is pinching in	

Meiosis: Sources of Genetic Variation

- Having **genetically different offspring** can be **advantageous** for natural selection
- **Meiosis** has several mechanisms that **increase the genetic diversity of gametes** produced
- Both crossing over and independent assortment (random orientation) result in **different combinations of alleles** in gametes

Crossing over

- Crossing over is the process by which **non-sister chromatids exchange alleles**
- Process:
    - During meiosis I homologous chromosomes pair up and are in very close proximity to each other
    - The **non-sister chromatids can cross over** and get entangled
    - These crossing points are called **chiasmata**
    - The entanglement places stress on the DNA molecules
    - As a result of this a section of chromatid from one chromosome may **break and rejoin** with the chromatid from the other chromosome
- This swapping of alleles is significant as it can result in a **new combination of alleles on the two chromosomes**
- There is usually at least one, if not more, chiasmata present in each bivalent during meiosis
- Crossing over is more likely to occur further down the chromosome away from the centromere

Independent assortment

- Independent assortment is the production of **different combinations of alleles in daughter cells** due to the **random alignment of homologous pairs along the equator of the spindle** during metaphase I
- The different combinations of chromosomes in daughter cells increases genetic variation between gametes
- In prophase I homologous chromosomes pair up and in metaphase I they are pulled towards the equator of the spindle
    - **Each pair can be arranged with either chromosome on top**, this is completely random
    - The **orientation of one homologous pair is independent** / unaffected by the orientation of any other pair
- The homologous chromosomes are then **separated** and pulled apart to different poles
- The combination of alleles that end up in each daughter cell depends on how the pairs of homologous chromosomes were lined up
- To work out the number of different possible chromosome combinations the formula 2^n can be used, where n corresponds to the number of chromosomes in a haploid cell
- For humans this is 2^{23} which calculates as 8 324 608 different combinations

❖ It is also worth remembering that genetic variation can occur on an even smaller scale than chromosomes. **Mutations** can occur within genes. A random mutation that takes place during DNA replication can lead to the production of new alleles and increased genetic variation.

Fusion of Gametes

- **Meiosis** creates genetic variation **between the gametes** produced by an individual through crossing over and independent assortment
- This means each gamete carries substantially **different alleles**
- During fertilization any male gamete can fuse with any female gamete to form a zygote
- This **random fusion of gametes** at fertilization creates genetic variation **between zygotes** as each will have a unique combination of alleles
- There is an almost zero chance of individual organisms resulting from successive sexual reproduction being genetically identical

❖ These sources of genetic variation explain why relatives can differ so much from each other. Even with the same parents, individuals can be genetically distinct due to the processes outlined above.

Key Terms in Genetics

Genes & alleles

- The DNA contained within chromosomes is essential for cell survival
- Every **chromosome** consists of a long DNA molecule which **codes for several different proteins**
- A length of DNA that codes for a single polypeptide or protein is called a **gene**
- The **position of a gene** on a chromosome is its **locus** (plural: loci)
- Each gene can exist in two or more different forms called **alleles**
- Different alleles of a gene have slightly **different nucleotide sequences** but they still occupy the **same position** (locus) on the chromosome

Genotype & phenotype

- The chromosomes of eukaryotic cells occur in **homologous pairs** (there are two copies of each chromosome)
- As a result cells have **two copies of every gene**
- As there are two copies of a gene present in an individual that means there can be **different allele combinations within an individual**
- The **genotype** of an organism refers to the **alleles of a gene** possessed by that individual. The different alleles can be represented by letters
- When the two allele copies are identical in an individual they are said to be **homozygous**
- When the two allele copies are different in an individual they are said to be **heterozygous**
- The genotype of an individual affects their phenotype
- A phenotype is the **observable characteristics** of an organism

Dominance

- Not all alleles affect the phenotype in the same way
- Some alleles are **dominant**: they are **always expressed** in the phenotype
    - This means they are expressed in both heterozygous and homozygous individuals
- Other are **recessive**: they are only **expressed** in the phenotype **if no dominant allele** is present
    - This means that it is only expressed when present in a homozygous individual

Codominance

- Sometimes **both alleles can be expressed** in the phenotype at the same time
- This is known as codominance
- When an individual is heterozygous they will express both alleles in their phenotype
- When writing the genotype for codominance the gene is symbolised as the capital letter and the alleles are represented by different superscript letters, for example $\mathbf{I^A}$

F_1, F_2 & test crosses

- When a **homozygous dominant** individual is crossed with a **homozygous recessive** individual the offspring are called the **F_1 generation**
    - All of the F_1 generation are **heterozygous**
- If two individuals from the F_1 generation are crossed, the offspring produced are called the F_2 generation
- A **test cross** can be used to try and **deduce the genotype of an unknown individual** that is expressing a dominant phenotype
    - The individual in question is crossed with an individual that is expressing **recessive phenotype**
    - The resulting phenotypes of the offspring provide sufficient information to suggest the genotype of the unknown individual
    - If there are any offspring expressing the recessive phenotype then the unknown individual must have a heterozygous genotype

Linkage

- There are two types of linkage in genetics: **sex linkage** and **autosomal linkage**
- Sex linkage:
    - There are two sex chromosomes: X and Y
    - Women have two copies of the X chromosome (XX) whereas men have one X chromosome and one shorter Y chromosome (XY)
    - Some **genes are found on a region of a sex chromosome that is not present on the other** sex chromosome
    - As the **inheritance of these genes is dependent on the sex** of the individual they are called sex-linked genes
    - Most often sex-linked genes are found on the longer X chromsome
    - Haemophilia is well known example of a sex-linked disease
    - Sex-linked genes are represented in the genotype by writing the alleles as superscript next to the sex chromosome. For example a particular gene that is found only on the X chromosome has two alleles **G** and **g**. The genotype of a heterozygous female would be written as X^GX^g. A males genotype would be written as X^GY
- Autosomal linkage:
    - This occurs on the autosomes (any chromosome that isn't a sex chromosome)
    - Two or more genes on the same chromosome **do not assort independently** during meiosis
    - These genes are linked and they stay together in the original parental combination

❖ When referring to the different alleles be careful about your notation. When describing a dominant allele use capitals (for example allele **B**) and when describing a recessive allele use lower case (for example allele **b**). Be careful when choosing the letters to represent the alleles when writing the genotype. Use letters that are easy to distinguish between the capital and the lower case (eg. B and b).

Predicting Inheritance: Monohybrid Crosses

- Monohybrid inheritance looks at how the **alleles for a single gene are passed on from one generation to the next**
- Known information about the genotypes, phenotypes and the process of meiosis are used to make **predictions** about the phenotypes of offspring that would result from specific breeding pairs
- When two individuals sexually reproduce there is an equal chance of either allele from their homologous pair making it into their gametes and subsequently the nucleus of the zygote
    - This means there is an **equal chance of the zygote inheriting either allele from their parent**
- Genetic diagrams are often used to present this information in a clear and precise manner so that predictions can be made
    - These diagrams include a characteristic table called a **Punnett square**
- The predicted genotypes that genetic diagrams produce are all based on **chance**
    - There is no way to predict which gametes will fuse so sometimes the observed or real-life results can differ from the predictions

Codominance

- When working with codominant alleles the genetic diagrams can be constructed in a similar way, however the genotypes are represented using a capital letter for the gene and superscript letters for the alleles (eg. I^AI^A)
- There will be **more possible phenotypes** and so the predicted ratios will be different

Sex-linkage

- Sex-linked genes are only **present on one sex chromosome and not the other**
- This means the sex of an individual affects what alleles they pass on to their offspring through their gametes
- If the gene is on the X chromosome **males (XY) will only have one copy** of the gene, whereas females (XX) will have two
- There are **three phenotypes** for **females** – normal, carrier and has the disease, whereas **males** have only **two** phenotypes – normal or has the disease

Predicting Inheritance: Dihybrid Crosses

- Monohybrid crosses look at how the alleles of one gene transfer across generations
- Dihybrid crosses look at how the alleles of **two genes** transfer across generations
- The genetic diagrams for both types of crosses are very similar
- There are several more genotypes and phenotypes involved
- When writing the different genotypes write the two alleles for one gene, followed immediately by the two alleles for the other gene. **Do not mix up the alleles from the different genes**
    - If there was a gene with alleles **Y** and **y** and another gene with alleles **G** and **g** an example genotype for an individual would be **YYGg**

Autosomal linkage

- Dihybrid crosses and their predictions rely on the assumption that the genes being investigated behave independently of one another during meiosis
- **Not all genes assort independently during meiosis**
- Some genes which are located on the **same chromosome** display autosomal linkage and **stay together in the original parental combination**

- Linkage between genes affects how parental alleles are passed onto offspring through the gametes
- When writing linked genotypes it can be easier to keep the linked alleles within a bracket
    - For example an individual has the genotype **FFGG** however if there is linkage between the two genes then it would be written as (**FG**)(**FG**)

Epistasis

- In some cases **one gene can affect the expression of another gene**
- Epistasis: when two genes on different chromosomes **affect the same feature**
- If epistasis is present it needs to be taken into account when determining the phenotypes of individuals
- The whole combination of alleles from the different genes dictates the phenotype

Test crosses involving autosomal linkage predict solely **parental type** offspring (offspring that have the same combination of characteristics as their parents). However in reality **recombinant** offspring (offspring that have a different combination of characteristics to their parents) are often produced. This is due to the **crossing over** that occurs during meiosis. The crossing over and exchanging of genetic material **breaks the linkage** between the genes and recombines the characteristics of the parents.

Predicting Inheritance: Test Crosses

- A test cross can be used to **deduce the genotype of an unknown individual that is expressing a dominant phenotype**
- The individual in question is crossed with an individual that is expressing the **recessive phenotype**
- This is because an individual with a recessive phenotype has a known genotype
- The resulting phenotypes of the offspring provides sufficient information to suggest the genotype of the unknown individual

Results

- For a monohybrid test cross:
    - If **no** offspring exhibit the recessive phenotype then the unknown genotype is **homozygous dominant**
    - If **at least one** of the offspring exhibit the recessive phenotype then the unknown genotype is **heterozygous**
- For a dihybrid test cross:
    - If no offspring exhibit the recessive phenotype for either gene then the unknown genotype is **homozygous dominant** for both genes
    - If at least one of the offspring exhibit the recessive phenotype for one gene but not the other, then the unknown genotype is **heterozygous for one gene and homozygous dominant for the other**
    - If at least one of the offspring exhibit the recessive phenotype for both genes then the unknown genotype is **heterozygous for both** genes

Predicting Inheritance: Chi-squared Test

- The **difference between expected and observed results** in experiments can be statistically significant or insignificant (happened by chance)
- If the difference between results is statistically significant it can suggest that **something else is happening** in the experiment that isn't being accounted for
    - For example linkage between genes

- A statistical test called the **chi-squared test** determines whether there is a significant difference between the observed and expected results in an experiment
- The chi-squared test is completed when the data is categorical (data that can be grouped)

Calculating chi-squared values

- Obtain the expected and observed results for the experiment
- Calculate the difference between each set of results
- Square each difference (as it is irrelevant whether the difference is positive or negative)
- Divide each squared difference by the expected value and get a sum of these answers to obtain the chi-squared value

$$\sum \frac{(O-E)^2}{E}$$

$$\Sigma = sum\ of$$
$$O = observed\ value$$
$$E = expected\ value$$

Analysing chi-squared values

- To work out what the chi-squared value means, a table that relates chi-squared values to probabilities is used
- If the chi-squared value r**epresents a larger probability** than the **critical probability** then it can be stated that the differences between the expected and observed results are **due to chance**
- If it represents a **smaller probability** than the critical probability then the differences in results are **significant** and something else may be causing the differences
- To determine the critical probability biologists generally use a probability of **05** (they allow that chance will cause five out of every 100 experiments to be different)
- The number of comparisons made must also be taken into account when determining the critical probability. This is known as the **degrees of freedom**

<u>Genes, Proteins & Phenotype</u>

- A gene can affect a phenotype of an organism
    - A gene codes for a single protein
    - The protein affects the phenotype through a particular mechanism
- The phenotype of an individual can also be affected by the **environment**

TYR gene & albinism

- Humans with albinism **lack the pigment melanin** in their skin, hair and eyes
- This causes them to have very pale skin, very pale hair and pale blue or pink irises in the eyes
- There is a metabolic pathway for producing melanin:
    1. The amino acid tyrosine is converted to DOPA by the **enzyme tyrosinase**

2. DOPA is converted to dopaquinone again by the **enzyme tyrosinase**
3. Dopaquinone is converted to melanin

tyrosine → DOPA → dopaquinone → melanin

- A **gene called *TYR*** located on chromosome 11 codes for the enzyme tyrosinase
- There is a **recessive allele** for the gene *TYR* that causes a lack of enzyme tyrosinase or the presence of inactive tyrosinase
- Without the tyrosinase enzyme tyrosine can not be converted into melanin

HBB gene & sickle cell anaemia

- **Sickle cell anaemia** is a condition that causes individuals to have frequent infections, episodes of pain and anaemia
- Humans with sickle cell anaemia have **abnormal haemoglobin** in their red blood cells
- β-globin is a polypeptide found in haemoglobin that is coded for by the **gene *HBB*** which is found on chromosome 11
- There is an **abnormal allele** for the gene *HBB* which produces a slightly different amino acid sequence to the normal allele
    - The change of a single base in the DNA of the abnormal allele results in an amino acid substitution (the base sequence CTT is replaced by CAT)
    - This **change in amino acid sequence** (the amino acid Glu is replaced with Val) results in an **abnormal β-globin** polypeptide
- The abnormal β-globin in haemoglobin affects the structure and **shape of the red blood cells**
    - They are pulled into a half moon shape
    - They are **unable to transport oxygen** around the body
    - They stick to each other and clump together blocking capillaries
- A homozygous individual that has two abnormal alleles for the *HBB* gene produces only sickle cell haemoglobin
    - They have sickle cell anaemia and suffer from the associated symptoms
- A heterozygous individual that has one normal allele and one abnormal allele for the *HBB* gene will produce some normal haemoglobin and some sickle cell haemoglobin
    - They are a carrier of the allele
    - They may have no symptoms

F8 gene & haemophilia

- **Factor VIII** is a coagulating agent that plays an essential role in blood clotting
- The gene ***F8*** codes for the Factor VIII protein
- There are **abnormal alleles** of the *F8* gene that result in:
    - Production of abnormal forms of factor VIII

- Less production of normal factor VIII
- No production of factor VIII

- A **lack of normal factor VIII prevents normal blood clotting** and causes the condition **haemophilia**
- The *F8* gene is located on the X chromosome
  - This means *F8* is a **sex-linked gene**
  - Haemophilia is a sex-linked condition
  - If males have an abnormal allele they will have the condition as they have only one copy of the gene
  - Females can be heterozygous for the *F8* gene and not suffer from the condition but act as a carrier

HTT gene & Huntington's disease

- Huntington's disease is a genetic condition that develops as a person ages
- Usually a person with the disease will not show symptoms until they are 30 years old +
- An individual with the condition experiences **neurological degeneration**; they lose their ability to walk, talk and think
- The disease is ultimately fatal
- It has been found that individuals with Huntington's disease have **abnormal alleles of the *HTT* gene**
  - The *HTT* gene codes for the protein **huntingtin** which is involved in neuronal development
  - People that have a large number (>40) of **repeated CAG triplets** present in the nucleotide sequence of their *HTT* gene suffer from the disease
- The **abnormal allele is dominant** over the normal allele
  - If an individual has one abnormal allele present they will suffer from the disease

Gene	Key molecules involved	Genotype	Phenotype
TYR	Tyrosinase enzyme and melanin	Homozygous for abnormal allele	Albinism
HBB	Haemoglobin	Heterozygous	Carrier
		Homozygous for abnormal allele	Sickle cell anaemia
F8	Factor VIII	Homozygous for abnormal allele	Haemophilia
		Heterozygous female	Carrier
HTT	Huntingtin	Homozygous for abnormal allele	Huntington's disease
		Heterozygous	Huntington's disease

<u>The Role of Gibberellin in Stem Elongation</u>

- In some plants species their height is partially controlled by their genes
- The ***Le* gene** dictates the height of some plants
- It has two alleles: *Le* and *le*

    - The dominant allele ***Le*** **produces tall plants** when present
    - The recessive allele ***le*** **produces shorter plants** when present (in a homozygous individual)
- The gene regulates the production of an **enzyme** that is involved in a pathway that forms **active gibberellin** GA_1
- Active gibberellin is a hormone that helps plants grow by **stimulating cell division and elongation** in the stem
- The recessive allele *le* results in **non-functional enzyme**
    - It is only **one nucleotide different** to the dominant allele
    - This causes a single amino acid substitution (threonine -> alanine) in the primary structure of the enzyme
    - This change in primary structure occurs at the **active site** of the enzyme, making it non-functional
- Without this enzyme **no active gibberellin is formed** and plants are unable to grow tall
- Plants that are **homozygous** for the recessive allele *le* are **dwarves**
- Some farmers apply active gibberellin to shorter plants to stimulate growth

<u>Gene Control</u>

- The nucleus of every cell in the human body contains the same genes
    - However **not every gene is expressed** in every cell
    - Not all of these genes are expressed all the time
- There are several mechanisms that exist within cells to make sure the **correct genes are expressed in the correct cell** at the right time
    - They involve regulatory genes

Structural & regulatory genes

- A structural gene codes for a protein that has a **function within a cell**
    - For example, the *F8* gene codes for the protein Factor VIII involved in blood clotting
- A regulatory gene codes for a protein that helps to **control the expression** of another gene
- Structural and regulatory genes that work together are usually found close together

Inducible & repressible enzymes

- Some genes code for proteins that form enzymes
- Some enzymes are required all the time and some are required only at specific times
- The expression of enzyme-producing genes can be controlled

- **Inducible** enzymes are only synthesized when their substrate is present
    - The presence of the **substrate induces** the synthesis of of the enzyme by causing the transcription of the gene for the enzyme to start
- **Repressible** enzymes are synthesized as normal until a **repressor protein** binds to an operator
    - The presence of the repressor protein **represses** the synthesis of the enzyme by causing the transcription of the gene for the enzyme to stop
- Controlling when enzymes are synthesized can be beneficial for cells as it **stops materials and energy being wasted**
    - For example, using materials and energy to synthesize an enzyme when its substrate is not present and it can't carry out its function would be highly wasteful

<u>Gene Control: Lac Operon</u>

- Regulatory genes control structural genes and their levels of protein production
- Regulatory genes sometimes have control over several structural genes at once
- Structural genes in **prokaryotes** can form an **operon**: a group or a cluster of genes that are controlled by the same promoter
- The **lac operon** found in some bacteria is one of the most well-known of these
- The lac operon controls the production of the enzyme **lactase** (also called β-galactosidase) and two other structural proteins
- Lactase breaks down the substrate lactose so that it can be used as an energy source in the bacterial cell
- It is an **inducible** enzyme that is **only synthesized when lactose is present**
- This helps prevent the bacteria from wasting energy and materials

Structure of the lac operon

- The components of the lac operon are found in the following order:
    - Promoter for structural genes
    - Operator
    - Structural gene ***lacZ*** that codes for **lactase**
    - Structural gene *lacY* that codes for permease (allows lactose into the cell)
    - Structural gene *lacA* that codes for transacetylase
- Located to the left (upstream) of the lac operon on the bacterium's DNA there is also the:
    - Promoter for regulatory gene
    - Regulatory gene ***lacI*** that codes for the ***lac*** **repressor protein**
- The lac repressor protein has **two binding sites** that allow it to bind to the **operator** in the lac operon and also to **lactose** (the **effector** molecule)
    - When it binds to the operator it **prevents the transcription** of the structural genes as RNA polymerase cannot attach to the promoter
    - When it binds to lactase the shape of the repressor protein distorts and it can **no longer bind to the operator**

When lactose is absent

- The following processes take place when lactose is **absent** in the medium that the bacterium is growing in:
    - The regulatory gene is transcribed and translated to produce *lac* repressor protein

- The *lac* **repressor protein binds to the operator** region upstream of *lacZ*
- Due to the presence of the repressor protein **RNA polymerase is unable to bind to the promoter region**
- Transcription of the structural genes does not take place
- **No lactase enzyme is synthesized**

When lactose is present

- The following processes take place when lactose is present in the medium that the bacterium is growing in:
  - There is an uptake of lactose by the bacterium
  - The **lactose binds to the second binding site on the repressor** protein, distorting its shape so that it cannot bind to the operator site
  - **RNA polymerase is then able to bind to the promoter region** and transcription takes place
  - The mRNA from all three structural genes is translated
  - **Enzyme lactase is produced** and lactose can be broken down and used for energy by the bacterium

In this mechanism an effector molecule also binds to a repressor protein produced by a regulatory gene. However this binding actually **helps the repressor bind to the operator region** and prevent transcription of the structural genes. So it's the opposite of the lac operon: when there is **less of the effector** molecule, the repressor protein cannot bind to the operator region and **transcription of the structural genes goes ahead,** meaning the enzyme is produced.

<u>Gene Control: Transcription Factors</u>

- Prokaryotes use operons to control the expression of genes in cells
- Eukaryotes also use **transcription factors** to control gene expression
- A transcription factor is a protein that **controls the transcription of genes** by binding to a specific region of DNA
- They ensure that genes are being expressed in the correct cells, at the correct time and to the right level
- It is estimated that ~10% of human genes code for transcription factors
  - There are several types of transcription factors that have varying effects on gene expression
  - This is still a relatively young area of research and scientists are working hard to understand how all the different transcription factors function
  - Transcription factors allow organisms to respond to their environment
  - Some hormones achieve their effect via transcription factors

How transcription factors work

- Some transcription factors **bind to the promoter** region of a gene
  - This binding can either **allow or prevent the transcription** of the gene from taking place
- The presence of a transcription factor will either **increase or decrease the rate** of transcription of a gene

- For example, **PIF** is a transcription factor found in plants that activates the transcription of the amylase gene

Gene Control: Gibberellin

- Plant cells use transcription factors in a similar way to animal cells
- Gibberellin is a **hormone** found in plants (e.g. wheat and barley) that **controls seed germination** by stimulating the synthesis of the enzyme amylase
- It does this by influencing **transcription of the amylase gene**
    - When gibberellin is applied to a germinating seed there is an increased amount of the mRNA for amylase present

Mechanism

- The **breakdown of DELLA protein by gibberellin** is necessary for the synthesis of amylase
- The following components are involved:
    - Repressor protein **DELLA**
    - Transcription factor **PIF**
    - **Promoter** of amylase gene
    - Amylase **gene**
    - **Gibberellin**
    - Gibberellin **receptor and enzyme**
- The process occurs as follows:
- DELLA protein is bound to PIF, **preventing it from binding to the promoter** of the amylase gene so no transcription can occur
- Gibberellin binds to a gibberellin receptor and enzyme which starts the **breakdown of DELLA**
- **PIF** is no longer bound to DELLA protein and so it **binds to the promoter** of the amylase gene
- Transcription of amylase gene begins
- Amylase is produced

17. Selection & Evolution

Variation: Phenotype

- The observable characteristics of an organism are its phenotype
- Phenotypic variation is the **difference in phenotypes** between organisms of the same **species**
- In some cases, **phenotypic variation** is explained by **genetic** factors
    - For example, the four different blood groups observed in human populations are due to different individuals within the population having two of **three** possible **alleles** for the single ABO gene
- In other cases, **phenotypic variation** is explained by **environmental** factors
    - For example, clones of plants with exactly the same genetic information (DNA) will grow to different heights when grown in different environmental conditions
- Phenotypic variation can also be explained by a **combination of genetic and environmental factors**
    - For example, the recessive allele that causes sickle cell anaemia has a high frequency in populations where malaria is prevalent due to heterozygous individuals being resistant to malaria
- The complete phenotype of an organism is determined by the expression of its genotype and the interaction of the environment on this:

Phenotype = Genotype + Environment

Genetic variation

- Organisms of the same species will have very similar genotypes, but two individuals (even twins) will have differences between their DNA base sequences
- Considering the size of genomes, these differences are small between individuals of the same species
- The small differences in DNA base sequences between individual organisms within a species population is called **genetic variation**
- Genetic variation is **transferred** from one generation to the next and it **generates phenotypic variation** within a species population
- Genetic variation is caused by the following processes as they result in a **new combination of alleles** in a gamete or individual:
    - **Independent assortment** of homologous chromosomes during metaphase I
    - **Crossing over** of non-sister chromatids during prophase I
    - **Random fusion** of gametes during fertilization
- Mutation results in the **generation of new alleles**
    - The new allele may be advantageous, disadvantageous or have no apparent effect on phenotype (due to the fact that the genetic code is **degenerate**
    - New alleles are not always seen in the individual that they first occur in
    - They can remain hidden (not expressed) within a population for several generations before they contribute to phenotypic variation
- Genes can have **varying effects** on an organism's phenotype
    - The phenotype may be affected by a single gene or by several
    - The effect that the gene has on the phenotype may be large, small and/or additive

Process	Mechanism	Consequences
Independent assortment of homologous chromosomes during metaphase I	Random alignment of chromosomes results in different combinations of chromosomes and different allele combinations in each gamete	Genetic variation between gametes produced by an individual
Crossing over of non-sister chromatids during prophase I	Exchange of genetic material between non-sister chromatids leads to new combinations of alleles on chromosomes. It can also break linkage between genes	Genetic variation between gametes produced by an individual
Random fusion of gametes during fertilization	Any male gamete is able to fuse with any female gamete (Random mating in a species population)	Genetic variation between zygotes and resulting individuals
Mutation	Random change in the DNA base sequence results in the generation of a new allele. Mutation must exist within gametes for it to passed onto future generations	Genetic variation between individuals within a species population

Environmental factors

- The **environment** that an organism lives in can also have an impact on its phenotype
- Different environments around the globe experience very different conditions in terms of the:
    - Length of sunlight hours (which may be seasonal)
    - Supply of nutrients (food)
    - Availability of water
    - Temperature range
    - Oxygen levels
- Changes in the factors above can affect how organisms **grow and develop**
    - For example, plants with a tall genotype growing in an environment that is depleted in minerals, sunlight and water will not be able to grow to their full potential size determined by genetics
- Variation in phenotype caused solely by environmental pressures or factors cannot be **inherited** by an organism's offspring
    - Only alterations to the genetic component of gametes will ever be inherited

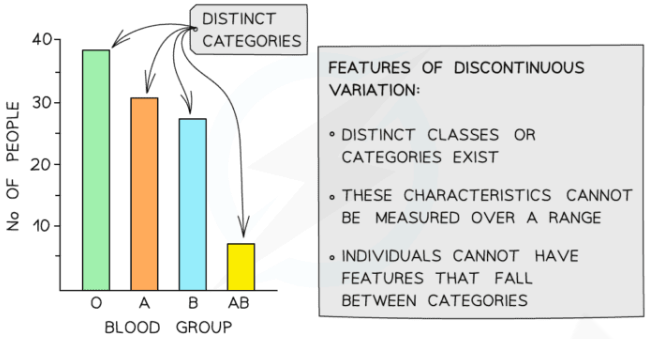
Why the variation in phenotype due to genetics is inherited but the variation in phenotype due to environmental factors is not. This is because genetic variation directly affects the DNA of the gametes but variation in phenotype caused by the environment does not.

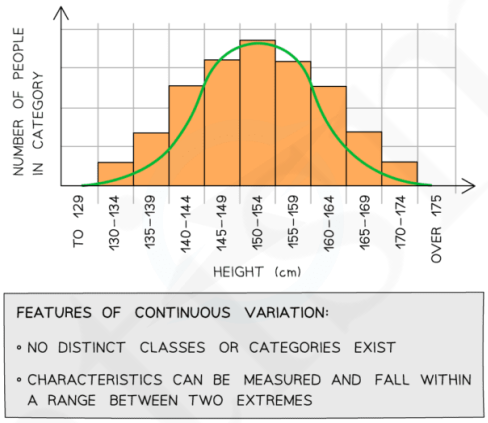
<u>Variation: Discontinuous & Continuous</u>

- The term **variation** refers to the differences that exist between at least two things (be it a level, amount, quantity or feature of something)
- In relation to natural selection, variation refers to the **differences that exist between individuals of a species**
    - This may also be referred to as **intraspecific** variation
- Variation observed in the **phenotypes** of organisms can be due to qualitative or quantitative differences

Discontinuous variation

- **Qualitative differences** in the phenotypes of individuals within a population give rise to **discontinuous variation**
- Qualitative differences fall into discrete and distinguishable **categories,** usually with no intermediates (a feature can't fall in between categories)
    - For example, there are four possible ABO blood groups in humans; a person can only have one of them
- It is easy to identify discontinuous variation when it is present in a table or graph due to the distinct categories that exist when data is plotted for particular characteristics





Continuous variation

- Continuous variation occurs when there are **quantitative differences** in the phenotypes of individuals within a population for particular characteristics
- Quantitative differences do not fall into discrete categories like in discontinuous variation
- Instead for these features, a **range of values** exist between two extremes within which the phenotype will fall
    - For example, the mass or height of a human is an example of continuous variation
- The lack of categories and the presence of a range of values can be used to identify continuous variation when it is presented in a table or graph

The Genetic Basis of Variation

- **Discontinuous variation** refers to the differences between individuals of a species where the differences are **qualitative** (categoric)
- **Continuous variation** is the differences between individuals of a species where the differences are **quantitative** (measurable)
- Each type of variation can be explained by **genetic** and / or **environmental factors**

Genetic basis of discontinuous variation

- This type of variation occurs solely due to **genetic factors**
- The environment has no direct effect
    - Phenotype = **genotype**
- At the genetic level:
    - Different **genes** have **different effects** on the phenotype
    - Different **alleles** at a single gene locus have a **large effect** on the phenotype
    - Remember diploid organisms will inherit two alleles of each gene, these alleles can be the same or different
- A good example of this is the *F8* gene that codes for the blood-clotting protein Factor VIII
    - The different alleles at the *F8* gene locus dictate whether or not normal Factor VIII is produced and whether the individual has the condition haemophilia

Genetic basis of continuous variation

- This type of variation is caused by an **interaction between genetics and the environment**
- Phenotype = **genotype + environment**
- At the genetic level:
    - Different **alleles** at a single locus have a **small effect** on the phenotype
    - Different **genes** can have the **same effect** on the phenotype and these add together to have an **additive effect**
    - If a large number of genes have a combined effect on the phenotype they are known as **polygenes**

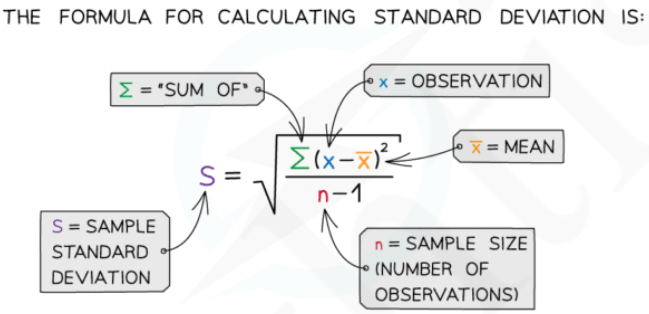
The additive effect of genes

- The height of a plant is controlled by two unlinked genes **H** / **h** and **T** / **t**
- The two genes have an **additive** effect
- The recessive alleles **h** and **t** contribute ***x*** cm to the plants height
- The dominant alleles **H** and **T** contribute 2***x*** cm to the plants height
- The following genotypes will have the following phenotypes:
    - **h h t t :** $x + x + x + x = 4x$ cm
    - **H H T T :** $2x + 2x + 2x + 2x = 8x$ cm
    - **H h T t :** $2x + x + 2x + x = 6x$ cm
    - **H H T t :** $2x + 2x + 2x + x = 7x$ cm
    - **H h T T :** $2x + x + 2x + 2x = 7x$ cm
    - **h h T t :** $x + x + 2x + x = 5x$ cm
    - **H h t t :** $2x + x + x + x = 5x$ cm

Variation: t-test Method

- A statistical test called the **t-test** can be used to **compare the means of two sets of data** and determine whether they are **significantly** different or not
    - The formula for the t-test will be provided in the exam, but formulate for how to calculate the number of **degrees of freedom** is not provided in the exam and must be learnt
- The sets of data must follow a rough **normal distribution**, be **continuous** and the **standard deviations** should be approximately equal
- The standard deviation (*s*) must be calculated for each data set before the t-test can be carried out
- A **null hypothesis** should also be given
- This is a statement of what we would expect if there is **no significant difference** between two means, and that any differences seen are due to change
- If there is a statistically significant difference between the means of two sets of data, then the observation is not down to chance and the **null hypothesis** can be **rejected**

Calculating the standard deviation



Using the t-test to compare two means

- The steps below outline the general steps in a *t* test; for a worked example see the next page
- Null hypothesis: there is no statistically significant difference between the means of sample 1 and sample 2
- **Step 1**: Calculate the mean for each data set:

$$\bar{x}_1 = \text{the mean for sample 1, and } \bar{x}_2 = \text{the mean for sample 2}$$

- **Step 2**: Calculate the **standard deviation** for each set of data, s_1 = standard deviation of sample 1 and s_2 = standard deviation of sample 2

$$s_n = \sqrt{\frac{\Sigma(x-\bar{x})^2}{n-1}}$$

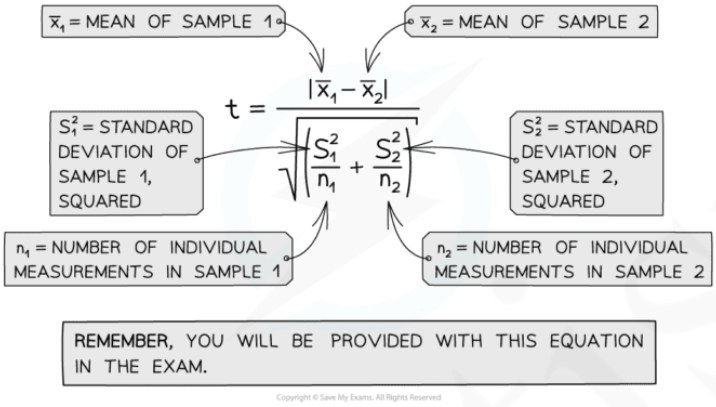
- **Step 3**: Square the standard deviation and divide by *n* (the number of observations) in each sample, for both samples:

$$\frac{s_1^2}{n_1} \text{ and } \frac{s_2^2}{n_2}$$

- **Step 4:** Add the values from step 3 together and take the square root:

$$\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}$$

- **Step 5:** Divide the difference between the two means (see step 1) with the value calculated in step 4 to get the *t* value:



- **Step 6:** Calculate the **degrees of freedom** (*v*) for the whole data set (remember the formulae for this will **not** be given in the exam):

$$\boldsymbol{v = (n_1 - 1) + (n_2 - 1)}$$

- **Step 7:** Look at a table that relates *t* values to the probability that the differences between data sets is due to chance to find where the *t* value for the degrees of freedom (*v*) calculated lies

Degrees of freedom	Value of t			
1	6.31	12.7	63.7	63.6
2	2.92	4.30	9.93	31.6
3	2.35	3.18	5.84	12.9
4	2.13	2.78	4.60	8.61
5	2.02	2.57	4.03	6.87
6	1.94	2.45	3.71	5.96
7	1.90	2.37	3.50	5.41
8	1.86	2.31	3.36	5.04
9	1.83	2.26	3.25	4.78
10	1.81	2.23	3.17	4.59
Probability that chance could have produced this value of t	0.10	0.05	0.01	0.001
Confidence level	10%	5%	1%	0.1%

- **Step 8**: The greater the *t* value calculated (for any degree of freedom), the lower the probability of chance causing any significant difference between the two sample means
    - Identify where the *t* value calculated lies with respect to the confidence levels provided
    - If the *t* value is **greater than the critical value** (obtained from the table at the critical probability of 0.05) then any difference between the two data sets is less likely to be **due to chance,** so the **null hypothesis can be rejected**
    - If the *t* value is less than the critical value given at a confidence of 5%/ the probability that any difference is down to chance is above 0.05; then an assumption can be made that the differences between the means of the two sets of data are not significant and the **null hypothesis is accepted**
- Using the table above, if a value of *t* was calculated to be 2.38 at 5 degrees of freedom, then it lies between 2.02 and 2.57, so the probability that chance produced any differences between the two means is between 10% and 5%; the null hypothesis would be accepted in this situation

<u>Natural Selection</u>

- Every individual within a species population has the **potential to reproduce** and have offspring which contribute to population growth
- If the offspring for every individual survived to adulthood and reproduced then the population would experience **exponential growth**
    - This type of growth only happens when there are **no environmental factors or population checks** acting on the population (for example, when there are plentiful resources and no disease)
    - One well known but rare example of exponential growth in a population is the introduction of 24 European rabbits into Australia in the 1800s. The rabbits had an abundance of resources, little or no competition and no natural predators. This meant the population increased rapidly and they became a major pest
- In reality, there are several **environmental factors** that prevent every individual in a population making it to adulthood and reproducing

Environmental factors

- **Environmental factors** limit population sizes by reducing the rate of population growth whenever a population reaches a certain size
- Environmental factors can be **biotic** or **abiotic**
- Biotic factors involve **other living organisms**
    - This includes things like predation, competition for resources and disease
- Abiotic factors involve the **nonliving parts** of an environment
    - Examples of abiotic factors include light availability, water supply and soil pH
- When biotic and abiotic factors come into play not all individuals within a population will survive
    - For example, if a food source is limited some animals within a population will not get enough to eat and will starve to death
- For most populations in the wild, the number of offspring produced is much higher than the number of individuals that make it adulthood

Population limitation by environmental factors

- For African lions living in the wild there are several environmental factors that limit their population growth rate:
    1. **Competition for food**
        - There is a limited supply of prey: other lions and carnivores will also be hunting the same prey. If a lion is not able to hunt and feed then they will die from starvation
    2. **Competition for a reproductive mate**
        - Female lions will often outnumber male lions in a population. This means the males compete with each other to mate with the females. When one male is in a contest with another male one (or both) could be injured or killed. Whoever loses the contest won't be able to mate with the females in a pride and so won't pass on his genes to any offspring
    3. **Supply of water**
        - African habitats can be very arid during the dry season. The water sources that the lions drink from can be miles apart. If a lake or source of water dries up then they can die due to dehydration
    4. **Temperature**
        - The extreme heat experienced in the lion's African habitat can cause them to overheat and die. It can also prevent them from hunting for long periods during the day, meaning they are less likely to get the food they need to survive
- The combined effect of all these environmental factors leads to a decrease in population growth as fewer individuals survive to adulthood and reproduce

Natural selection & survival

- **Variation** exists within a species population
- This means that some individuals within the population possess **different phenotypes** (due to genetic variation in the alleles they possess; remember members of the same species will have the same genes)
- Environmental factors affect the chance of survival of an organism; they, therefore, act as a **selection pressure**
- Selection pressures **increase the chance** of individuals with a **specific phenotype** surviving and reproducing over others
- The individuals with the favoured phenotypes are described as having a higher **fitness**
    - The fitness of an organism is defined as its ability to **survive** and **pass on its alleles** to offspring
    - Organisms with higher fitness posses **adaptations** that make them **better suited to their environment**
- When selection pressures act over several generations of a species they have an effect on the **frequency of alleles** in a population through **natural selection**
    - Natural selection is the process by which individuals with a fitter phenotype are more likely to survive and pass on their alleles to their offspring so that the **advantageous alleles increase in frequency over time and generations**

Natural selection in rabbits

- **Variation in their fur colour** exists within rabbit populations
- At a single gene locus, normal **brown fur** is produced by a dominant allele whereas **white fur** is produced by a recessive allele in a homozygous individual
- Rabbits have natural predators like foxes which act as a selection pressure
- Rabbits with a white coat do not camouflage as well as rabbits with brown fur, meaning predators are more likely to see white rabbits when hunting
- As a result, rabbits with white fur are less likely to survive than rabbits with brown fur
- The **rabbits with brown fur** therefore have a selection advantage, so they are **more likely to survive** to reproductive age and be able to pass on their alleles to their offspring
- **Over many generations,** the **frequency** of **alleles** for **brown** fur will **increase** and the frequency of alleles for white fur will decrease

Remember that organisms better suited to their environments are more likely to survive, but survival is not guaranteed. Organisms that are less suited to an environment are still able to survive and potentially reproduce within it, but their chance of survival and reproduction is lower than their better-suited peers. Also, it is important to be aware that an environment, and the selection pressures it exerts on an organism, **can change** over time. When a change occurs then a **different phenotype may become fitter**. Finally, remember that all organisms (not just animals) experience selection pressures as a result of the environment they are in!

<u>Natural Selection: Types of Selection</u>

- Environmental factors that affect the chance of survival of an organism are **selection pressures**
    - For example, there could be high competition for food between lions if there is not plentiful prey available; this environmental factor 'selects' for faster, more powerful lions that are better hunters
- These selection pressures can have different effects on the **allele frequencies** of a population through **natural selection**
- There are three types of selection:
    - **Stabilising**
    - **Disruptive**
    - **Directional**

Stabilising selection

- **Stabilising** selection is natural selection that keeps allele frequencies relatively **constant** over generations
- This means things stay as they are unless there is a change in the environment
- A classic example of stabilising selection can be seen in human birth weights
    - Very-low and very-high birth weights are selected against leading to the **maintenance of the intermediate** birth weights

Directional selection

- **Directional** selection is natural selection that produces a **gradual change** in allele frequencies over several generations
- This usually happens when there is a **change in environment** / selection pressures or a **new allele** has appeared in the population that is advantageous
- For example: A recent finding has shown that climate change is having an effect on fish size in certain habitats
    - The increase in temperature is **selecting for a smaller body size** and against a larger body size
    - Warmer seas cause fish metabolism to speed up and so increases their need for **oxygen**; oxygen levels are lower in warmer seas
    - Larger fish have greater **metabolic** needs than smaller fish, and so they feel the effect of increased temperatures more strongly
    - Organisms are **sensitive** to changes in **temperature** primarily because of the effect that temperature can have on **enzyme** activity
    - **Fish** with a **smaller** body size are therefore fitter and **better adapted** to living in seas experiencing increased temperatures
    - Fish body size is determined by both genetic and environmental factors
    - Fish of a smaller size are **more likely** to reproduce and pass on their alleles to offspring
    - Over **generations,** this leads to an **increase** in the **frequency** of **alleles** that produce a small body size and a decrease in the frequency of alleles that produce a larger body size

Disruptive selection

- **Disruptive** selection is natural selection that **maintains high frequencies of two different sets of alleles**
    - In other words, individuals with intermediate phenotypes or alleles are selected against
- Disruptive selection causes **polymorphism**: the continued existence of two or more distinct phenotypes in species
- This can occur in an environment that shows **variation**
- For example, birds that live on the Galapagos Islands use their beaks to forage for different sized seeds
    - The size of the bird's beaks are either small or large with the intermediate medium-sized beak selected against
    - The reason for this is that the different types of seed available are more efficiently foraged by a shorter or longer beak

<u>Natural Selection: Changes in Allele Frequencies</u>

- **Natural selection** causes a change in allele frequencies over time
    - Selection pressures (caused by the environment an organism is in) increase the likelihood that certain individuals with specific alleles survive to reproductive age, enabling them to pass on their alleles to their offspring
- There are other factors or processes that can affect allele frequencies in a population:
    - **The founder effect**
    - **Genetic drift**
    - **The bottleneck effect**

Natural selection

- When a **new allele** arises in a population or a **change in the environment** occurs then directional selection can happen
- Directional selection produces a **gradual change** in allele frequencies over several generations
    - There is always **phenotypic variation** within a population
    - There is a **selection pressure** that favours a particular phenotype
    - The phenotype is **produced by particular alleles**
    - Individuals with the favoured phenotype are fitter and so more likely to reproduce and **pass on the advantageous alleles** to their offspring
    - Those who do not possess the advantageous allele or phenotype are less likely to survive and pass on their alleles to their offspring
    - So over time and several generations the **frequency of the advantageous allele increases** and the frequency of other alleles decreases

The Founder effect

- The Founder effect occurs when only a small number of individuals from a large parent population start a **new population**
- As the new population is made up of only a few individuals from the original population **only some of the total alleles from the parent population** will be present
- In other words, **not all of the gene pool is present** in the smaller population
    - A gene pool is the **complete range of DNA sequences** (alleles) that exist in all the individuals of a population or species
- Which alleles end up in the new founding population is completely up to **chance**
- As a result, the changes in allele frequencies may occur in a **different direction** for the new small population vs the larger parent population

The founder effect in lizards

- Anole lizards inhabit most Caribbean Islands and they can travel from one island to another via floating debris or vegetation
- The **individual lizards** that arrive on an island, as well as the alleles they carry, is completely up to **chance**
- They may only carry a small selection of alleles, with many more alleles present in the lizard population on the original island
- The lizards on the original island could display a range of scale colours from white to yellow and the two individual lizards that arrived on the island have white scales
    - This means that the whole population that grows on that island might only have individuals with white scales
    - In comparison, the original island population has a mixture of white and yellow scaled individuals. This **difference between the two populations is completely due to chance**

Genetic drift

- When a population is significantly small, **chance** can affect which alleles get passed onto the next generation
- Over time some alleles can be lost or favoured purely by chance
- When there is a gradual change in allele frequencies in a small population due to chance and not natural selection then **genetic drift** is occurring

Example of genetic drift in plants

- In a small population of five plants growing near a playground with a rubber floor; three of the plants have blue-and-white flowers and two of the plants have pink-and-white flowers
- By chance, most of the seeds from the pink-and-white flowered plants end up on the rubber floor of the playground, whereas all the seeds from the blue-and-white flowered plants land on fresh fertile soil where they are able to germinate and grow
- Over several generations, the **allele for the pink-and-white flowers may disappear** from this population due to chance (because the seeds carrying pink-and-white alleles for flower colour cannot germinate on rubber)

Bottleneck effect

- The b**ottleneck** effect is similar to the Founder effect
- It occurs when a previously large population suffers a **dramatic fall in numbers**
- A major environmental event can massively reduce the number of individuals in a population which in turn **reduces the genetic diversity** in the population as **alleles are lost**
- The surviving individuals end up breeding and **reproducing with close relatives**

Example of the bottleneck effect

- A clear example of a genetic bottleneck can be seen in **cheetahs** today
- Roughly 10,000 years ago there was a large and genetically diverse cheetah population
- Most of the population was **suddenly killed** off when the **climate changed** drastically at the end of the Ice Age
- As a result, the surviving cheetahs were isolated in **small populations** and lots of **inbreeding** occurred
- This meant that the cheetah population today has a serious **lack of genetic variation**
- This is problematic for **conservation** as genetic variation within a species increases the likelihood that the species is able to **respond** (survive) in the event of any environmental changes
    - Remember the environment exerts a selection pressure on organisms

Process	Result
Natural Selection	**Selection pressures** produce a **gradual change** in allele frequencies over several generations.
Founder effect	Changes in allele frequencies occur in a **different direction** for the newly isolated small population in comparison to the larger parent population due to **chance**.
Genetic drift	Gradual change in allele frequencies in a small population due to chance and not natural selection.
Bottleneck effect	**Reduction in the gene pool** of a population due to a dramatic decrease in population size.

Natural Selection: Antibiotic Resistance

- When humans experience a pathogenic bacterial infection they are often prescribed **antibiotics** by a healthcare professional
- Antibiotics are chemical substances that **inhibit or kill bacterial cells** with little or no harm to human tissue
    - Antibiotics are derived from naturally occurring substances that are harmful to prokaryotic cells (structurally or physiologically) but usually do not affect eukaryotic cells
    - The aim of antibiotic use is to aid the body's immune system in fighting a bacterial infection
- **Penicillin** is a well-known example; it was the first antibiotic to be discovered in 1928 by Sir Alexander Fleming
- Antibiotics are either described as being **bactericidal** (they kill) or **bacteriostatic** (they inhibit growth processes), they target prokaryotic features but can affect both pathogenic and mutualistic bacteria living on or in the body
- However, like in all species, there exists **genetic diversity** within populations, and the same applies to disease-causing bacteria
- Individual bacterial cells may possess **alleles** that confer **resistance to the effects** of the antibiotic
    - These alleles are generated through random **mutation** and are not caused by antibiotic use, but antibiotic use exerts selection pressures that can result in the increase in their frequency
- Bacteria have a single loop of DNA with only **one copy of each gene** so when a new allele arises it is immediately displayed in the phenotype
- When **an antibiotic is present:**
    - Individuals with the allele for antibiotic resistance have a massive **selective advantage** so they are more likely to survive, reproduce and pass genome (including resistance alleles)
    - Those without alleles are less likely to die and reproduce
    - Over several generations, the **entire population** of bacteria may be antibiotic-resistant
- Antibiotic resistance is an important example of natural selection

Staphylococcus

- There are known populations of the bacterium *Staphylococcus* that possess alleles which make them resistant to the effects of penicillin
    - These are known as **resistant strains**
- Due to the **rapid reproduction rate** of bacteria (generations of 20-30 minutes for some species in optimal conditions) a single resistant bacterium can produce 10 000 million resistant descendants within a day

The future of antibiotic resistance

- Antibiotic-resistant strains are a major problem in human medicine
- New resistant strains are constantly emerging due to the **overuse of antibiotics**
    - By using antibiotics frequently, humans exert a **selective pressure** on the bacteria, which supports the evolution of antibiotic resistance
- Scientists are trying hard to find **new antibiotics** that bacteria have not yet been exposed to, but this process is expensive and time-consuming
- Some strains of bacteria can be **resistant to multiple antibiotics** and they create infections and diseases which are very difficult to treat
- When antibiotics were discovered, scientists thought they would be able to **eradicate** bacterial infections, but less than a century later a future is being imagined where many bacterial infections cannot be treated with current medicines

Bacteria pass on alleles for antibiotic resistance through reproduction (vertical gene transfer) but they can also do it in another way. Bacterium possess plasmids which are a small circular piece of DNA that is not the main chromosome. Alleles for antibiotic resistance are often found on these plasmids. **Plasmids can be easily transferred from one bacterium to another,** even between different species. This is an example of horizontal gene transfer. This means that alleles for antibiotic resistance can be passed one from species of bacteria to another species.

<u>Natural Selection: Hardy-Weinberg Principle</u>

- The Hardy-Weinberg principle states that if certain conditions are met then the **allele frequencies of a gene within a population will not change from one generation to the next**
- There are seven **conditions or assumptions** that must be met for the Hardy-Weinberg principle to hold true
- The Hardy-Weinberg **equation** allows for the **calculation of allele and genotype frequencies** within populations
- It also allows for predictions to be made about how these frequencies will change in future generations

Conditions for the Hardy-Weinberg principle

- Organisms are **diploid**
- Organisms reproduce **by sexual reproduction** only
- There is **no overlap** between generations
- **Mating** is **random**
- The **population** is **infinitely large**
- There is **no migration**, **mutation** or **selection**
- Allele **frequencies** are **equal** in both sexes

Hardy-Weinberg equations

- If the phenotype of a trait in a population is determined by a single gene with only two alleles (we will use **B / b** as examples throughout this section**)**, then the population will consist of individuals with three possible genotypes:
    - **Homozygous** dominant **(BB)**
    - **Heterozygous (Bb)**
    - Homozygous recessive (**bb)**
- When using the Hardy-Weinberg equations the **frequency of a genotype** is represented as a **proportion** of the population
    - For example, the **BB** genotype could be 0.40
    - Whole population = 1
- The letter ***p*** represents the frequency of the dominant allele (**B)**
- The letter ***q*** represents the frequency of the recessive allele (**b)**
- As there are only two alleles at a single gene locus for this phenotypic trait in the population:

$$\boldsymbol{p + q = 1}$$

- The chance of an individual being homozygous dominant is $\boldsymbol{p^2}$
    - In this instance, the offspring would inherit dominant alleles from both parents ($\boldsymbol{p} \times \boldsymbol{p} = \boldsymbol{p}^2$ *)*
- The chance of an individual being heterozygous is **2*pq***
    - Offspring could inherit a dominant allele from the father and a recessive allele from the mother ($\boldsymbol{p} \times \boldsymbol{q}$) **or** offspring could inherit a dominant allele from the mother and a recessive allele from the father ($\boldsymbol{p} \times \boldsymbol{q}$) = 2***pq***
- The chance of an individual being homozygous recessive is $\boldsymbol{q^2}$
    - In this instance, the offspring would inherit recessive alleles from both parents ($\boldsymbol{q} \times \boldsymbol{q} = \boldsymbol{q}^2$ *)*
- As these are all the possible genotypes of individuals in the population the following equation can be constructed:

$$\boldsymbol{p^2 + q^2 + 2pq = 1}$$

When you are using Hardy-Weinberg equations, start your calculations determining the proportion of individuals that display the **recessive phenotype** – you will always know the genotype for this: **homozygous recessive**. Remember that the dominant phenotype is seen in both homozygous dominant, and heterozygous individuals. Also, don't mix up the **Hardy-Weinberg equation**s with the **Hardy-Weinberg principle**. The **equations** are used to **estimate** the allele and genotype **frequencies** in a population.
The **principle** suggests that there is an **equilibrium** between allele frequencies and there is no change in this between generations.

Artificial Selection

- Artificial selection is the process by which **humans choose** organisms with **desirable traits** and selectively breed them together to enhance the expression of these desirable traits over time and many generations
- This practice is also known as **selective breeding**
- Humans have been selectively breeding organisms for thousands of years, long before scientists understood the genetics behind it
- Knowledge of the alleles that contribute to the expression of the desired traits are not required as individuals are selected by their **phenotypes,** and not their genotypes
- As the genetics is not always understood, breeders can accidentally **enhance other traits that are genetically linked** to the desirable trait
    - These other traits can sometimes negatively affect the organism's health
- Examples of artificial selection include:
    - Increased milk **yield** from cattle
    - Faster racehorses
    - Disease-resistant crops
- There are always biological **limitations** to how extreme a trait can become in an organism

Principles of selective breeding

1. The population shows **phenotypic variation** – there are individuals with different phenotypes / traits
2. Breeder selects an **individual with the desired phenotype**
3. Another individual with the desired phenotype is selected. The two selected individuals should not be closely related to each other
4. The two selected individuals are **bred** together
5. The offspring produced reach maturity and are then **tested for the desirable trait.** Those that display the desired phenotype to the greatest degree are selected for further breeding
6. The process continues for many generations: the best individuals from the offspring are chosen for breeding until all offspring display the desirable trait

Artificial selection in racing horses

- Selective breeding has been a major part of the horseracing industry for many years. Breeders have found that horses tend to have one of the three following phenotypes:
    - Good at sprinting short distances
    - Good endurance over long distances
    - All-rounder
- If a breeder wanted to breed a horse for a sprinting event they are likely to do the following:
    - Select the fastest sprinting female horse they have
    - Select the fastest sprinting male horse they have
    - Breed the two selected horses
    - Allow their offspring to reach maturity and test their sprinting speeds to find the fastest horse (male or female)
    - The breeder could then use this horse for racing, or they could continue the process of selective breeding by breeding this horse with another horse that is fast or descended from fast-sprinters
    - Over several generations, it would be hoped that the offspring are all fast-sprinters (but remember there are biological limitations to this)

Selective breeding can be used to enhance a single desired trait but it can also be used to **combine several desired traits** together in a single individual. A lot of this type of selective breeding is seen in plants. Farmers are constantly trying to breed plants with a high yield, disease resistance and the ability to grow in poor soil.

Examples of Selective Breeding

- Selective breeding (or artificial selection) is the process by which **humans choose** individuals with **desired traits** to reproduce, with the aim of producing offspring with the desired traits also
- Most selective breeding is done with the aim of **increasing the yield of a sellable product**
- It is not done with the organism's survival in mind, and unlike natural selection, it can lead to organisms that are poorly adapted to their environments
- Unless the genetic mechanism behind a trait is fully understood, is highly likely that **other traits could also be accidentally enhanced**
- Some examples of selective breeding in **agriculture and livestock** include:
    - **Disease-resistance** in wheat and rice varieties
    - **Hybridization** in maize
    - **Milk yield** in cattle

Disease-resistance in wheat & rice

- **Wheat** plants have been selectively bred for hundreds of years as a crop
- Wheat crops can be badly affected by fungal diseases: *Fusarium* is a fungus that causes "head blight" in wheat plants
- **Fungal diseases** are highly problematic for farmers as they destroy the wheat plant and **reduce crop yield**
- By using selective breeding to introduce a fungus-resistant allele from another species of wheat, the hybrid wheat plants are **not susceptible to infection, and so yield increases**
    - Introducing the allele into the crop population can take **many generations** and collaboration with researchers and plant breeders
- **Rice** is another crop that has been subject to large amounts of selective breeding
- Rice plants are prone to different **bacterial and fungal diseases**
    - Examples include "bacterial blight" and "rice blast" caused by the *Magnaporthe* fungus
- These diseases all **reduce the yield** of the crop as they damage infected plants
- Scientists are currently working hard to create varieties of rice plants that are resistant to several bacterial and fungal diseases

Inbreeding & hybridization in maize

- Maize (also known as corn) is a staple crop in many countries around the world; it is grown to feed both livestock and people
- In the past, maize plants have been heavily inbred (bred with plants with similar genotypes to their own)
- This has resulted in **small and weaker** maize plants that have less vigour
- This is **inbreeding depression** which:
    - Increases the chance of **harmful recessive alleles combining** in an individual and being expressed in the phenotype
    - **Increases homozygosity** in individuals (paired alleles at loci are identical)
    - Leads to **decreased growth and survivability**
- A farmer can prevent inbreeding depression by **outbreeding**
    - This involves breeding individuals that are **not closely related**
    - Outbreeding produces taller and healthier maize plants
    - It **decreases** the chance of **harmful** recessive alleles combining in an individual and being expressed in the phenotype
    - **Increases heterozygosity** (paired alleles at loci are different)
    - Leads to increased growth and survivability (known as **hybrid vigour**)
    - Crops of these plants have a **greater yield**
- **Uniformity** is important when growing a crop:
    - If outbreeding is carried out completely randomly, it can produce **too much variation** between plants within one field
    - A farmer needs the plants to ripen at the same time and be of a similar height; the more variation there is, the less likely this is
- In order to achieve **heterozygosity and uniformity,** farmers buy sets of homozygous seeds from specialised companies and cross them to produce an **F1 generation**
- Different hybrids of maize are constantly being created and tested for **desirables traits** such as: resistance to pests / disease, higher yields and good growth in poor conditions

In selective breeding, selection pressure is applied by humans who desire certain traits in animals or plants – this is why it's described as **artificial selection**.
In **natural selectio**n, the environment applies selection pressure on populations / species – but not to achieve a desirable outcome. Selection pressures in natural selection are simply driven by the environment in which organisms live and which features within a population or species are best suited (adapted) to that environment.

<u>Theory of Evolution</u>

- A species can be defined as a group of organisms that are able to **interbreed** and **produce fertile offspring**
- Members of one species are reproductively isolated from members of another species
- In reality, it is quite hard to define 'species' and the determination of whether two organisms belong to the same species is dependent on investigation
- Individuals of the same species have similar behavioural, morphological (structural) and physiological (metabolic) features
- A common example used to illustrate this concept are mules; the infertile offspring produced when a male donkey and a female horse mate

The gene pool

- The phenotype of all organisms is dependent on its genotype and environmental influence on this
- Members of the same species will have the same genes, of which there may exist alleles (alternate versions)
- A **gene pool** is the collection of **genes** within an interbreeding population
- A **gene pool** can be thought of as the sum of all the alleles at all of the **loci** within the **genes** of a population of a single species or a population
- The gene pool (or **allele frequencies)** in a species population can **change** over time due to processes such as:
    - Natural selection
    - Genetic drift
    - The founder effect
- When the **gene pool within a species population changes** sufficiently over time, the characteristics of the species will also change
- The **change can become so great that a new species forms**
- This is **evolution**

Evolution is the formation of new species from pre-existing species over time, as a result of changes to gene pools from generation to generation

- In order for evolution to occur the new species population must be **genetically and reproductively isolated** from the pre-existing species population
    - When this happens, there can no longer be an exchange of genes between the two populations
- Reproductive isolation can occur for a number of reasons, such as when a population splits and geographical separation (isolation) occurs, preventing mixing, or the incompatibility of gametes
- The evolution of a new species can take a **very long time** and many generations
- For organisms with a short generation time (such as bacteria), evolution can be observed far more quickly

Genetic isolation

- Two groups, when reproductively isolated from each other, become **genetically isolated**
- If two groups are no longer reproducing with each other, then they **do not interchange genes** with each other in the production of offspring
- Changes that occur in the **allele frequencies** of each group are not shared, so they **evolve independently** of each other which can lead to the formation of two groups that are no longer successfully able to interbreed

Evidence of Evolutionary Relationships in DNA

- DNA found in the nucleus, mitochondria and chloroplasts of cells can be sequenced and used to show evolutionary relationships between species
- The **differences between the nucleotide sequences** (DNA) of different species can provide a lot of information:
    - The **more similar** the sequence the **more closely related** the species are
    - Two groups of organisms with very similar DNA will have separated into separate species more recently than two groups with less similarity in their DNA sequences
- DNA sequence analysis and comparison can also be used to create family trees that show the evolutionary relationships between species

DNA Analysis and Comparison

- **DNA is extracted** from the nuclei of cells taken from an organism
    - DNA can be extracted from blood or skin samples from living organisms or from fossils
- The extracted DNA is processed, analysed and the **base sequence is obtained**
- The **base sequence is compared** to that of other organisms to determine evolutionary relationships
    - The more similarities there are in the DNA base sequence, the more closely related (in that the less distant the species separation) members of different species are
- In 2005, the chimpanzee genome was sequenced, and when compared to the human genome it was discovered that humans and chimpanzees share almost 99% of their DNA sequences, making them our closest living relatives
    - In 2012, the sequencing of the bonobo genome also revealed that humans and bonobos also share 98% of their genome (with slight differences to the differences seen in chimpanzees)

Mitochondrial DNA

- When analysing DNA from the mitochondria is is important to remember that:
    - A zygote only contains the mitochondria of the egg and none from the sperm so **only maternal mitochondrial DNA is present in a zygote**
    - There is **no crossing over** that occurs in mtDNA so the base sequence can only change by mutation
- The lack of crossing over in mtDNA has allowed scientists to research the origins of species, genetic drift and migration events
- It has even been possible to estimate how long ago the first human lived and where
    - Mitochondrial Eve is thought to have lived in Africa ~200,000 years ago
    - The estimation of this date relies on the **molecular clock theory** which assumes there is a **constant rate of mutation** over time
    - The greater the number of differences there are between nucleotide sequences, the longer ago the common ancestor of both species existed
    - The molecular clock is calibrated by using **fossils and carbon dating**
    - A fossil of a known species is carbon-dated to estimate how long ago that organism lived
    - This mtDNA of this species is then used as a **baseline for comparison** with the mtDNA of other species
- Although for your exams you should say that only maternal mitochondrial DNA can be passed on or inherited by the zygote, recent research suggests that paternal mDNA may also be present in zygotes

<u>Allopatric & Sympatric Speciation</u>

- **Evolution** causes **speciation**: the formation of **new species** from pre-existing species over time, as a result of **changes to gene pools** from generation to generation
- **Genetic isolation** between the new population and the pre-existing species population is necessary for speciation
- There are two different situations when speciation can take place:
    - Two groups of a species are separated by a **geographic** barrier
    - Two groups of species are **reproductively isolated** but still living in the same area (experiencing similar environmental selection pressures)

Allopatric Speciation

- Allopatric speciation occurs as a result of **geographical isolation**
- It is the most common type of speciation
- A species population splits into one or more groups which then become separated from each other by geographical **barriers**
    - The barrier could be natural like a body of water, or a mountain range
    - It can also be man-made (like a motorway)
- This separation creates two populations of the same species who are isolated from each other, and as a result, **no genetic exchange** can occur between them
- If there is sufficient **selection pressure or genetic drift** acting to change the gene pools within both populations then eventually these populations will **diverge and form separate species**
    - The changes in the alleles/genes of each population will affect the phenotypes present in both populations
    - Over time, the two populations may begin to differ physiologically, behaviourally and morphologically (structurally)

Example of Allopatric Speciation in Trees

- Imagine there is a population of trees that are all one species
- A new mountain range forms that divides the population into two
- The natural barrier prevents the two groups from interbreeding, so there is no gene flow between them
- The two populations experience **different selection pressures and genetic drift**
- Over thousands of years the divided populations **form two distinct species** that can no longer interbreed

Sympatric Speciation

- Sympatric speciation takes place with **no geographical barrier**
- A group of the same species could be living in the same place but in order for speciation to take place there must exist two populations within that group and **no gene flow** occurs between them
- Something has to happen that splits or separates the population:
    - **Ecological** separation: Populations are separated because they **live in different environments within the same area**
        - For example, soil pH can differ greatly in different areas. Soil pH has a major effect on plant growth and flowering
    - **Behavioural** separation: Populations are separated because they have **different behaviours**
        - For example differences in feeding, communication or social behaviour

Example of Sympatric Speciation in Fish

- A species of fish lives in a lake
- Some individuals within the population feed on the bottom while others remain higher up in the water
- The different feeding behaviours separates the population into different environments
    - **Behavioural separation leads to ecological separation**
- The separated groups experience **different selection pressures**
    - Long jaws are advantageous for bottom-feeding whereas shorter jaws are advantageous for mid-water feeding
- Over time natural selection causes the populations to **diverge** and evolve **different courtship displays**
- They can no longer interbreed; they are separate species

When looking at cases of sympatric speciation try not to confuse the factors that **originally caused a separation** between the populations vs the factors that then **prevent them from breeding** after genetic isolation. For the example of the fish: the difference in feeding behaviour is what originally causes separation but it is a difference in courtship displays (which is caused by genetic isolation) that prevents them breeding them together. Also do not forget that speciation is reliant on **mutation**! Without mutation, there are no new alleles or genes for selection to act on. The change in genetic material by mutation is important as it is what produces the differences in physiology, behaviour and morphology between species.

18. Classification, Biodiversity & Conservation

Definitions of Species

- Scientists have been classifying organisms into species for hundreds of years, in order to investigate the diversity of life that exists today and in the past
- There is difficulty in determining whether new organisms discovered belong to an existing species, or a new one
- This is because the most widely accepted definition of a species is:
    - A group of organisms with similar **morphological** and **physiological** features that able to breed together and **produce fertile offspring**
- This is the **biological species concept**, and is reliant on determining whether interbreeding produces fertile offspring – this is difficult and time-consuming to determine in practice
- However there are other discriminating factors that scientists can use to group similar organisms together

Morphological species concept

- In the past, most scientists described organisms by their **physical features** (morphology) as these can be more easily observed
- They group together organisms that **share many physical features** that **distinguish them from other species**
- This is the **morphological species concept**

Ecological species concept

- When there is a population of similar organisms **living in the same area at the same time,** they can be described as an **ecological** species
- This is the **ecological species concept**

Naming species

- Species are often given common names, but in order to avoid confusion about what group of organisms scientists are talking about, all species are given a two-part scientific name using the **binomial system**
- This naming convention was developed and established by the Swedish scientist Carl Linnaeus in the 18th Century
- The first part of the name is the genus that the species belongs to; this is a group of very similar organisms
- The second part of the name is specific and unique to a single group of organisms that are identified as a species (and occasionally there may be a third name)
- The binomial name is always italicized in writing (or underlined if it is not possible to italicise)
- For example:
    - The most commonly known yeast is ***Saccharomyces cerevisiae***
    - It is common to abbreviate the genus name: ***S. cerevisiae***
    - ***Saccharomyces paradoxus*** is another species of that is a member of the same genus as *cerevisiae*

The Three Domains: Archaea, Bacteria & Eukarya

- Taxonomy is the practice of biological classification
- It involves placing organisms into a series of categories or taxa
- By grouping organisms into taxa it can make them easier to understand and remember
- There are several **different ranks** or levels within the hierarchical classification system used in biology
- The highest rank is the **domain**
- **Cell type** has a major role in the classification of organisms into the three domains; but do not confuse cell types and domain
    - **Prokaryotic** cells are easily distinguishable in that they lack a nucleus
    - **Eukaryotic** cells have compartmentalised structures, with at least their genetic material segregated from the rest of the cell in a nucleus
- Based upon molecular analysis of RNA genes in particular, scientists have realised that using cell type to classify organisms is insufficient, and that **prokaryotes** could be divided into two separate groups (domains)
- The **three domains** are:
    - Archaea (prokaryotes)
    - Bacteria (prokaryotes)
    - Eukarya (eukaryotes)

Archaea

- Organisms within this domain are sometimes referred to as the extremophile **prokaryotes,** archaea were first discovered living in **extreme environments,** but not all archaea do
- Archael cells have **no nucleus** (and so are **prokaryotic**)
- They were initially classified as bacteria until several unique properties were discovered that separated them from known bacteria, including:
    - Unique lipids being found in the membranes of their cells
    - No **peptidoglycan** in their cell walls
    - Ribosomal structure (particularly that of the small subunit) are more similar to the eukaryotic ribosome than that of the bacteria
- Archaea a similar size range as bacteria (and in many ways metabolism is similar between the two groups)
- DNA transcription is more similar to that of eukaryotes
- Example: *Halobacterium salinarum* are a species of the archaea domain that can be found in environments with high salt concentrations like the Dead Sea

Bacteria

- These are organisms that have **prokaryotic cells** which contain no nucleus
- They vary in size over a wide range: the smallest are bigger than the largest known-viruses and the largest are smaller that the smallest known single-celled eukaryotes
- Bacterial cells divide by binary fission
- Example: *Staphylococcus pneumoniae* is a bacteria species that causes pneumonia

Eukarya

- Organisms that have **eukaryotic cells** with nuclei and membrane-bound organelles are placed in this domain
- They vary massively in size from single-celled organisms several micrometres across to large multicellular organisms many-metres in size, such as blue whales
- Eukaryotic cells divide by mitosis
- Eukaryotes can reproduce sexually or asexually
- Example: *Canis lupus* also known as wolves

Differences between Archaea & Bacteria

- Domains are the highest taxonomic rank that exist within the hierarchical classification system of organisms
- Initially, all organisms within the Archaea domain were classified as Bacteria
- Then several unique features possessed by Archaea were discovered that separated them from both Bacteria and Eukarya
- The main differences between Archaea and Bacteria are seen in:
    - **Membrane lipids**
    - **Ribosomal RNA**
    - **Cell wall composition**

Membrane lipids

- The membrane lipids found in the cells of Archaea organisms are completely **unique**
- They are not found in any **bacterial** or **eukaryotic** cells
- The membrane lipids of Archaea consist of **branched** hydrocarbon chains bonded to glycerol by **ether** linkages
- The membrane lipids of Bacteria consist of **unbranched** hydrocarbon chains bonded to glycerol by **ester** linkages

Ribosomal RNA

- Both Archaea and Bacteria possess 70S ribosomes
- The 70S ribosomes in Archaea possess a smaller subunit that is **more similar to the subunit found in Eukaryotic** ribosomes than subunits in Bacterial ribosomes
    - The **base sequences of ribosomal RNA** in Archaea show more similarity to the rRNA of Eukarya than Bacteria
    - The **primary structure of ribosome proteins** in Archaea show more similarity to the ribosome proteins in Eukarya than Bacteria

Composition of cell walls

- Organisms from the **Bacteria** domain have cells that always possess cell walls **with peptidoglycan**
- Organisms from the **Archaea** domain also have cells that always possess cell walls, however these **do not contain peptidoglycan**

Feature	Archaea	Bacteria	Eukarya
Cell type	Prokaryotic	Prokaryotic	Eukaryotic
DNA	Circular chromosome	Circular	Linear chromosomes + circular mtDNA and cpDNA
Nucleus in cells	No	No	Yes
Plasmids	Sometimes	Yes	No
Membrane bound organelles	No	No	Yes
Ribosomes	70S ribosomes	70S ribosomes	Larger 80S ribosomes in cytosol and 70S ribosomes in mitochondria and chloroplasts
Cell walls	Always present (without peptidoglycan)	Always present (with peptidoglycan)	No
Histones	No	No	Yes
Cell division	Cells divide by binary fission	Cells divide by binary fission	Cells divide by mitosis

Eukarya

- The hierarchical classification system of organisms in biology is used to organise and **group similar organisms together** so that they can be more easily understood
- There are several taxonomic ranks that exist
- **Species is the lowest taxonomic rank** in the system
    - Similar species can be grouped in a **genus**
    - Similar genuses can be grouped in a **family**
    - Similar families can be grouped into an **order**
    - Similar orders can be grouped into a **class**
    - Similar classes can be grouped into a **phylum**
    - Similar phyla can be grouped into a **kingdom**
    - Similar kingdoms can be grouped into a **domain**
- **Domains are the highest taxonomic rank** in the system

Classification of an organism in the Eukarya domain

- Just like the other domains, **Eukarya** contains the taxonomic hierarchy of kingdom, phylum, class, order, family, genus and species
- A wolf is an example of an organism in the Eukarya domain
- It can be classified further into its kingdom, phylum, class, order, genus and species

Taxonomic Rank	Wolf	Hibiscus
Domain	Eukarya	Eukarya
Kingdom	Animalia	Plantae
Phylum	Chordata	Angiospermae
Class	Mammalia	Dicotyledonae
Order	Carnivora	Malvales
Family	Canidae	Malvaceae
Genus	Canis	Hibiscus
Species	Canis Lupus	Hibiscus Rosa–sinensis

The name of a species always consists of two words: the **genus and species**. This means when provided with the Latin name of a species you are automatically provided with information about the last two taxonomic ranks that the organism belongs to. Remember this when being asked to show or explain the classification of an organism in the exam.

<u>Kingdoms</u>

- The domain Eukarya can be divided into 4 kingdoms:
    - **Protoctista**
    - **Fungi**
    - **Plantae**
    - **Animalia**
- Organisms from each of the four kingdoms have **distinct characteristics** and features, but share similarities in that they have cells with membrane-bound nuclei separating genetic material from the cytoplasm, and compartmentalisation within their cells as a result of the presence of other organelles

Kingdom Protoctista

- All Protoctista are **eukaryotic,** and this broad group of cellular life encompasses all eukaryotic cells that do not belong to the other three eukaryotic kingdoms
- Members of this kingdom show great diversity in all aspects of life including structure, life cycle, feeding and trophic levels and well as modes of locomotion
- Protoctists can exist as **single-celled organisms** or as a **group of similar cells**
- A group of Protoctista known as protozoa possess cells similar to animal cells
    - Their cells have **no cell wall**
- Another group of Protoctista known as algae possess cells similar to plant cells
    - Their cells have **cellulose cell walls and chloroplasts**
- *Stentor roseli* is a protoctist that has flagella all over its body which help it feed and move

Kingdom Fungi

- The oldest organism in the world is thought to be a fungus aged somewhere between 1500 – 10,000 years old
- All fungi are **eukaryotic** cells
- The cells of fungi:
    - Possess non-cellulose **cell walls** (often made of the polysaccharides **chitin** and **glucans**
    - Don't have cilia
- Fungi are **heterotrophs:**
    - They use organic compounds made by other organisms as their source of energy and molecules for metabolism
    - They obtain this energy and carbon by **digesting dead/decaying matter** extracellularly or from being **parasites** on living organisms
- Fungi **reproduce using spores** that disperse onto the ground nearby
- Fungi have a simple body form:
    - They can be unicellular (like the common baker's yeast *Saccharomyces cerevisiae*
    - Some consist of long threads called hyphae that grow from the main fungus body (mycelium)
    - Larger fungi possess fruiting bodies that release large numbers of spores
- The mould found on bread is actually a fungus: bread mould fungus *Rhizopus nigricans*

Kingdom Plantae

- Plants are **multicellular eukaryotic** organisms
- Plant cells:
    - All have **cell walls** composed of cellulose
    - Possess large (and usually permanent) **vacuoles** that provide structural support
    - Are able to differentiate into **specialized cells** to form **tissues and organs**
    - Possess **chloroplasts** that enable **photosynthesis** (not all plant cells have chloroplasts)
    - Can sometimes have **flagella**
- They are **autotrophs**
    - This means they can synthesize their organic compounds and molecules for energy use and building biomass from inorganic compounds
- Plants have **complex body forms**
    - They have branching systems above and below the ground
- Bristlecone pines are found in the USA, it is estimated that some of them could be 3000 years old

Kingdom Animalia

- Animals are also **multicellular eukaryotic** organisms
- Animal cells:
    - Are able to differentiate into **many different specialised cell types** that can form **tissues and organs**
    - Have **small temporary vacuoles** (for example, lysosomes)
    - Have **no cell walls**
    - Sometimes have cilia
- They are **heterotrophs** (they have a wide range of feeding mechanisms)
- They have a wide range of body forms:
    - Communication within their complex body forms takes place through a **nervous system and chemical signalling**

Viruses

- Viruses are **microorganisms** that can only be seen using an electron microscope
- They have no cellular structure (and so are **acellular** and **no metabolism)**
- Viruses **hijack the DNA replication machinery** in host cells
- The energy viruses need for replication is provided by **respiration** in the host cell
- Viruses possess none of the characteristic features used for classifying organisms so they sit **outside** of the three-domain classification system
- There is a wide-ranging debate as to whether viruses should be classified as 'living' or 'non-living' based on their inability to carry out the defining features of life outside of a host cell

Classifying viruses by their genetic material

- Viruses are classified according to the **type of nucleic acid** (RNA or DNA) their genome is made from, and whether it is single-stranded or double-stranded
- In **cellular organisms** like animals and plants, **DNA is always double-stranded** and **RNA is usually always single-stranded**
- However, in **viruses,** DNA and RNA can be **either single-stranded or double-stranded**
- As a result, there are four groups of viruses that exist:
    - DNA single-stranded viruses
    - DNA double-stranded viruses
    - RNA single-stranded viruses (this is the type of genome of SARS-CoV-2, the virus responsible for the COVID-19 pandemic)
    - RNA double-stranded viruses

Nucleic acid	Single or Double stranded	Virus	Host Organism	Disease
DNA	Single	Canine parvovirus type 2	Dog	Canine parvovirus
DNA	Double	Varicella zoster virus (VZV)	Human	Chickenpox
RNA	Single	Morbillivirus	Human	Measles
RNA	Double	Human immunodeficiency virus	Human	AIDS

Ecosystem & Niches

Ecosystems

- **Species** do not exist by themselves in their own isolated environment, they interact with other species forming **communities**
- These communities interact with each other and the **environment** they live in, forming ecosystems
- An **ecosystem** is a relatively self-contained community of interacting organisms and the environment they live in, and interact with
- There is a **flow of energy** within an ecosystem and nutrients within it are recycled
- There are both **living** (biotic) **components** and **non-living** (abiotic)**components** within an ecosystem
- Ecosystems **vary greatly in size and scale**
    - Both a small pond in a back garden and the open ocean could be described as ecosystems
    - A human being could also be described as an ecosystem; there are thousands of species of bacteria living on and in every person
- Ecosystems **vary in complexity**:
    - A desert is a relatively simple ecosystem
    - A tropical rainforest is a very complex ecosystem
- No ecosystem is completely self-contained as organisms from one ecosystem are often linked to organisms from another
    - For example, birds are able to fly long distances to feed from multiple ecosystem

Example of an ecosystem

A forest is a perfect example of a complex ecosystem. There is a large community of organisms including trees, birds, small and large mammals, insects and fungi. The non-living components of the ecosystem include: the soil, dead leaves, water from the rain and streams, the rocks and any other physical or chemical factors. The non-living components of the ecosystem influence the community of organisms.

Niche

- The place where a species lives within an ecosystem is its **habitat**
- The role that species plays within an ecosystem is its **niche**
    - It encompasses **where** in the environment the organism is, how it gets its **energy** and how it **interacts** with other species and its physical environment
    - This is how an organism fits into the ecosystem

Example of a niche

A dung beetle occupies a very specific niche within its ecosystem. Dung beetles have learned to exploit the dung of animals as a resource and they have a characteristic behaviour of rolling the dung into balls before transporting it to their underground burrow for storage as food. Their behaviour within their ecosystem has many knock-on effects on the environment and other organisms living in it. The burrows and tunnels that they create turns over and aerates the soil and the buried dung releases nutrients into the soil both of which can benefit other organisms like plants. The transportation of the dung underground by the beetles also helps to keep fly populations under control.

Biodiversity

- Biodiversity can be thought of as a study of all the **variation** that exists within and between all forms of life
- Biodiversity looks at the range and variety of genes, species and habitats within a particular region
- It can be assessed at three different levels:
    - The number and range of different **ecosystems** and habitats
    - The number of **species** and their relative abundance
    - The **genetic** variation within each species
- Biodiversity is very important for the **resilience of ecosystems**, in that it allows them to **resist changes** in the environment

Ecosystem or habitat diversity

- This is the **range** of different ecosystems or **habitats** within a **particular area or region**
- If there is a large number of different habitats within an area, then that area has high biodiversity
    - A good example of this is a coral reef. They are very complex with lots of microhabitats and **Error: you must enter a valid popover post ID** to be exploited
- If there is only one or two different habitats then an area has low biodiversity
    - Large sandy deserts typically have very low biodiversity as the conditions are basically the same throughout the whole area

Species diversity

- An ecosystem such as a tropical rainforest that has a very high number of different species would be described as **species-rich**
    - **Species richness** is the **number** of species within an ecosystem
- Species diversity looks at the number of different species in an ecosystem, and also the **evenness of abundance** across the different species present
    - The greater the number of species in an ecosystem, and the more evenly distributed the number of organisms are among each species, then the greater the **species diversity**
    - For example, an ecosystem can have a large number of different species but for some species, there may only be 3 or 4 individuals. As a result, this ecosystem does not necessarily have high species diversity
- Ecosystems with **high species diversity** are usually **more stable** than those with lower species diversity as they are **more resilient** to environmental changes
    - For example in the Pine forests of Florida, the ecosystem is **dominated by one or two** tree species. If a pathogen comes along that targets one of the two dominant species of trees, then the whole population could be wiped out and the ecosystem it is a part of could collapse

Genetic diversity

- The genetic diversity within a species is the **diversity of alleles and genes in the genome** of species
- Although individuals of the same species will have the same genes they will not necessarily have the same **alleles** for each gene
- Genetic diversity is measured by working out the proportion of genes that have more than one form (allele) and how many possible alleles each gene has
- There can be genetic differences or diversity **between populations** of the same species
    - This may be because the two populations occupy slightly different ranges in their habitat and so are subject to slightly different selection pressures that affect the allele frequencies in their populations
- Genetic diversity **within** a single population has also been observed
    - This diversity in a species is important as it can help the population **adapt** to, and survive, **changes in the environment**
    - The changes could be in **biotic factors** such as new predators, pathogens and competition with other species
    - Or the changes could be through **abiotic factors** like temperature, humidity and rainfall

<u>Random Sampling</u>

- Measuring the different levels of biodiversity within an ecosystem can be a tasking job
- Finding out which species live in an ecosystem and the size of the populations requires the **identification and cataloguing** of all organisms present to build a **species list**
- This is possible for areas that are very small or where the species are very large like trees
- However, for larger and more complex ecosystems like rainforests, it is simply **impossible** to find, identify and count every organism that exists there
- When this is the case different **samples** of the area can be taken and used to make an **estimate for the total** species numbers in the area

Sampling

- Sampling is a method of investigating the **abundance and distribution of species and populations**
- There are two different types of sampling:
    - **Random**
    - **Systematic**
- In random sampling the positions of the **sampling points** are completely random or **due to chance**
    - This method is beneficial because it means **there will be no bias** by the person that is carrying out the sampling that may affect the results
- In systematic sampling the positions of the **sampling points are chosen** by the person carrying out the sampling
    - There is a possibility that the person choosing could show bias towards or against certain areas
    - Individuals may deliberately place the quadrats in areas with the least species as these will be easier and quicker to count
    - This is unrepresentative of the whole area
- When a sampling area is **reasonably uniform** or has **no clear pattern** to the way the species are distributed then **random sampling** is the best choice

Testing for Distribution & Abundance

- The **distribution** of a species describes how it is spread throughout the ecosystem
- The **abundance** of a species is the number of individuals of that species
- The distribution and abundance of a species in an area can be assessed using different practical methods:
    - Frame **Quadrats**
    - Line and Belt **Transects**
    - **Mark-release-capture**

Frame quadrats

- Some ecosystems are very complex with large numbers of different species of different sizes
- For the sake of logistics, **sampling** is often used to estimate the **distribution** and **abundance** of species
- When carrying out sampling, square frames called **quadrats** can be used to mark off the area being sampled
- Quadrats of different sizes can be used depending on what is being measured and what is most suitable in the space the samples are being made in
- Quadrats must be laid **randomly** in the area to **avoid sampling bias**
    - This random sampling can be done by converting the sampling area into a **grid format** and labelling each square on the grid with a number
    - Then a random number generator is used to pick the sample points
- Once the quadrat has been laid on the chosen sample point the **abundance** of all the **different species** present can be recorded

Results from quadrats

- The results from the quadrats can be used to calculate the predicted frequency and density of a species within an area
- **Species frequency** is the probability that the species will be found within any quadrat in the sample area
    - The number of **quadrats** that the species was present in is divided by the total number of quadrats and then multiplied by 100
    - For example, if bluebells were found in 18 out of 50 quadrats the species frequency would be (18/50) x 100 = 36%
- **Species density** indicates how many individuals of that species there are per unit area
    - The number of individuals counted across all **quadrats** is divided by the total area of all the quadrats
    - For example, if 107 bluebells were found across 50 quadrats that are $1m^2$ each the species density would be 107/50 = 2.14 individuals per m^2
- It can sometimes be difficult to count individual plants or organisms. When this is the case **percentage cover** of the species within the quadrat can be estimated instead
    - The quadrat is divided into 100 smaller squares. The number of squares the species is found in is equivalent to its percentage cover in that quadrat
    - For example, if grass is found in 89 out of 100 squares in the quadrat then it has a percentage cover of 89%

Line & belt transects

- Throughout some areas, there can be **changes in the physical conditions**
    - For example, there may be changes in altitude, soil pH or light intensity
- When investigating the species distribution in these kinds of areas **systematic sampling** is more appropriate
- Methods using **transects** can help show how species distribution changes with the different physical conditions in the area
    - A transect is a line represented by a measuring tape, along which sample are taken
- For a **line transect:**
    - Lay out a measuring tape in a straight line across the sample area
    - At **equal distances** along the tape **record the identity of the organisms that touch the line.** For example, every 2m
    - This produces qualitative data
- For a **belt transec**t:
    - Place quadrats at **regular intervals** along the tape and **record the abundance of each species within each quadrat**
    - This produces quantitative data

Mark-release-capture

- The methods above are only useful for stationary organisms
- Different methods are required for estimating the number of individuals in a population of **mobile animals**
- The **mark-release-capture** method is used in conjunction with **the Lincoln Index**
- For a single species in the area:
    - The **first large sample is taken**. As many individuals as possible are caught, counted and **marked** in a way that won't affect their survival
    - They are **returned to their habitat** and allowed to randomly mix with the rest of the population
    - When a sufficient amount of time has passed **another large sample is captured**
    - The number of marked and unmarked individuals within the sample are **counted**
    - The proportion of marked to unmarked individuals is used to calculate an **estimate of the population size**
    - The formula for the calculation is:

$$\mathbf{N = n_1 \times n_2 / m_2}$$

- Where:
    - **N** = population estimate
    - $\mathbf{n_1}$ = number of marked individuals released
    - $\mathbf{n_2}$ = number of individuals in the second sample (marked and unmarked)
    - $\mathbf{m_2}$ = number of marked individuals in the second sample

Pearson's Linear Correlation

- When recording the abundance and distribution of species in an area different trends may be observed
- Sometimes **correlation** between two variables can appear in the data
    - **Correlation** is an association or relationship between variables
    - There is a clear distinction between **correlation** and **causation**: a correlation does not necessarily imply a causative relationship
    - **Causation** occurs when one variable has an influence or is influenced by, another
- There may be a correlation **between species;** for example, two species always occurring together
- There may be a correlation **between a species and an abiotic factor**, for example, a particular plant species and the soil pH
- The apparent correlation between variables can be analysed using **scatter graphs** and different **statistical tests**

Correlation between variables

- In order to get a broad overview of the correlation between two variables the data points for both variables can be plotted on a **scatter graph**
- The correlation coefficient (r) indicates the **strength of the relationship** between variables
- Perfect correlation occurs when **all of the data points lie on a straight line** with a **correlation coefficient of 1 or -1**
- Correlation can be **positive or negative**
    - Positive correlation: as variable A increases, variable B increases
    - Negative correlation: as variable A increases, variable B decreases
- If there is **no correlation** between variables the **correlation coefficient will be 0**
- The **correlation coefficient (*r*)** can be calculated to determine whether a linear relationship exists between variables and how strong that relationship is

Pearson linear correlation

- Pearson's linear correlation is a statistical test that determines whether there is **linear correlation** between two variables
- The data must:
    - Be **quantitative**
    - Show **normal distribution**
- Method:
    - **Step 1:** Create a scatter graph of data gathered and identify if a linear correlation exists
    - **Step 2:** State a null hypothesis
    - **Step 3:** Use the following equation to work out Pearson's correlation coefficient *r*
- If the correlation coefficient *r* is close to 1 or -1 then it can be stated that there is a strong linear correlation between the two variables and the null hypothesis can be rejected

$$r = \frac{\Sigma xy - n\overline{x}\,\overline{y}}{(n-1)s_x s_y}$$

r = correlation coefficient

x = no. of species A

y = no. of species B

n = no. of readings

$\mathbf{S_x}$ = standard deviation of species A

$\mathbf{S_y}$ = standard deviation of species B

$\bar{x}$ = mean no. of species A

$\bar{y}$ = mean no. of species B

Spearman's Rank Correlation

- If there is an apparent relationship between two variables but the **data does not show a normal distribution**, Pearson's linear correlation coefficient should not be used
- **Spearman's rank** correlation determines whether there is correlation between variables that don't show a normal distribution
- Method:
    - **Step 1:** Create a scatter graph and identify possible linear correlation
    - **Step 2:** State a null hypothesis
    - **Step 3:** Use the following equation to work out Spearman's rank correlation coefficient *r*

$$r_s = 1 - \left(\frac{6 \times \Sigma D^2}{n^3 - n} \right)$$

    - Where:
        - $\mathbf{r_s}$ = spearman's rank coefficient
        - **D** = difference in rank
        - **n** = number of samples
    - **Step 4:** Refer to a table that relates critical values of r_s to levels of probability
- If the value calculated for Spearman's rank is greater than the critical value for the number of samples in the data (*n*) at the 0.05 **probability level** *(p)*, then the null hypothesis can be rejected, meaning there is a correlation between two variables

Correlation does not always mean causation. Just because there is a correlation between the abundance of species A and species B it does not mean that the presence of species A causes the presence of species B.

Simpson's Index

- Once the abundance of different species in an area has been recorded the results can be used to calculate the **species diversity** or biodiversity for that area
- Species diversity looks at the number of different species in an area but also the **evenness of abundance** across the different species
- **Simpson's index of diversity (D)** can be used to quantify the biodiversity of an area
- The formula is:

$$D = 1 - (\sum \left(\frac{n}{N}\right)^2)$$

- Where:
    - ***n*** = total no. of organisms for a single species
    - ***N*** = total no. of organisms for all species
- To calculate Simpson's Index:
    - **Step 1:** First step is to calculate n / N for each species
    - **Step 2:** Square each of these values
    - **Step 3:** Add them together and subtract the total from 1
- The possible values of **D** are significant:
    - The value of **D** can fall between 0 and 1
    - Values near 1 indicate high levels of biodiversity
    - Values near 0 indicate low levels of biodiversity

19. Genetic Technology

Recombinant DNA

- The genetic code is **universal**, meaning that almost every organism uses the same four nitrogenous bases – A, T, C & G. There are a few exceptions
- This means that the **same codons code for the same amino acids in all living things** (meaning that genetic information is transferable between species)
- Thus scientists have been able to artificially change an organism's DNA by combining lengths of nucleotides from different sources (typically the nucleotides are from different species)
- The altered DNA, with the introduced nucleotides, is called **recombinant DNA** (rDNA)
- If an organism contains nucleotide sequences from a different species it is called a **transgenic** organism
- Any organism that has introduced genetic material is a **genetically modified organism** (GMO)
- It is because of the universal genetic code that recombinant DNA can be formed. All forms of life use the same genetic code, which is the strongest piece of **evidence for evolution**. Remember, the genetic code is the basis for storing instructions that, alongside environmental influences, dictate the behaviour of cells and as a result, the behaviour of the whole organism.

Genetic Engineering

- Genetic engineering is a technique used to deliberately modify a specific characteristic (or characteristics) of an organism. The technique involves **removing a gene** (or genes) with the desired characteristic from **one organism** and **transferring** the gene (using a **vector**) **into another** organism where the **desired gene is then expressed**
- The genetically engineered organism will then contain recombinant DNA and will be a genetically modified organism (GMO)
- In order for an organism to be genetically engineered the following steps must be taken:
    - **Identification** of the **desired gene**
    - **Isolation** of the desired gene by:
        - Cutting from a chromosome using enzymes (**restriction endonucleases**)
        - Using **reverse transcriptase** to make a single strand of complementary DNA (**cDNA**) from mRNA
        - Creating the gene artificially using nucleotides
    - **Multiplication** of the gene (using polymerase chain reaction – PCR)
    - **Transfer** into the organism using a **vector** (e.g. plasmids, viruses, liposomes)
    - **Identification** of the cells with the new gene (by using a **marker**), which is then cloned
- Genetic engineers need the following to modify an organism:
    - **Enzymes** (restriction endonucleases, ligase and reverse transcriptase)
    - **Vectors** – used to deliver genes into a cell (eg. plasmids, viruses and liposomes)
    - **Markers** – genes that code for identifiable substances that can be tracked (eg. GFP – green fluorescent protein which fluoresces under UV light or GUS – β-glucuronidase enzyme which transforms colourless or non-fluorescent substrates into products that are coloured or fluorescent)
- Genetic engineering is being used in the new field of science called **synthetic biology**
    - This is an area of research that studies the design and construction of different biological pathways, organisms and devices, as well as the redesigning of existing natural biological systems

Isolating the Desired Gene

- The gene with the specific characteristic that is required can be obtained in the following ways:
    - Extracting the gene from the DNA of a donor organism using enzymes (**restriction endonucleases**)
    - Using **reverse transcriptase** to synthesise a single strand of complementary DNA (**cDNA**) from the mRNA of a donor organism
    - Synthesising the gene artificially using nucleotides

Extraction of gene

- The extraction of the gene (containing the desired nucleotide sequence) from the donor organism occurs using **restriction endonucleases**
- Restriction endonucleases are a class of enzymes found in bacteria. They are used as a defence mechanism by bacteria against bacteriophages (viruses that infect bacteria, also known as phages)
- The enzymes **restrict** a viral infection by cutting the viral genetic material into smaller pieces at specific nucleotide sequences **within** the molecule. This is why they are called restriction endonuclease ('endo' means within)
- They are also referred to as **restriction enzymes**
- There are many different restriction endonucleases because they bind to a **specific restriction site** (specific sequences of bases) on DNA, eg. *Hind*III will always bind to the base sequence AAGCTT
- The restriction endonucleases are named according to the bacteria they are sourced from and which numbered enzyme it is from that source (eg. *Hind*III comes from *Haemophilus influenzae* and it is the third enzyme from that bacteria)
- Restriction endonucleases will separate the two strands of DNA at the specific base sequence by 'cutting' the sugar-phosphate backbone in an uneven way to give **sticky ends** or straight across to give **blunt ends**
- Sticky ends result in one strand of the DNA fragment being longer than the other strand
- The sticky ends make it easier to insert the desired gene into another organism's DNA as they can easily form hydrogen bonds with the complementary base sequences on other pieces of DNA that have been cut with the **same** restriction enzyme
- When using genes isolated by restriction endonucleases that give blunt ends nucleotides can be added to create sticky ends

mRNA & reverse transcriptase

- Another method to isolate the desired gene is to use the mRNA that was transcribed for that gene
- Once isolated, the mRNA is then combined with a **reverse transcriptase** enzyme and nucleotides to create a **single strand** of **complementary DNA** (**cDNA**)
- Reverse transcriptase enzymes are sourced from retroviruses and they catalyse the reaction that reverses transcription. The **mRNA is used as a template to make the cDNA**
- **DNA polymerase** is then used to convert the single strand of cDNA into a **double-stranded DNA** molecule which contains the desired code for the gene
- This technique for isolating the desired gene is considered advantageous as it is easier for scientists to find the gene because specialised cells will make very specific types of mRNA (eg. β-cells of the pancreas produce many insulin mRNA) and the **mRNA** (therefore the cDNA) **does not contain introns**

Artificial synthesis

- As scientists are becoming more familiar with the base sequences for our proteins (proteome) it is possible to synthesise genes artificially
- With the knowledge of the genetic code (that is, which amino acids are required) scientists **use computers to generate the nucleotide sequence** (rather than an mRNA template) to produce the gene
- Short fragments of DNA are first produced which are joined to make longer sequences of nucleotides and then inserted into vectors (eg. plasmids)
- This method is being used to create novel genes being used to make vaccines and even to synthesise new bacteria genomes

<u>Genetic Engineering: Enzymes</u>

- Genetic engineering is the deliberate modification of a specific characteristic (or characteristics) of an organism. The technique involves **removing a gene** (or genes), with the desired characteristic, from **one organism** and **transferring** the gene (using a **vector**) **into another** organism where the **desired gene is then expressed**
- In order to genetically engineer an organism there are a number of enzymes required:
    - **Restriction endonucleases** (enzymes) – cuts the DNA strands so that the desired gene can be isolated or spliced (inserted) into a vector
    - **Reverse transcriptase** – reverses transcription to produce a single-strand complementary DNA (cDNA) from an mRNA strand with the code for the desired gene
    - **DNA polymerase** – used to convert the single-stranded cDNA into a double-stranded DNA molecule of the desired gene
    - **DNA ligase** – is used to splice (insert) the gene into the vector

Restriction endonucleases

- The role of **restriction endonucleases** (or restriction enzymes) in the transfer of a gene into an organism is to:
    - Isolate the desired gene
    - Separate the DNA strands (**at the same base sequence**) in a vector so the desired gene can be inserted
- There are many different restriction endonucleases because they bind to a **specific restriction site** (specific sequences of bases) on DNA, eg. *Hind*III will always bind to the base sequence AAGCTT
- Restriction endonucleases will separate the two strands of DNA at the specific base sequence by **'cutting' the sugar-phosphate backbone** in an uneven way to give **sticky ends** or straight across to give **blunt ends**
- Sticky ends result in one strand of the DNA fragment being longer than the other strand
- The sticky ends make it easier to insert the desired gene into another organism's DNA or **into a vector** as they can easily form hydrogen bonds with the complementary base sequences on other pieces of DNA that have been cut with the **same restriction endonucleases**

Reverse transcriptase

- The role of **reverse transcriptase** in the transfer of a gene into an organism is to produce a **single-strand complementary DNA molecule** (**cDNA**) that contains the code for the desired characteristic, this will then be inserted into a vector (after being converted into a double-stranded DNA molecule)
- **Reverse transcriptase** enzymes are sourced from retroviruses and they catalyse the reaction that reverses transcription. The mRNA (with the genetic code for the desired gene) is used as a template to synthesise a single strand of complementary DNA (cDNA)
- Reverse transcriptase enzymes are often used as it is easier for scientists to find mRNA with the specific characteristic because specialised cells make very specific types of mRNA (eg. β-cells of the pancreas produce many insulin mRNA) and **mRNA does not contain introns**

DNA polymerase

- **DNA polymerase** is used to **convert the single strand of cDNA into a double-stranded DNA molecule** which contains the desired code for the gene
- The enzyme builds the second strand by pairing free nucleotides with the **complementary bases** on the cDNA strand

DNA ligase

- **DNA ligase** catalyses the formation of phosphodiester bonds in the DNA sugar-phosphate backbone
- This enzyme enables the isolated desired gene to be spliced into a vector (generally a plasmid) so that it can be transferred to the new organism

<u>Genetic Engineering: Vectors</u>

- Vectors are used to **transfer the desired genes** into a foreign cell
- Plasmids are the most commonly used vector but viruses and liposomes (a small vesicle with a phospholipid layer) can also be used to transfer genes

Plasmids

- Plasmids are small, circular rings of double-stranded DNA
- They occur naturally in bacteria, but can also been found in archaea and eukaryotic organisms (eg. yeast and fungi) and can contain genes for antibiotic resistance
- Plasmids are used as they can self replicate
- A plasmid is used to transfer the desired gene to a new organism
- To insert the desired gene into the circular DNA of the plasmid it is 'cut' open. The **same restriction endonuclease** that was used to isolate the desired gene is used to 'cut' open the plasmid. This results in the plasmid having **complementary sticky ends** to the sticky ends on the desired gene fragment
- **DNA ligase** forms phosphodiester bonds between the sugar-phosphate backbone of the DNA fragment and the plasmid to form a **recombinant** plasmid (a closed circle of double-stranded DNA containing the desired gene)
- Scientists can **modify** bacterial plasmids or artificially produce them. One benefit of this is that the plasmids can have one or more **marker genes** so that cells that have the **recombinant plasmids can be identified**
- Plasmids are transferred into host cells (usually bacteria) by a process called **transformation**. Only a small proportion of bacteria will become transformed and therefore markers are used to identify these. Transformation can occur by:

- Bathing the plasmids and bacteria in an ice-cold calcium chloride solution and then briefly incubating at 40°C. This makes the bacteria membrane permeable
- Electroporation – where the bacteria is given a small electrical shock making the membranes very porous (this technique can be used to get DNA fragments into eukaryotic cells)

Viruses

- Viruses are commonly used as vectors in the process of gene therapy, which is currently used to treat genetic diseases such as cystic fibrosis
- The viruses are genetically modified to carry non-mutated genes into host cells
- Different types of viruses have been used; retroviruses, lentiviruses and adeno-associated viruses

Liposomes

- Liposomes are small spherical vesicles with a phospholipid layer
- These vesicles can also be used in gene therapy to carry non-mutated genes into host cells
- The advantage of using liposomes as a vector is that they can fuse with the cell surface membrane

Genetic Engineering: Promoter

- The **promoter** (an example of a length of non-coding DNA that has a specific function) is the **region of DNA that determines which gene will be expressed**. This is because it is the **site where RNA polymerase binds** to in order to begin transcription
- The promoter also ensures that RNA polymerase can recognise which is the DNA template strand. RNA polymerase recognises the template strand as the promoter contains the transcription start point (the first nucleotide of the gene to be transcribed) which is where the enzyme will bind
- Thus the promoter is used to **regulate gene expression** because only if it is present will transcription and therefore the expression of the gene occur
- If genetic engineers want to ensure the desired gene is expressed when modifying the plasmid they have to **add an appropriate promoter**
- As with eukaryotic cells bacteria have many different genes coding for many different proteins although not all genes are switched on at once. Bacteria will only express genes (to make proteins) if the growing conditions require a certain protein (eg. *coli* bacteria only make β-galactosidase enzymes when their growing medium contains lactose but lacks glucose)
- Scientists used this knowledge when first genetically engineering bacteria to produce insulin. In this case they added the insulin gene along with the β-galactosidase gene to share a promoter (which switched on the gene when the bacteria needed to metabolise lactose)
    - So when the scientists grew the bacteria in a medium containing lactose but no glucose, the bacteria produced the β-galactosidase and human insulin

Genetic Engineering: Marker Genes

- A **marker** is a gene that is transferred with the desired gene to enable scientists to **identify** which **cells** have been **successfully altered** and now contain recombinant DNA
- **Antibiotic-resistant genes** were once commonly used as marker genes. Scientists genetically modified the bacteria so that the plasmid contained the desired gene along with a specific antibiotic-resistant gene (and promoter) and then grew the bacteria on agar plates embedded with that antibiotic. The bacteria that contained the recombinant plasmids could be identified as these were the bacteria that grew
- Using antibiotic-resistant genes as marker genes concerns scientists as:
    - There is a risk that the antibiotic-resistant genes could be **accidentally transferred** to other bacteria including pathogenic strains creating pathogenic antibiotic-resistant bacteria
    - If the resistance spread to other bacteria this could **make antibiotics less effective**
- The spread of the antibiotic-resistant genes can occur due to the **conjugation** (the transfer of genetic material from one bacterium to another) or due to **transduction** (the transfer of genetic material from one bacterium to another via a virus)
- So **genes** that express proteins that are **fluorescent** are now commonly used as **markers**
- The fluorescence is due to the presence of a **green fluorescent protein** (GFP)
- The GFP gene along with the desired gene are linked to a specific promoter and once this promoter is activated, and the protein is expressed, the recombinant bacteria are detected when they glow green under exposure to ultraviolet light
- The use of fluorescent genes as markers is preferable because:
    - They are **easier to identify** (all that is required is the ultraviolet light)
    - **More economical** (do not need to grow the bacteria on plates of agar infused with antibiotics)
    - **No risk of antibiotic resistance** being passed onto other bacteria
    - There are antibiotics that are no longer effective and therefore would not stop any bacteria from growing

<u>Gene Editing</u>

- **Gene genome editing** (or editing) allows genetic engineers to alter the DNA of organisms by **inserting**, **deleting** or **replacing DNA** at specific sites in the genome known to cause disease. It is a form of genetic engineering where foreign DNA is **not** introduced into the genome
- Gene editing enables the scientists to be more accurate in their manipulation of the genome. In the past, inaccurate methods using vectors were used. These included:
    - Modifying viruses to insert DNA into the gene causing the disease. However this resulted in DNA being inserted into other genes causing unforeseen consequences
    - Liposomes (small spheres of lipid molecules) containing the normal gene which was sprayed into noses. This was only a short-term solution as the epithelial cells lining the nasal passageway were short lived
- Today scientists have developed new gene editing techniques, the most commonly used one being CRISPR (**C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats). This technique involves using the natural defense mechanism bacteria (and some archaea) have evolved to cut the DNA strands at a specific point as determined by a guide RNA attached to an enzyme (Cas9). Once cut scientists can then either insert, delete or replace the 'faulty' DNA with normal DNA
- Gene editing is involved in gene therapies (e.g. developing treatments for cystic fibrosis and sickle cell anaemia). **Gene therapy** is the **treatment of a genetic disease by altering the person's genotype**

- As scientists learn more about the human genome (from the Human Genome Project) and the **proteome**, and have the technology to process, large quantities of data through computational biology, they are gain a better understanding of which genes are responsible for genetic diseases and where they are located and therefore what base changes need to occur to treat or cure the disease

Polymerase Chain Reaction (PCR)

- **Polymerase chain reaction** (PCR) is a common molecular biology technique used in most applications of gene technology, for example, DNA profiling (eg. identification of criminals and determining paternity) or genetic engineering
- It is used to produce **large quantities** of specific fragments of DNA or RNA from very small quantities (even just one molecule of DNA or RNA). By using PCR scientists can have billions of **identical copies** of the DNA or RNA sample within a few hours
- The PCR process involves **three key stages** per cycle. In each cycle the DNA is doubled so in a standard run of 20 cycles a million DNA molecules are produced. The three stages are undertaken in a PCR instrument (or **thermal cycler**) which automatically provides the **optimal temperature** for each stage and controls the **length of time** spent at each stage
- Each PCR reaction requires:
    - **Target DNA** or RNA being amplified
    - **Primers** (forward and reverse) – these are short sequences of single-stranded DNA that have base sequences complementary to the 3' end of the DNA or RNA being copied. They define the region that is to be amplified by identifying to the DNA polymerase where to begin building the new strands
    - **DNA polymerase** – is the enzyme used to build the new DNA or RNA strand. The most commonly used polymerase is ***Taq* polymerase** as it comes from a thermophilic bacterium *Thermus aquaticus* which means it **does not denature** at the **high temperature** involved during the first stage of the PCR reaction and secondly, its optimum temperature is high enough to prevent annealing of the DNA strands that have not been copied yet
    - **Free nucleotides** – used in the construction of the DNA or RNA strands
    - **Buffer solution** – to provide the optimum pH for the reactions to occur in
- The three stages are:
    - **Denaturation** – the double-stranded DNA is heated to 95°C which breaks the hydrogen bonds that bond the two DNA strands together
    - Error: you must enter a valid popover post ID – the temperature is decreased to between 50 – 60°C so that primers (forward and reverse ones) can anneal to the ends of the single strands of DNA
    - **Elongation / Extension –** the temperature is increased to 72°C for at least a minute, as this is the optimum temperature for *Taq* polymerase to build the complementary strands of DNA to produce the new identical double-stranded DNA molecules

Gel Electrophoresis

- Gel electrophoresis is a technique used widely in the analysis of DNA, RNA and proteins. During electrophoresis the **molecules** are **separated** according to their **size / mass** and their **net (overall) charge**
- The separation occurs because:
    - Of the electrical charge molecules carry – positively charged molecules will move towards the cathode (negative pole) whereas negatively charged molecules will move towards the anode (positive pole) eg. **DNA is negatively charged** due to the **phosphate** groups and thus when placed in an electric field the molecules **move towards the anode**
    - Different sized molecules move through the gel (agarose for DNA and polyacrylamide – PAG for proteins) at different rates. The tiny pores in the gel result in **smaller molecules** moving **quickly**, whereas **larger molecules** move **slowly**
    - Of the type of gel – different gels have different sized pores which affects the speed the molecules can move through them

DNA separation

- DNA can be collected from almost anywhere on the body, e.g. the root of a hair or saliva from a cup. After collection DNA must be prepared for gel electrophoresis so that the **DNA** can be **sequenced** or analysed for **genetic profiling** (**fingerprinting**)
- To prepare the fragments scientists must first increase the number of DNA molecules by the polymerase chain reaction (PCR). Then restriction endonucleases (enzymes) are used to cut the DNA into fragments
- Different restriction enzymes cut the DNA at different base sequences. Therefore scientists use enzymes that will cut close to the **variable number tandem repeat** (VNTR) regions
- **Variable number tandem repeats** (VNTRs) are regions found in the non-coding part of DNA. They contain **variable numbers of repeated DNA sequences** and are known to **vary between different people** (except for identical twins). These VNTR may be referred to as 'satellite' or 'microsatellite' DNA
- To separate the DNA fragments in gel electrophoresis the scientists :
    - Create an agarose gel plate in a tank. Wells (a series of groves) are cut into the gel at one end
    - Submerge the gel in an electrolyte solution (a salt solution that conducts electricity) in the tank
    - Load (insert) the fragments into the wells using a micropipette
    - Apply an electrical current to the tank. The negative electrode must be connected to the end of the plate with the wells as the DNA fragments will then move towards the anode (positive pole) due to the attraction between the negatively charged phosphates of DNA and the anode
    - The smaller mass / shorter pieces of DNA fragments will move faster and further from the wells than the larger fragments
    - The fragments are not visible so must be transferred onto absorbent paper or nitrocellulose which is then heated to separate the two DNA strands. **Probes** are then added, after which an X-ray image is taken or UV-light is shone onto the paper producing a pattern of bands which is generally compared to a control fragment of DNA
- **Probes** are **single-stranded DNA sequences** that are **complementary** to the **VNTR** regions sought by the scientists. The probes also contain a means by which to be identified. This can either be:
    - A radioactive label (eg. a phosphorus isotope) which causes the probes to emit radiation that makes the X-ray film go dark, creating a pattern of dark bands
    - A fluorescent stain / dye (eg. ethidium bromide) which fluoresces (shines) when exposed to ultraviolet (UV) light, creating a pattern of coloured bands

Protein separation

- The different amino acids (because of the different R groups) determine the charge of proteins. The charge of the R groups depends on the pH and therefore buffer solutions are used during the separation of proteins to keep the pH constant
- Gel electrophoresis is used to separate polypeptide chains produced by different alleles eg. the haemoglobin variants (α-globin, β-globin and the sickle cell anaemia variant of β-globin)

Microarrays

- Microarrays are laboratory tools **used to detect the expression** of thousands of genes at the same time and to **identify the genes present** in an organism's genome
- Microarrays are used in medical diagnosis and treatment (e.g. comparison between healthy cells and diseased cells to find the characteristics of the disease), biotechnology (eg. in agriculture to identify insect pests), as well as crime (forensic analysis)
- As **large numbers of genes** can be studied in a short period of time microarrays have been very valuable to scientists
- The microarray consists of a small (usually $2cm^2$) piece of glass, plastic or silicon (also known as chips) that have **probes** attached to a spot (called a gene spot) in a grid pattern. There can be 10 000 or more spots per cm^2
- Probes are **short lengths of single-stranded DNA** (oligonucleotides) or RNA which are synthesised to be complementary for a specific base sequence (this sequence depends on the purpose of the microarray)
- When a microarray is used to **analyse genomes**:
    - DNA is collected from the species going to be compared
    - Restriction enzymes are used to cut the DNA into fragments
    - These fragments are denatured to create single-stranded DNA molecules
    - These DNA fragments are labelled using **fluorescent tags** (the fragments from the different sources are tagged different colours, usually red and green)
    - Once these fragments are mixed together they are then allowed to **hybridise** with the probes on the microarray
    - After a set period of time any DNA that did not hybridise with the probes is washed off
    - The microarray is then examined using ultraviolet light (which causes the tags to fluoresce) or scanned (colours are detected by the computer and the information is analysed and stored)
    - The presence of the colour indicates where hybridisation has occurred, as the DNA fragment is complementary to the probe. If red and green fluorescent spots appear then only one species of DNA has hybridised, however, if the spot is yellow then both species have hybridised with that DNA fragment, which suggests that both species have that gene in common
    - If a spot lacks colour that indicates the gene is not present in either species
- When genes are being expressed or are in their active state, many copies of mRNA are produced by transcription. The corresponding proteins are then produced from these mRNAs during translation. Thus scientists can indirectly, by assessing the quantity of mRNAs, determine which genes are being expressed in the cells
- Microarrays can be used to detect whether a gene is being expressed (a method used to research cancerous vs non-cancerous cells) by detecting the quantity of mRNA present

- To compare which **genes are being expressed** using microarrays the following steps occur:
    - **mRNA** is collected from both types of cells and **reverse transcriptase** is used to convert mRNA into **cDNA**
    - PCR may be used to increase the quantity of cDNA (this occurs for all samples to remain proportional so a comparison can be made when analysis occurs)
    - **Fluorescent tags** are added to the cDNA
    - The cDNA is then **denatured** to produce **single-stranded DNA**
    - The single-stranded DNA molecules are allowed to **hybridise** with the **probes** on the microarray
    - When the ultraviolet light is shone on the microarray the spots that fluoresce indicate that gene was transcribed (expressed) and the **intensity of the light** emitting from the spots indicates the **quantity of mRNA** produced (i.e. how active the gene is). If the light being emitted is of **high intensity** then **many mRNA** were present, while a **low intensity** emission indicates **few mRNA** are present

<u>Bioinformatics</u>

- The various technologies (eg. microarrays and gene sequencing) being used today to analyse genes and proteins generate enormous quantities of data
- The data being collected ranges from the sequences of genomes, when genes are being expressed during an organism's life to the structure (amino acid sequence) and functions of proteins
- To analyse all of this data scientists are using **bioinformatics**
- Bioinformatics is an interdisciplinary science (incorporating biology with computer technology and statistics) where biological data is collected, organised, manipulated, analysed and stored
- Large databases are created containing information ranging from gene sequences to amino acid sequences of proteins. The databases are available online and can perform analysis of the data selected. As this data needs to be accessed and searched software developers play an important role. Some of the databases that exist are:
    - The European Molecular Biology Laboratory – Nucleotide sequence database
    - Array Express – a microarray database with the level and types of mRNA expressed in different cells
    - Protein Data Bank at Europe – Protein sequence searches
    - BLAST (**B**asic **L**ocal **A**lignment **S**earch **T**ool) – used by researchers to find similarities between sequences they are studying with those already in the database
- Once a genome is sequenced bioinformatics allows scientists to make comparisons with the genomes of other organisms using the many databases available. This can help to find the degree of similarity between organisms which then gives an indication of how closely related the organisms are and whether there are organisms that could be used in experiments as a model for humans (eg. the fruit fly *Drosophila*)
- The nematode *Caenorhabditis elegans* is an animal that has been used as a model organism for studying the genetics of organ development, neurone development and cell death. It was the first multicellular organism to have its genome fully sequenced and as it has few cells (less than 1000) and is transparent it has been a useful model
- One of the applications for bioinformatics includes using databases with the genome of *Plasmodium* to determine which genes and or proteins could be altered or affected to control the parasite (eg. finding a vaccine for malaria)

Recombinant Human Proteins

- DNA that has been altered by introducing nucleotides from another source is called **recombinant DNA** (rDNA)
- If the organism contains nucleotides from a different species it is called a **transgenic** organism
- Any organism that has introduced genetic material is a **genetically modified organism** (GMO)
- Recombinant DNA has been used to produce **recombinant proteins** (RP), thus recombinant proteins are manipulated forms of the original protein
- Recombinant proteins are generated using microorganisms such as bacteria, yeast, or animal cells in culture. They are used for research purposes and for treatments (eg. diabetes, cancer, infectious diseases, haemophilia)
- Most recombinant human proteins are **produced using eukaryotic cells** (eg. yeast, or animal cells in culture) rather than using prokaryotic cells, as these cells will carry out the **post-translational modification** (due to presence of Golgi Apparatus and / or enzymes) that is required to produce a suitable human protein
- The **advantages** of genetic engineering organisms to produce recombinant human proteins are:
    - More **cost-effective** to produce large volumes (i.e. there is an unlimited availability)
    - **Simpler** (with regards to using prokaryotic cells)
    - **Faster** to produce many proteins
    - **Reliable** supply available
    - The proteins are engineered to be **identical** to human proteins or have **modifications** that are **beneficial**
    - It can solve the issue for people who have **moral** or **ethical** or **religious** concerns against using cow or pork produced proteins

Insulin

- In 1982, **insulin** was the first recombinant human protein to be approved for use in **diabetes** treatment
- Bacteria plasmids are modified to include the human insulin gene
    - Restriction endonucleases are used to cut open plasmids and DNA ligase is used to splice the plasmid and human DNA together
- These recombinant plasmids are then inserted into *Escherichia coli* by transformation (bath of calcium ions and then heat or electric shock)
- Once the transgenic bacteria are identified (by the markers), they are isolated, purified and placed into fermenters that provide optimal conditions
- The transgenic bacteria multiply by binary fission, and express the human protein – insulin, which is eventually extracted and purified
- The advantages for scientists to use recombinant insulin are:
    - It is **identical to human insulin**, unless modified to have different properties (eg. act faster, which is useful for taking immediately after a meal or to act more slowly)
    - There is a **reliable supply available** to meet demand (no need to depend on availability of meat stock)
    - **Fewer ethical**, **moral** or **religious** concerns (proteins are not extracted from cows or pigs)
    - **Fewer rejection problems** or **side effects** or **allergic reactions**
    - **Cheaper** to produce in large volumes
    - That it is useful for people who have **animal insulin tolerance**

Factor VIII

- **Factor VIII** is a blood-clotting protein that **haemophiliacs** cannot produce
- **Kidney** and **ovary hamster cells** have been genetically modified to produce Factor VIII
- Once modified these recombinant cells are placed into a fermenter and cultured
- Due to the optimal conditions in the fermenter, the hamster cells constantly express Factor VIII which can then be extracted and purified, and used as an injectable treatment for haemophilia
- The advantages for scientists to use recombinant Factor VIII are:
    - **Fewer ethical**, **moral** or **religious** concerns (proteins are not extracted from human blood)
    - **Less risk of transmitting infection** (eg. HIV) or disease
    - **Greater production rate**

Adenosine deaminase

- **Adenosine deaminase** (ADA) is an enzyme used to treat the inherited condition called **Adenosine Deaminase Deficiency**
- ADA Deficiency is a common cause of **Severe Combined Immunodeficienc**y (SCID)
- This is because the immune system is damaged
- The larva of the cabbage looper moth has been genetically modified (using a virus vector) to produce the enzyme adenosine deaminase so that it can be used as a treatment whilst the patients wait for gene therapy or when gene therapy is not possible
- The advantages for scientists to use recombinant adenosine deaminase are:
    - **Fewer ethical**, **moral** or **religious** concerns (proteins are not extracted from cows)
    - **Less risk of transmitting infection** or disease (from cows)
    - More reliable production of enzyme
    - Faster to produce many proteins

<u>Genetic Screening</u>

- In certain circumstances (eg. in the pregnancy in an older woman, or pregnancy where there is a family history of a genetic disease) may require individuals to determine if they have a particular allele present in their genome. This can be determined by **genetic screening**
- Genetic screening can help identify individuals who are carrying an allele at a gene locus for a particular disorder
- Genetic screening is the **testing of an embryo, fetus or adult to analyse the DNA**
- The sample of DNA to be analysed can be obtained by:
    - Taking **tissue samples from adults** or embryos produced by in-vitro fertilisation
    - **Chorionic villus sampling** or **amniocentesis** of embryos and fetuses in the uterus
- As genetic screening can leave future parents with many questions, **genetic counsellors** are available to help. The counsellors will read the results and explain them. Counsellors can also be seen before screening has occurred. They may discuss the following with the prospective parents:
    - The chances of the couple having a child with a certain disease
    - Termination of the pregnancy
    - Therapeutic treatments possible for the child
    - Financial implications of having the child
    - Effect on existing siblings
    - Ethical issues

Breast cancer (BRCA1 and BRCA2)

- BRCA1 and BRCA2 are genes that produce tumour suppressor proteins and thus they play an important role in regulating cell growth
- Faulty alleles of these particular genes exist which increase the risk of an individual developing breast and ovarian cancers during their lifetime
- Faulty BRCA1 and BRCA2 alleles can be inherited from either parent
- The **advantages** of genetic screening for an adult who has a family history of **BRCA1** and **BRCA2** gene mutations are:
    - That the person may decide to take **preventative measures** (e.g. by having an elective mastectomy – breast removal – to reduce the risk of developing cancer)
    - **Screening** for breast cancer may begin from an **earlier age** or **more frequently,** and the individual (if female) will have more frequent clinical examinations of the ovaries
    - That it enables the person to **participate in research and clinical trials**

Huntington's disease

- Huntington's is a progressive (gets worse with time) inherited disease that affects the brain
- Signs of the disease typically appear in affected individuals after reaching their 40's and include **uncontrolled movements**, **lower cognitive** (thinking) **ability** and emotional problems
- There is no cure for the Huntingdon's disease, with treatments available only alleviating the symptoms but not curing it
- Huntington's is an **autosomal dominant** disease (therefore if the person has an allele for Huntington's they will get the disease)
- The advantage of genetic screening for Huntington's is it enables:
    - People to **plan for the future** (how they will live and be cared for)
    - Couples to make **informed reproductive decisions** (as the risk that their children may inherit the disease is 50%)
    - People to **participate in research and clinical trials**

Cystic fibrosis

- Cystic fibrosis is an **autosomal recessive genetic disorder** that is caused by a mutation of the gene that codes for a transported protein called **CFTR**
- It is a **progressive** disease that causes **mucus** in various organs (lungs, pancreas, lungs) to become thick and sticky. This is because the faulty CFTR protein no longer transports chloride ions across the cell plasma membrane and therefore water does not move by osmosis across the membrane either (the presence of water would normally make the mucus thinner enabling cilia to remove it)
- There is no cure for cystic fibrosis, although there are many different treatments that help alleviate symptoms. The common cause of death is bacterial infection in the lungs
- The advantage of genetic screening for cystic fibrosis is:
    - It enables couples to make **informed reproductive decisions** (as both may be carriers and therefore not display any symptoms)
    - That people can participate in **research and clinical trials**

Gene Therapy

- Gene therapy involves using various mechanisms to **alter a person's genetic material** to treat, or cure, diseases
- As scientists gain a better understanding of the human genome and therefore the location of genes that cause genetic disorders, the possibilities of gene therapy being able to **replace a faulty gene**, **inactivate a faulty gene** or **insert a new gene** are growing
- Experimental techniques are being used to treat and research treatments for genetic diseases such as **severe combined immunodeficiency** (**SCID**), Leber congenital amaurosis – a rare form of blindness, β-thalassaemia and haemophilia B
- Most gene therapies are still in the clinical trial stage because scientists are having difficulty finding delivery systems that can transfer normal alleles into a person's cells and how to ensure the gene is correctly expressed once there
- Finding an appropriate delivery system has been one of the problems. **Vectors** are currently used as the delivery system, with **viruses** being the most commonly used, but **non-viral vectors** are also being researched (eg. **liposomes** and **'naked' DNA**)
- Viruses (eg. retroviruses and lentiviruses) are the most commonly used vectors as they have the mechanisms needed to recognise cells, and deliver the genetic material into them
- Currently all gene therapies have targeted and been tested on **somatic** (body) Changes in genetic material are **targeted to specific cells** and so will not be inherited by future generations (as somatic gene therapy does not target the gametes)
- Often the effects of changing the somatic cells are **short-lived**
- There are two types of s**omatic gene therapy**:
    - **Ex vivo** – the new gene is inserted via a virus vector into the cell **outside the body**. Blood or bone marrow cells are extracted and exposed to the virus which inserts the gene into these cells. These cells are then grown in the laboratory and returned to the person by an injection into a vein
    - **In vivo** – the new gene is inserted via a vector into cells **inside the body**
- There is the potential for new genetic material to be inserted into **germ cells** (cells involved in sexual reproduction eg. gametes or an early embryo)
- However, this is illegal in humans as any changes made to the genetic material of these cells is potentially **permanent** and could therefore be **inherited** by future generations

Severe combined immunodeficiency (SCID)

- **Severe combined immunodeficiency** (SCID) is caused by the body's inability to produce **adenosine deaminase (ADA)**, an enzyme that is key to the **functioning of the immune system**. Without this enzyme children can die from common infections and therefore need to be keep isolated often inside plastic 'bubbles'
- To treat SCID scientists have used **ex vivo somatic gene therapy.** During this therapy, a virus transfers a **normal allele for ADA into T-lymphocytes** removed from the patient and the cells are then returned via an injection
- This is **not a permanent cure** as the T-lymphocytes are replaced by the body over time and therefore the patient requires **regular transfusions** every three to five months to keep their immune systems functioning
- Originally **retroviruses** were used as the vectors, however these viruses insert their genes randomly into a host's genome which means they could insert the gene into another gene or into a regulatory sequence of a gene (which could result in cancer)
- Initial treatments did cause cases of **leukaemia** in children, so researchers switched to using lentiviruses or adeno-associated viruses as vectors. **Lentiviruses** also randomly insert their genes into the host genome however they can be modified to not replicate, whereas **adeno-associated viruses** do not insert their genes into the host genome and therefore the genes are not passed onto the daughter cells when a cell divides. This is an issue with short-lived cells like lymphocytes but has not been a problem when used with longer living cells such as liver cells

Inherited eye diseases

- An example of a group of inherited eye diseases that causes blindness due to damage to the light receptors in the retina are **Leber Congenital Amaurosis**. It begins to affect children from birth and by their 20s or 30s the person is totally blind. There is no cure for these diseases
- Using **in vivo somatic gene therapy,** doctors injected into the retina **adeno-associated viruses** that contained the normal alleles of one of the genes that caused damage to the photoreceptors (there are at least 18 known mutated genes causing this group of diseases). All patients that have had the injections have shown improvement in their eyesight

<u>Social & Ethical Considerations</u>

- The use of **gene technology** (genetic screening and gene therapy) in medicine is becoming more common
- Genetic screening in medicine is being used to:
    - Allow people with a **family history** of a genetic disease to have their DNA analysed to determine if they are at risk
    - Carry out **pre-implantation genetic diagnosis (PGD)** – embryos that are created outside the body (with the IVF procedure) have their DNA analysed, which allows for embryos that are not carrying a harmful allele that would cause the disease, to be chosen for implantation
- Gene therapy is being used in medicine for introducing corrected copies of genes into patients with genetic diseases (eg. **cystic fibrosis**, **haemophilia**, **severe combined immunodeficiency**)

Genetic screening

- There are many social and ethical considerations for genetic screening, which include:
    - Being able to **take preventative measures** (e.g. elective mastectomy when BRCA1 and BRCA2 are detected) – giving individuals control to prevent illness
    - Using **pre-implantation genetic diagnosis** to **select embryos** that do not carry faulty disease-causing alleles. This could lead to the fear of "**designer babies**' being created (this includes creating/choosing embryos with tissue matches to older siblings). Pre-implantation genetic diagnosis can be carried out during **in-vitro fertilisation (IVF)**; cells are extracted from the embryo in an **embryo biopsy** and genetically screened in order to **preselect** the embryos without faulty alleles
    - Using **genetic counsellors** to help people understand their choices and make informed decisions (eg. financial costs, whether termination of fetus is appropriate if quality of life is poor)
    - **Risk of miscarriage** (which has emotinal consequences) due to the procedures used to collect DNA which are not 100% risk-free
        - **Amniocentesis** – is used to obtain a sample of amniotic fluid using a hypodermic needle at 15 to 16 weeks of pregnancy
        - **Chorionic villus sampling** – is used to obtain a small sample of the placenta using a needle between 10 and 13 weeks of pregnancy
- Choosing to terminate a pregnancy (**therapeutic abortion**) because the embryo has a genetic disorder (eg. Thalassaemia or cystic fibrosis) or even terminating the embryo due to a minor 'defect' that could have seen the child lead an almost normal life
- Being able to make **informed reproductive decisions** (eg. Thalassaemia)
- Determining whether it is best to know the **risk of having a disease**, especially when there is no cure (eg. Huntington's)
- Deciding at what age screening should begin eg. whether parents should be able to choose for their children to be screened
- The possibility of **stigmatization and discrimination**. The person may feel stigmatized if they have the disease or discriminated against by health insurers or employers
- Confidentiality of the data collected – who will have the right to view the results obtained

Social & ethical considerations of using gene therapy

- The social and ethical considerations of using gene therapy include:
    - The **potential for side effects** that could cause death (eg. the children who were treated for **SCID** developed leukaemia)
    - Whether **germline gene therapy** (the alteration of genes in egg and sperm cells which results in the alteration being passed onto future generations) should be allowed – it could be a cure for a disease or it could create long-term side effects
    - The **commercial viability** for pharmaceutical companies – if it is a rare disease, the relative small number of patients may not mean that the companies will make a profit (eg. Glybera – a gene therapy for lipoprotein lipase deficiency is no longer produced as there were too few patients)
    - The **expense of treatments** as multiple injections of the genes may be required if the somatic cells are short-lived (eg. severe combined immunodeficiency). This may make the cost of gene therapy accessible to a limited number of people
    - The possibility that people will become **less accepting of disabilities** as they become less common
    - Who has the right to determine **which genes can be altered** and which cannot (eg. should people be allowed to enhance intelligence or height)
    - Another method of enhancing sporting performances unfairly through **gene doping**. This is where the genes are altered to give an unfair advantage eg. to provide a source of erythropoietin (the hormone that promotes the formation of red blood cells)

<u>Genetic Engineering: Use in Agriculture</u>

- Genetic engineering is a technique used to deliberately modify a specific characteristic (or characteristics) of an organism
- The technique involves **removing a gene** (or genes) with the desired characteristic from **one organism** and **transferring** the gene (using a **vector**) **into another** organism where the **desired gene is then expressed**
- The **genetically engineered organism** will then contain **recombinant DNA** and will be a genetically modified organism (GMO)
- Although plants and animals have been genetically engineered to produce proteins used in medicine, the main purpose for genetically engineering them is to meet the global demand for food
- Crop plants have been genetically modified to be:
    - **Resistant to herbicides** – increases productivity / yield
    - **Resistant to pests** – increases productivity / yield
    - **Enriched in vitamins** – increases the nutritional value
- Farmed animals have been genetically modified to grow faster. It is rarer for animals to be modified for food production due to **ethical concerns** associated with this practice
- Scientists have genetically modified many organisms including bacteria (eg. to produce insulin), sheep (eg. to produce a human blood protein known as AAT), maize (eg. to be resistant to insect attacks), rice (eg. to produce β-carotene to provide vitamin A)
- The benefits of using genetic engineering rather than the more traditional selective breeding techniques to solve the global demand for food are:
    - Organisms with the **desired characteristics** are **produced more quickly**
    - **All organisms** will contain the desired characteristic (no chance that recessive allele may arise)
    - The desired characteristic may **come from a different species / kingdom**

GM salmon

- In 2015 Aqua Adventure Salmon was approved by the US Food and Drug Authority (FDA) for human consumption
- This salmon has been genetically modified (GM) to **grow more rapidly** than non-GM salmon as a result of growth hormone being produced in the salmon throughout the year, instead of just in spring and summer. The producer therefore has a product to sell in half the time, which increases their yield
- Scientists combined a **growth hormone** gene from a chinook salmon with the promoter gene from an ocean pout, a cold-water fish. The ocean pout fish can grow in near-freezing waters, thus the promoter gene ensured the growth hormone was continually being expressed
- To prevent the GM salmon from reproducing in the wild, all the salmon are female and sterile

Herbicide resistance in soybean

- Growing **herbicide-resistant soybeans** allows farmers to spray a herbicide on the crop after germination to kill weeds that would otherwise compete with the growing soybeans for light, water and minerals, therefore decreasing the yield
- The resistant gene comes from a strain of the bacterium *Agrobacterium*
- This gene allows an enzyme in the soybean to continue to synthesise three amino acids (**phenylalanine**, **tyrosine** and **tryptophan**) needed to produce proteins required in the growing tips of plants
- The herbicide **glyphosate** inhibits the enzyme in plants without the resistant gene; without the proteins being synthesised, the plants die

Insect resistance in cotton

- Cotton has been genetically modified with a gene for the **Bt toxin**, which is taken from the bacterium *Bacillus thuringiensis*
- Cotton plants modified with the Bt toxin gene produce their own insecticide
- When an insect ingests parts of the cotton plant, the alkaline conditions in their guts activate the toxin (the toxin is harmless to vertebrates as their stomach is highly acidic), killing the insect
- Different strains of ***thuringiensis*** produce different toxins which are toxic to different insect species
- Insect populations have developed resistance to the genes for Bt toxin, reducing effectiveness as a means of protecting crops

GMOs in Food: Social & Ethical Implications

- The genetic modification of microorganisms for the production of medicines, antibiotics and enzymes raises little debate compared to the use of genetically modified organisms (GMOs) for food production
- The use of GMOs in food production has been proposed as a solution to feeding the increasing world population, the decreasing arable land and decreasing the impact on the environment, however concerns such as the development of resistance in insects and weeds and costs of seeds have meant that countries are not allowing GMOs to be grown
- The solution could be integrated pest-management systems that could help avoid the development of resistance and increased population of secondary pests

Ethical implications

- The ethical implications of using GMOs in food production are:
    - The lack of long-term research on the effects on human health – should GM food be consumed if it is unknown whether it will cause allergies or be toxic over time (although there has been no evidence to suggest this would occur to date)
    - Making choices for others:
        - That without appropriate labelling the consumer cannot make an informed decision about the consumption of GM foods
        - As the pollen from the GM crop may contaminate nearby non-GM crops that have been certified as organic
        - By reducing the biodiversity for future generations

Social implications

- The social implications of growing GMOs for food evolve around whether the crops are safe for human consumption and for the surrounding environment
- The possible implications are:
    - The GM crops may become weeds or invade the natural habitats bordering the farmland
    - The development of resistance for the introduced genes in the wild relative populations
    - Potential ecological effects (e.g. harm to non-targeted species like the Monarch butterflies)
    - Cost to farmers (new seed needs to purchase each year)
    - Could cause allergic reactions
    - The ability to provide enriched foods to those suffering from deficiencies (eg. Golden Rice) and therefore decrease in diseases
    - Reduced impact on the environment due to there being less need to spray pesticides (eg. less beneficial insects being harmed)
    - Reduction in biodiversity which could affect food webs
    - The herbicides that are used on the GM crops could leave toxic residues

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