RAFFLES INSTITUTION

H2 BIOLOGY LECTURE NOTES

Syllabus 9744

2018 – 2019

3

CORE IDEA 1: THE CELLS & MOLECULES OF LIFE
CORE IDEA 2: GENETICS & INHERITANCE
CORE IDEA 3: ENERGY & EQUILIBRIUM

Topics

1. Respiration
2. Photosynthesis
3. Genetic Basis for Variation I
4. Genetic Basis for Variation II
5. Molecular Biology Techniques
6. Homeostasis
7. Cell Signalling
8. Stem Cells

Name: **Class:**

CORE IDEA
(3) Energy and Equilibrium

RESPIRATION

Content
- Respiration as an energy-releasing process
- Aerobic Respiration
- Anaerobic Respiration

Learning Outcomes
Candidates should be able to:
(f) outline the process of glycolysis, highlighting the location, raw materials used and products formed (knowledge of details of the intermediate compounds and isomerisation is not required)
(g) outline the processes of the link reaction and Krebs cycle, highlighting the location, raw materials used and products formed (in terms of dehydrogenation and decarboxylation)
(h) outline the process of oxidative phosphorylation including the role of oxygen and the electron transport chain in aerobic respiration (names of complexes in the ETC are not required)
(i) explain the production of a small yield of ATP from respiration in anaerobic conditions in yeast and in mammalian muscle tissue
(j) explain the significance of the formation of ethanol in yeast and lactate in mammals in the regeneration of NAD
(k) investigate the effect of factors such as substrate concentration, type of substrate and temperature on the rate of respiration
(l) outline chemiosmosis in photosynthesis and respiration (names of complexes in the ETC are not required). Use the knowledge gained in this section in new situations or to solve related problems.

References
Main reference:
- Campbell, Reece *et al.* (2015) Biology, 10th edition. Benjamin-Cummings Publishing Co.
- Raven, Johnson, Losos, Mason and Singer (2008). Biology, 8th edition. McGraw-Hill.

*** This handout is the effort of several Biology teachers at RI. It has been and will continue to be updated.**
**** Any content given in a double-lined box is for your information only.**

(1) RESPIRATION - OVERVIEW

- Cellular respiration is defined as 'the process by which chemical energy in organic molecules is released by oxidation' i.e. it involves metabolic processes which allow organisms to obtain energy from organic molecules.
- Under this broad definition, there are 2 main energy-producing pathways:
  (1) **aerobic respiration** which occurs in the **presence of oxygen.**
  (2) **anaerobic respiration** which occurs in the **absence of oxygen.**

- Functions of respiration:
  Respiration is an energy-releasing process. As ATP is the primary energy currency of the cell, main function of respiration is production of ATP.
  Some examples where ATP is required:
  (1) muscle contraction, beating of cilia (e.g. on cells lining trachea), beating of flagella (e.g. in sperm, bacteria)
  (2) active transport of substances into or out of cells (e.g. Na^+-K^+ pump in cell membranes)
  (3) synthesis of substances for growth and repair (e.g. translation)
  (4) electrical transmission of nerve impulses
  (5) maintenance of constant body temperature i.e. in homoeothermic/ warm-blooded animals
  (6) bioluminescence by fireflies, glow-worms and some deep sea animals

- Locations of respiration:
  - Aerobic: Partly in **cytosol** and partly in **mitochondria** of the cell.
  - Anaerobic: In **cytosol** of the cell.

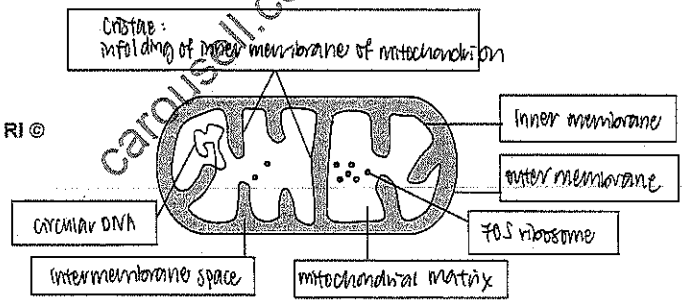


Figure 1. Diagram of a mitochondrion

(2) AEROBIC RESPIRATION

- In a eukaryotic cell, aerobic respiration involves 4 main stages *(see Fig. 2 for overview)*:
  (1) **glycolysis** in cytosol
  (2) **link reaction** in mitochondrial matrix
  (3) **Krebs cycle** in mitochondrial matrix
  (4) **oxidative phosphorylation** involving electron transport chain on cristae

in mitochondria

- All 4 stages require many different **enzymes**.

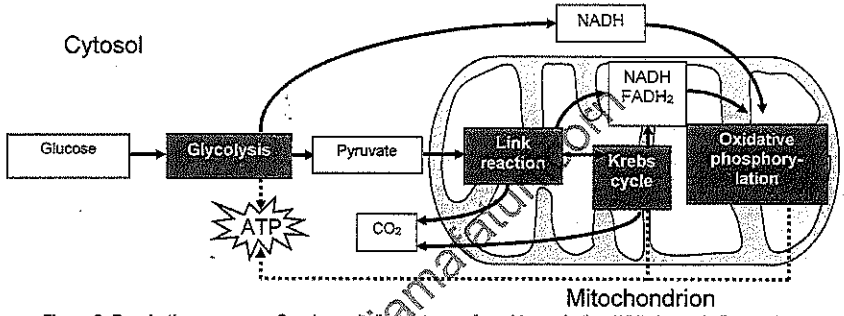


Figure 2. Respiration summary. Grey boxes indicate stages of aerobic respiration. White boxes indicate substrates and products.

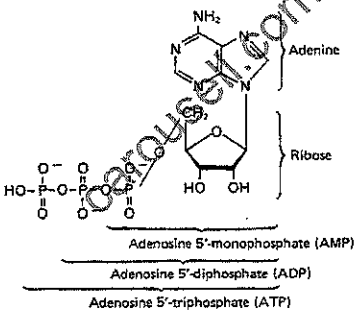


Figure 3. Structure of ATP. Loss of terminal phosphate group results in an ADP molecule, as occurs during the first stage of glycolysis. Loss of 2nd phosphate group will produce AMP.
Note: ATP is a ribonucleotide.

Suitability of ATP as an energy source:

- Universally used – all cells and organisms use it as an energy source.
- Soluble, highly mobile and can be transported readily/ diffuses readily to point of need.
- Easy interconversion: ADP + P_i forms ATP, and ATP can be hydrolysed to ADP + P_i, leading to energy release. (easily interconverted between ADP & ATP)

Cellular respiration

Aerobic respiration
- $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O +$ ATP
  (complete oxidation of glucose)

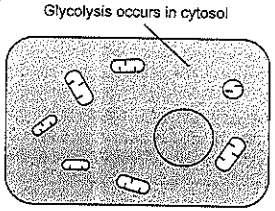
Anaerobic respiration
- $C_6H_{12}O_6 \rightarrow$ 2 lactic acid + ATP
- $C_6H_{12}O_6 \rightarrow$ 2 ethanol + $2CO_2$ + ATP
  (incomplete oxidation)

- ▶ High energy levels → high ATP, low ADP
- ▶ Low energy levels → low ATP, high ADP

2.1 Glycolysis

- The first stage of respiration involves glycolysis – oxidation of **glucose (6C)** to form **2 pyruvate (3C)**. In effect, a 6 carbon sugar is split into two 3-carbon sugars. (Glycolysis = splitting of sugar)

very stable, requires many enzymes to be hydrolysed into pyruvate



- It **does not require** O_2 (it occurs whether or not O_2 is present) and **does not release** CO_2.
- It can be divided into 4 stages:

(1) Phosphorylation of glucose

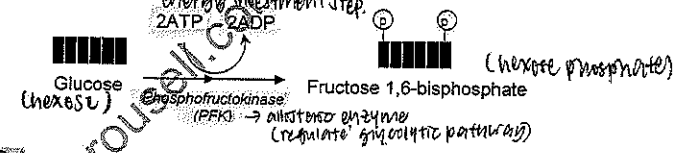
- ▶ Firstly, it involves **initial investment of 2 ATP molecules** – 1 phosphate groups from each of the 2 ATP are used to phosphorylate glucose to produce **fructose 1,6-bisphosphate.**
- ▶ This phosphorylation activates the sugar, making it more reactive and committing it to the glycolytic pathway. Phosphorylation also confers a negative charge to on glucose, making it impermeable, cannot diffuse across cell membrane hence it is trapped within the cytosol.

Why invest energy?
1. So lysis of glucose can proceed
2. To commit glucose to pathway (when glucose is phosphorylated, it is unable to pass through channel protein to exit the cell)

- ▶ **Phosphofructokinase (PFK)** catalyses addition of the 2nd phosphate group.
  - This enzyme is **inhibited by excess ATP and/or citrate** in the cell. Since the ultimate goal of glycolysis is to produce ATP and too much of this end product (ATP) inhibits the enzyme, this is an example of **end product inhibition** (allosteric inhibition).
  - Conversely, PFK is **stimulated by AMP and ADP (allosteric activators)**. Thus, rate of glycolysis is regulated according to the energy demands of the cells.
    (Note: Hexokinase catalyses addition of the first phosphate)

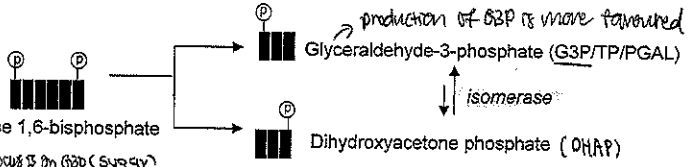
-kinase → phosphorylates something

energy investment step



(2) Lysis

- ▶ Next, phosphorylated 6C sugar (fructose 1,6-bisphosphate) is **split** into two 3C sugar phosphates: **glyceraldehyde-3-phosphate (G3P)** and dihydroxyacetone phosphate. G3P is also known as triose phosphate (TP) and phosphoglyceraldehyde (PGAL).
- ▶ The 2 sugar phosphates (G3P and dihydroxyacetone) are isomers of each other, and can be converted from one form to the other through the action of an isomerase. The reaction favours formation of G3P, since it is constantly removed by the next step of the reaction.



production of G3P is more favoured

(3) Oxidation by dehydrogenation

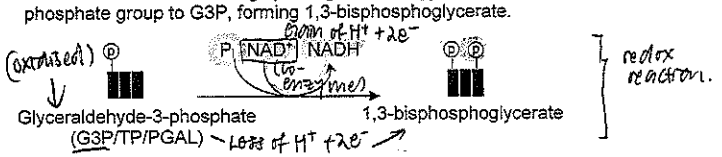
focus is on G3P (sugar)

- ▶ Oxidation can be defined in 3 ways:
  (a) Addition of oxygen
  (b) Removal of hydrogen **(dehydrogenation)**

process ($H^+ + e^-$)

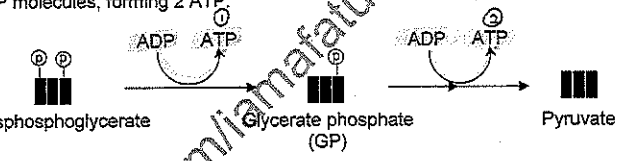
(c) Removal of electrons

- During glycolysis, oxidation of respiratory substrate occurs by **dehydrogenation.**
- The substrate G3P is oxidised by dehydrogenation. At the same time, the **coenzyme NAD⁺ is reduced to NADH** (remember: oxidation must always be coupled to a reduction). *(for details about NAD⁺, refer to pg 10)*
- As this redox reaction is highly exergonic, energy released is used to add a second phosphate group to G3P, forming 1,3-bisphosphoglycerate.



(4) Substrate-level phosphorylation

- Next, 1,3-bisphosphoglycerate is dephosphorylated to form the final product of glycolysis, **pyruvate.**
- The 2 phosphate groups on 1,3-bisphosphoglycerate are transferred by enzymes to 2 ADP molecules, forming 2 ATP.



- **Since each G3P yields 2 ATP and 1 NADH, 2 G3P molecules will yield 4 ATP and 2 NADH.**
- This process whereby an enzyme transfers a phosphate group directly from a substrate molecule (i.e. an organic molecule generated during the oxidation of glucose) to ADP is known as **substrate-level phosphorylation** *(as opposed to oxidative phosphorylation, refer to pg 10).* [refer to pg 6]

Considering the 2 ATP expended earlier as an initial investment, there is a **net gain of 2 ATP** and **2 NADH per glucose.**

Overall equation for glycolysis would be:

Glucose + 2 ADP + 2 P_i + 2 NAD^+ → 2 pyruvate + 2 ATP + 2 NADH

What happens to pyruvate at the end of glycolysis?

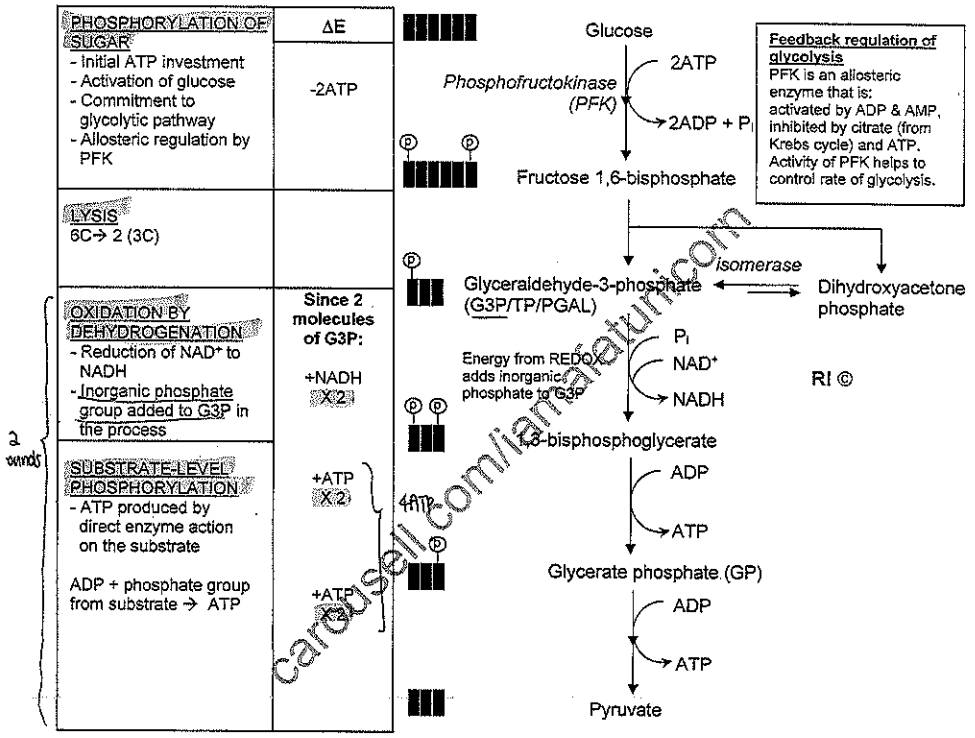
(1) If O_2 unavailable → pyruvate converted into ethanol or lactate (anaerobic respiration)

(2) If O_2 available → pyruvate enters mitochondrion into next stage in aerobic respiration — link reaction

▮ - C atom

Ⓟ - phosphate group

Glycolysis Flowchart



Summary of Glycolysis

Energy is transferred from glucose to pyruvate, ATP and NADH

For each glucose molecule:

No. of ATP used up: 2

No. of ATP produced: 4

Net ATP produced: 2 No. of NADH produced: 2

Overall equation: Glucose + 2ADP + 2Pi + 2NAD^+ → 2 pyruvate + 2ATP + 2NADH

enzymes (for oxidative decarboxylation)
↳ dehydrogenases & decarboxylases.

2.2 Link Reaction / Oxidative Decarboxylation

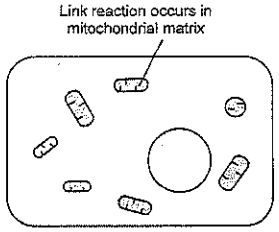
A transport protein embedded in the mitochondrial membrane translocates pyruvate from cytosol into mitochondrion via active transport.

① ► Within matrix, each pyruvate (3C) is **decarboxylated.** This involves removal of C from pyruvate, through the **loss of CO_2.**

② ► **Oxidation by dehydrogenation** occurs, yielding **NADH** and a 2C compound.

③ ► This 2C compound combines with coenzyme A to form **acetyl coenzyme A (acetyl CoA).**

► Acetyl CoA moves on into Krebs cycle, which also takes place in mitochondrial matrix



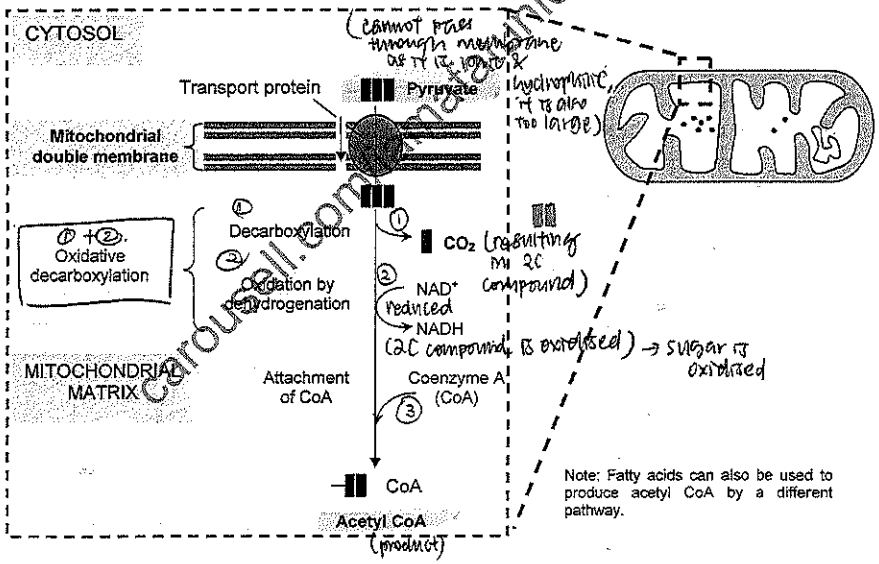
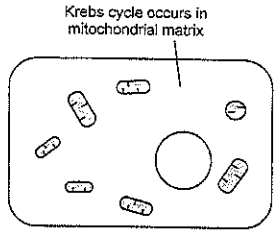


Figure 4. Link reaction. Each pyruvate is converted to acetyl CoA, with production of one molecule of CO_2 and NADH.

stage	location	start compound & end product	for 1 molecule of glucose: No. of ATP	No. of NADH	No. of $FADH_2$	No. of CO_2
Glycolysis	cytoplasm	Glucose → 2 pyruvate	2 (net)	2	–	–
Link reaction	mitochondrion matrix	2 pyruvate → 2 acetyl CoA	–	2	–	2
Krebs cycle	mitochondrial matrix	–	2	6	2	4

2.3 Krebs Cycle (citric acid cycle (TCA cycle))

- Occurs in the mitochondrial matrix.
- Krebs cycle involves 3 main stages:
  - (1) **Acetyl CoA (2C)** enters the cycle by combining with **oxaloacetate (4C)** to form **citrate (6C).**
  - (2) Citrate is decarboxylated and dehydrogenated to form **α-ketoglutarate** (5C) and NADH.
    - A decarboxylation step results in a loss of carbon in the form of CO_2. This process involves **oxidative decarboxylation.** (↗ 2× cycle repeats)
  - (3) **Oxaloacetate** (4C) is regenerated. This involves
    - 1 decarboxylation step to yield 1 CO_2
    - 3 dehydrogenation steps, to yield 2 NADH, 1 $FADH_2$ (reduced **F**lavin **A**denine **D**inucleotide, another coenzyme), and
    - 1 substrate-level phosphorylation to yield 1 ATP



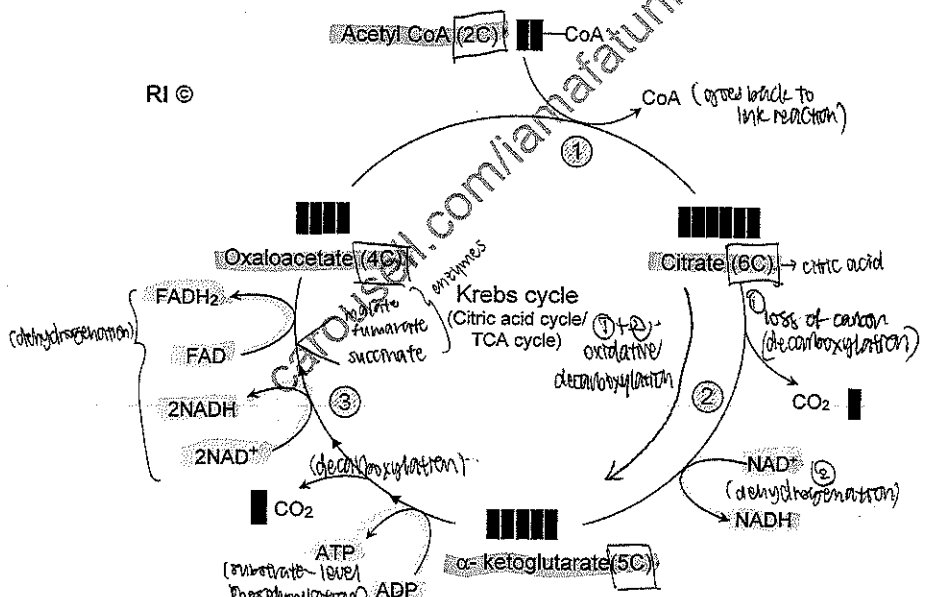


Figure 5. Krebs Cycle. It involves 3 main stages: (1) formation of citrate, (2) oxidation of the carbon substrate and (3) regeneration of oxaloacetate.

Note: Acids exist as salts in physiological systems and their names end with –ate. Citric acid is thus referred to as citrate.

- Since for 1 glucose molecule, glycolysis yields 2 pyruvate and hence 2 acetyl CoA, **two rounds of Krebs cycle** are needed to completely oxidise one molecule of glucose.
- All the 6 carbons in glucose are lost as 6 CO_2. For each 6C glucose molecule, 4 CO_2 are released through Krebs cycle while 2 CO_2 are released in link reaction.

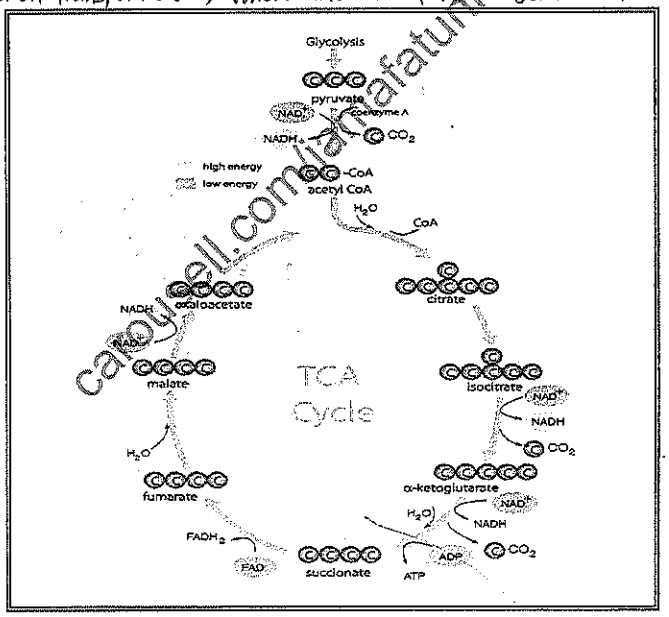
Summary of Krebs Cycle

Product	No. of molecules per acetyl CoA fed in	No. of molecules per glucose molecule
CO_2		
NADH		
$FADH_2$		
ATP		

Note: ATP produced in Krebs cycle is obtained from substrate-level phosphorylation.

Does Krebs cycle produce the bulk of the ATP from a glucose molecule?

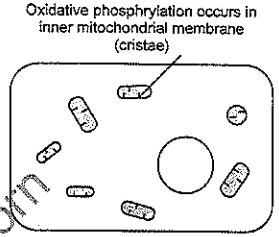
Not directly. Most of the energy released in Krebs cycle is actually carried on the NADH and $FADH_2$ produced. The mobile electron carriers (NADH & $FADH_2$) with their reducing power will next be transported to the electron transport chain, where the bulk of ATP is generated.



- Nicotinamide adenine dinucleotide (NAD)

2.4 Oxidative Phosphorylation

- NADH and $FADH_2$ generated in previous processes (glycolysis, Link reaction and Krebs cycle) will now take part in oxidative phosphorylation *(refer to pg 12 for definition)*.
- Location: inner mitochondrial membrane (cristae), which is highly folded to increase surface area for the accommodation of:
  (1) Many **electron transport chains** (ETC) – a series of electron carriers (mainly proteins), with increasing electronegativity.
    - Each electron carrier has an energy level that is lower than the one preceding it.
    - The electron carriers undergoes reduction oxidation as they accept and donate electrons respectively.
  (2) Many **ATP synthases** (also referred to as: stalked particles) - enzyme complexes involved in ATP synthesis.



- Oxidative phosphorylation occurs in the **presence of oxygen**.
- **Function of NAD⁺ and FAD:**
  - Through glycolysis, link reaction and Krebs cycle, organic food such as carbohydrates, fats and proteins are oxidised to yield high-energy electrons.
  - These electrons (together with protons) are transferred to NAD⁺ and FAD forming NADH (reduced NAD) and $FADH_2$ (reduced FAD).
  - NAD⁺ and FAD serve as **mobile electron carriers**, which transport the high-energy electrons from organic molecules to the electron transport chain in mitochondria.
  - As electrons are passed down the electron transport chain, energy is released and coupled to the formation of ATP *(under a process called Oxidative Phosphorylation, refer to pg 12)*.
  - By passing their electrons to the electron transport chain
    - NADH and $FADH_2$ are oxidised,
    - the coenzymes NAD⁺ and FAD are regenerated, allowing them to pick up more electrons (and protons) from glycolysis, link reaction and Krebs cycle.

What are the components of oxidative phosphorylation?

(1) flow of electrons down the electron transport chain release energy which is used to pump H^+ from mitochondrial matrix, across inner mitochondrial membrane into intermembrane space

(2) production of ATP from ADP and P_i via chemiosmosis

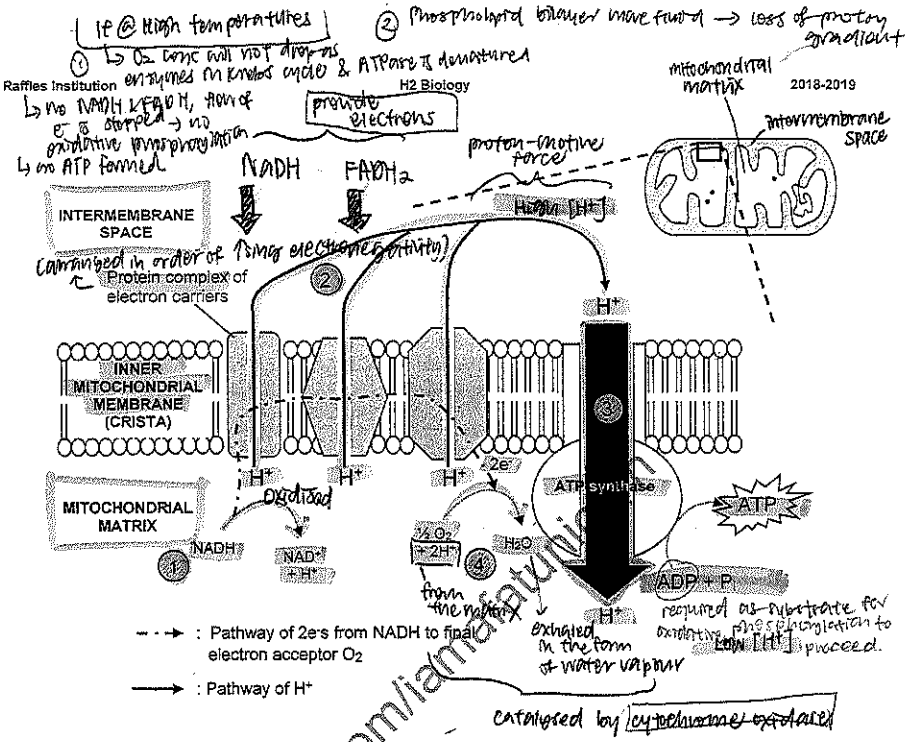


Figure 6. Oxidative phosphorylation involving electron transport chain

- Process of oxidative phosphorylation:

(1) NADH molecules from glycolysis, link reaction and Krebs cycle donate electrons to the first electron carrier of electron transport chain (i.e. first electron carrier become reduced). It then passes its electrons to the next carrier, and so on.
NADH → NAD+ + 2e- (fed into electron transport chain) + H+ (matrix)

(2) As the high-energy electrons travel down the ETC, energy released is coupled to the pumping of H+ ("pumping" = energy is required) from mitochondrial matrix into intermembrane space via some of the electron carriers of ETC.
- Concentration of H+ is thus higher in the intermembrane space. This builds up a proton-motive force across cristae.
- Proton-motive force is potential energy stored in the form of a proton chemical gradient, generated by pumping of H+ across a biological membrane during chemiosmosis.

(3) As H+ diffuses down its concentration gradient through ATP synthase, ADP is phosphorylated to form ATP. This process is called chemiosmosis, where energy stored in the form of a proton gradient across a membrane is used to drive cellular work (e.g. ATP synthesis).

(4) O_2 (molecular oxygen) acts as final electron acceptor, accepts the electrons and combines with H+ producing H_2O.

Note: H_2O produced this way is called metabolic water, which is used by desert animals to supplement their water needs. This step is catalysed by cytochrome oxidase.

Although ½ O_2 is equivalent to a single O atom, the symbol O_2 is deliberately used to emphasise that it is molecular oxygen, not the O atom, which is reduced to H_2O.
Most of the electron carriers of the chain are grouped into 4 main protein complexes. But only 3 complexes are represented in the above diagram.

- Phospholipid bilayer of mitochondrial membranes, like any other membrane, is impermeable to ions like H+. Therefore, a proton gradient can be generated across the inner mitochondrial membrane. The only passage where H+ can traverse the membrane is through ATP synthase.
- 90% of the ATP produced during aerobic respiration is produced via **chemiosmosis**.
- Importance of oxygen:
  - Presence of O_2 (molecular oxygen), the final electron acceptor, allows the electron carriers NADH and $FADH_2$ to continue donating their electrons to the chain, thereby generating ATP.
    - **Overall, presence of O_2 allows oxidative phosphorylation to continue, to generate ATP via chemiosmosis.**
  - The coenzymes NAD^+ and FAD are regenerated when NADH and $FADH_2$ donate electrons to electron transport chain. This allows NAD^+ and FAD to pick up more electrons and protons from glycolysis, link reaction and Krebs cycle.
  - Reduction of oxygen to water removes H+ from matrix, contributing to the generation of a proton gradient across inner mitochondrial membrane.
- Electron transport chain serves two important functions:
  (1) generating a proton motive force to produce ATP
  (2) regeneration of coenzymes NAD^+ and FAD. Without the regeneration of these coenzymes, glycolysis, Link reaction and Krebs cycle (the rest of the respiratory processes) would not be able to continue efficiently.

Summary of Oxidative Phosphorylation

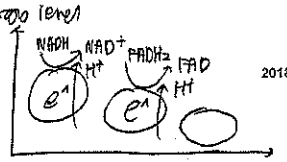
In aerobic respiration, **Oxidative Phosphorylation**

relates to
- reduced coenzyme molecules (i.e. NADH and $FADH_2$) being reoxidised to NAD^+ and FAD in the presence of O_2 and with energy released in the process.

relates to
- addition of a phosphate group to ADP to form ATP. This ATP synthesis is driven by the electrochemical proton gradient.

Thus, oxidative phosphorylation is the process by which the exergonic reoxidation of NADH and $FADH_2$ by oxygen is coupled to the formation of ATP, with an electrochemical proton gradient as an intermediate.

Features of oxidative phosphorylation



2.5 ATP produced per glucose molecule

- Each NADH entering electron transport chain can yield 3 ATP.
  ↳ greater concentration gradient of proton motive force
- Each $FADH_2$ entering electron transport chain can yield 2 ATP. This is because $FADH_2$ enters the chain at a lower energy potential i.e. it passes its electrons to a protein that is further down the chain. Hence, the electrons 'fall' down a smaller energy difference, and less energy is released to pump (less) H^+ across the membrane

Summary on the number of various products per glucose molecule:

	CO_2	ATP	NADH	$FADH_2$
Glycolysis	-	2 (net)	2	-
Link reaction	2	-	1X2 = 2	-
Krebs cycle	4	2	3X2 = 6	2
Sub-total	$6CO_2$	4ATP	10 NADH	$2FADH_2$
Oxidative phosphorylation	-	From NADH: 10X3ATP = 30 From FADH: 2X2 ATP = 4 34 ATP	-	-
Total ATP:		38		

Note: Depending on cell type, less than **38 ATP per glucose molecule** may be produced. This is because mitochondrial membrane is impermeable to the NADH generated by glycolysis. Electrons and protons of NADH are passed to either NAD^+ or FAD inside mitochondrion via a shuttle system. If passed to FAD, then only 2 ATP will be produced instead of 3.

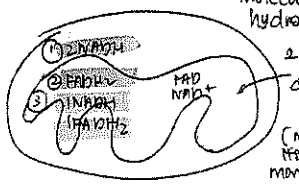
- For every glucose molecule,
  - 2 ATP in glycolysis + 2 ATP in Krebs cycle are formed through **substrate-level phosphorylation.**
  - 10 NADH and 2 $FADH_2$ enter electron transport chain to produce 34 ATP via **oxidative phosphorylation.**
  - In total, a potential 34 + 2 + 2 = 38 ATP are generated (i.e. 38 moles of ATP are generated from 1 mole of glucose).
- Oxidative phosphorylation thus, contributes to a whopping 90% of the total ATP generated.

per 1 molecule of glucose

	SLP	NADH	$FADH_2$
Glycolysis	net 2	2	2
Link Reaction	0	2	0
Krebs cycle	2	6	2
	4	8	4
		8X3 = 24	4X2 = 8

38 ATP

36 - 38 ATP



(Larger molecule; hydrophilic)
2 NADH donate e-
(NADH itself cannot move through as an entire molecule into mitochondrial matrix)

structure of phospholipid membrane

(3) Anaerobic Respiration

- What happens to the processes involved in aerobic respiration when oxygen is absent?
  - In the **absence of oxygen**, there is **no final electron acceptor** to accept electrons from the ETC and oxidative phosphorylation cannot proceed.
  - Electron carriers within the ETC remain reduced, and NADH and $FADH_2$ can no longer donate electrons to the ETC.

What happens if NADH and $FADH_2$ remain reduced?

- How do some cells continue to produce ATP?
  - In the absence of oxygen, anaerobic respiration takes place.
  - Anaerobic respiration takes place in **cytosol** and it involves **glycolysis, followed by fermentation**.
  - Fermentation processes are pathways to regenerate NAD^+ from NADH, thereby ensuring a steady supply of NAD^+ for glycolysis to continue to produce 2 ATP per glucose.
  - Two common fermentation pathways to regenerate NAD^+ from NADH:
    (1) **alcohol fermentation**
    (2) **lactic acid fermentation**
    ↳ yield same amounts of ATP (2 ATP)
  - Since oxygen is no longer present, **pyruvate** or its derivative **ethanal**, becomes the **final electron acceptor** to regenerate NAD^+ via lactate and alcohol fermentation respectively.
- Functions of anaerobic respiration:
  (1) **To produce a small yield of energy.**
  2 ATP are produced per glucose molecule, through glycolysis. It produces 2/38 = 1/19 the amount of ATP generated in aerobic respiration.
  (2) **To regenerate NAD^+ from NADH.**
  During glycolysis, oxidation of glucose to yield 2 ATP leads to reduction of NAD^+ to form NADH. In order for glycolysis to continue, NAD^+ needs to be regenerated. Alcohol and lactic acid fermentation include processes that recycle NAD^+ concentrations in the cytosol.

Where does anaerobic respiration occur within the cell? Does it involve the mitochondria?

3.1 Alcohol fermentation

- Following glycolysis, **pyruvate decarboxylase** converts **pyruvate** (3C) to **ethanal/acetaldehyde** (2C) through decarboxylation (CO_2 is released).
- **NADH** produced during glycolysis has to be **recycled / regenerated to NAD$^+$** so that glycolysis can continue to produce 2 ATP each round.
- **Alcohol dehydrogenase** reduces ethanal/ acetaldehyde **(final electron acceptor)** to **ethanol**, while removing H^+ from NADH to reform NAD$^+$. → oxidation.
- Alcohol fermentation occurs in yeast and certain bacteria. It is used in wine and beer production, through anaerobic action of yeast.

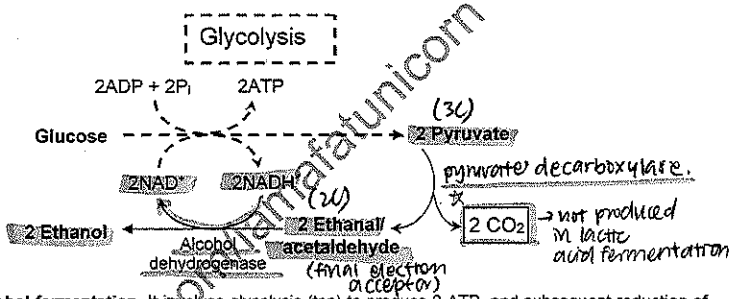


Figure 7. Alcohol fermentation. It involves glycolysis (top) to produce 2 ATP, and subsequent reduction of ethanal (bottom) to regenerate NAD$^+$. NAD$^+$ is fed back into glycolysis so that ATP production in glycolysis can continue. Ethanol and CO_2 are the end products.

3.2 Lactic acid fermentation

- When a muscle contracts, the exceptionally high demand for ATP is met by a dramatic increase in rate of glycolysis. This rapidly depletes the limited supply of NAD$^+$.
  - ► Oxidative phosphorylation, which normally replenishes NAD$^+$ supply, may not be able to replenish the NAD$^+$ quickly enough. Hence, muscle cells have to rely on an **additional** system to regenerate NAD$^+$: anaerobic respiration (glycolysis followed by fermentation, a process that regenerates NAD$^+$).
  - ► Thus, anaerobic respiration can occur concurrently with aerobic respiration. The two pathways do not have to be mutually exclusive.
- **Pyruvate** is the **final electron acceptor** and is reduced to become **lactic acid**/lactate. NAD$^+$ is regenerated in the process.
- **Lactate dehydrogenase** catalyses the reaction.
- The end products are 2 ATP and 2 lactate per glucose molecule.
  - ► **No CO_2 is produced** since no decarboxylase is involved (unlike in alcohol fermentation).

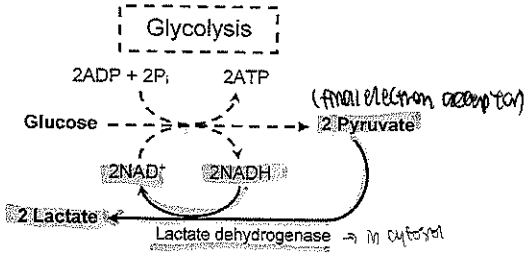


Figure 8. Lactic acid fermentation. This involves both glycolysis (top) followed by lactic acid fermentation (bottom)

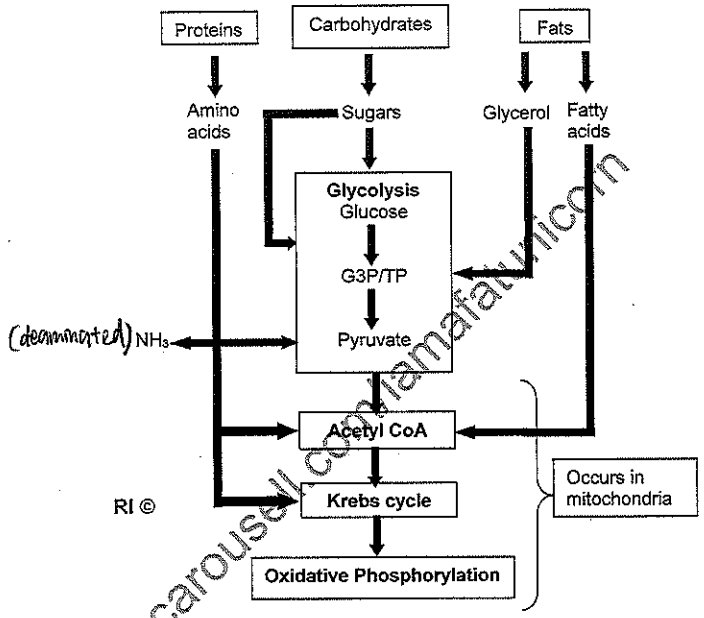
Note: Only 2 ATP per glucose are produced during anaerobic respiration, compared to 38 ATP produced during aerobic respiration!

- During strenuous exercise, lactic acid accumulates in the muscle faster than it can be removed.
  - ► As excessive lactic acid accumulates, muscle fatigue eventually sets in.
- As with ethanol, lactate still contains a large part of the energy originally contained in glucose.
  - ► Lactate is eventually carried from muscle cells via blood to liver, where it can be converted back into pyruvate by liver cells. This pyruvate can then enter link reaction and Krebs cycle during aerobic respiration to produce more ATP.
- Lactic acid fermentation is used in cheese and yoghurt production, through anaerobic action of certain bacteria and fungi.

Greater efficiency if respiration occurs in steps

(4) Other substrates used in respiration

Apart from glucose, other sugars, proteins and fats can also be used as respiratory substrates. These enter the respiratory cycle at various points. Note that amino acids are first deaminated (amino group is removed) before the remaining acid enters the cycle:



5) Summary of Important Terms

H^+ movement across a partially-permeable membrane, down its gradient, to phosphorylate ADP to ATP

1. **Chemiosmosis** – mechanism by which energy stored in the proton gradient drives ATP synthesis.
2. **Oxidative phosphorylation** – mechanism by which the exergonic re-oxidation of reduced coenzyme molecules (NAD^+ and FAD) by O_2 is coupled to the formation of ATP, with an electrochemical proton gradient as an intermediate.
3. **Proton motive force** – potential energy stored in the form of a proton gradient, generated by by pumping of H^+ across a membrane.
4. **Substrate-level phosphorylation** – synthesis of ATP by direct enzyme action. An enzyme transfers a phosphate group directly from a substrate molecule to ADP to form ATP.

KEYWORDS include

Processes	Molecules	Organelles
chemiosmosis	ATP	cristae
electron transport chain	ATP synthase	cytosol
glycolysis	carbon dioxide	matrix
Krebs cycle	electrons (and protons)	mitochondria
link reaction	final electron acceptor	
lysis	NAD^+/ FAD	
oxidation by dehydrogenation	NADH / $FADH_2$	
oxidative phosphorylation	oxygen / molecular oxygen	
phosphorylation	water	
proton motive force	lactate	
proton gradient	lactate dehydrogenase	
regenerate NAD^+ and FAD		

TRUE OR FALSE?

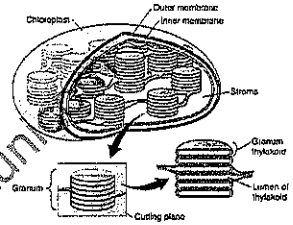
- ☐ Glycolysis produces 4 ATP.
- ☐ The mitochondrion is the organelle where all respiratory processes take place.
- ☐ During respiration, O_2 is reduced to form CO_2.
- ☐ Through respiration, chemical energy in organic food is converted to heat energy + energy stored in the form of ATP
- ☐ Water formed during respiration is called metabolic water.
- ☐ Microorganisms such as yeast can carry out anaerobic respiration; large mammals such as humans cannot.
- ☐ Proteins and fats can also be used to produce energy through respiration. However, they first need to be converted to glucose, as glucose is the primary respiratory substrate.
- ☐ Oxygen is a double-edged sword – while we need it to respire and produce ATP, it is also a cause of ageing in our cells.

CORE IDEA
(3) Energy and Equilibrium

PHOTOSYNTHESIS

Content

- The need for energy in living organisms.
- Photosynthesis as an energy-trapping process.



Learning Outcomes:

Candidates should be able to:

(a) Identify components of chloroplasts and mitochondria in drawings, photomicrographs and electron micrographs.
(b) Explain the absorption and action spectra of photosynthetic pigments.
(c) With reference to the chloroplast structure, describe and explain how light energy is harnessed and converted into chemical energy during the light-dependent reactions of photosynthesis.
(d) Outline the three phases of the Calvin cycle in C3 plants: (i) CO_2 fixation, (ii) PGA reduction and (iii) ribulose bisphosphate (RuBP) regeneration, indicating the roles of rubisco, ATP and reduced NADP in these processes that ultimately allow synthesis of sugars.
(e) Discuss limiting factors in photosynthesis and carry out investigations on the effect of limiting factors such as temperature, light intensity and carbon dioxide concentration on the rate of photosynthesis.
(f) Outline chemiosmosis in photosynthesis (names of complexes in the ETC are not required).

Use the knowledge gained in this section in new situations or to solve related problems.

References:

- Reece, J. B., Urry, L. A., Cain, M. L., Wasserman, S. A., Minorsky, P. V., & Jackson, R. B. (2011). *Campbell Biology* (9th ed.). San Francisco: Pearson Benjamin Cummings.
- Garrett, R. H., & Grisham, C. M. (2010). *Biochemistry* (4th ed.). Belmont, CA: Brooks/Cole, Cengage Learning.

*** This handout is the effort of several Biology teachers at RI (Yr 5-6). It has and will continue to be updated.**
**** Any information given in a double-lined box is for your information only.**

TABLE OF CONTENTS

1) Introduction

- Organisms derive all their metabolic energy needs in this world from the sun. Consumers such as carnivores and herbivores get their energy ultimately from producers and producers in turn derive their energy from the sun by the process called photosynthesis.
- Photosynthesis converts carbon (inorganic CO_2) into organic compounds, a form that we can utilize, to fulfill our energy needs. It is also a source of carbon, which is needed for the synthesis of other organic compounds e.g. proteins, lipids and nucleic acids.
- Energy is needed for many processes in the living organism:
  1. Drive chemical reactions e.g. metabolic processes, regulation of internal environment such as maintaining body temperature.
  2. Active transport systems e.g. nerve action, absorption
  3. Synthesize new material (i.e. anabolism) e.g. growth
  4. Muscular contraction e.g. locomotion, blood circulation
  5. Reproduction
- An autotroph is an organism that makes organic compounds from simple molecules. Photoautotrophs are able to convert light energy into chemical energy, which can be used to drive the above-mentioned processes.
- The table below summarizes the various modes of nutrition that different types of organisms adopt.

Energy source / Carbon Source	Chemical (Chemotrophic)	Light (Phototrophic)
Inorganic CO_2 (Autotrophic)	**(Chemoautotrophic)** e.g. nitrifying bacteria that feed on NO_2^- such as nitrobacter	**(Photoautotrophic)** e.g. green plants, blue-green algae (cyanobacteria)
Organic (Heterotrophic)	**(Chemoheterotrophic)** e.g. mammals, fungi and most bacteria	**(Photoheterotrophic)** e.g. non-sulfur purple bacteria

- ***Definition of photosynthesis:***
  The **conversion of light energy to chemical energy** that is stored in glucose and other organic compounds.

PHOTOSYNTHESIS

relates to
- **light-dependent reaction**
- Photochemical events whereby energy from visible light is converted into high energy intermediates and ATP. These products are then used to drive the reactions in the next stage of photosynthesis.

relates to
- **light-independent reaction**
- Fixing atmospheric carbon in the form of CO_2 into sugar molecules using the high energy intermediates and ATP from light-dependent reaction.
  This will continue to take place so long as the products of the light-dependent reactions have not run out.

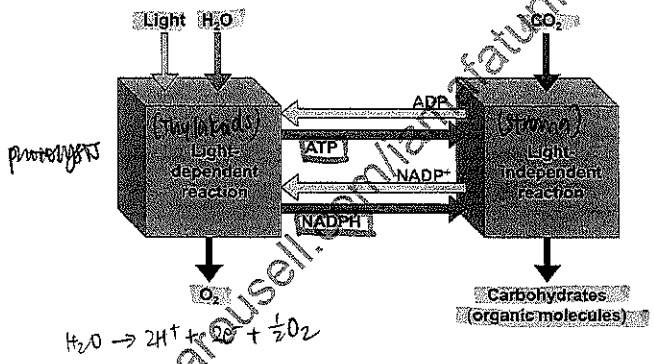
- **ATP (adenosine triphosphate)** is often described as a universal energy currency. The reason for saying so is that it is a highly mobile energy carrier because of its small size. ATP can easily release its energy via a single step reaction – hydrolysis. It is involved in diverse reactions and it can easily be reformed from ADP and P_i according to demand.
- Biochemistry of photosynthesis:

  A simplified equation:

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

  From the equation, you can see that CO_2 is **fixed** into organic molecules while O_2 is liberated as a by-product. (A by-product is an incidental or secondary product made in the manufacture or synthesis of something else.) Thus the composition of our atmosphere is influenced by photosynthesis.

- In summary, the light-dependent and the light-independent reactions operate in unison, as shown in the following diagram:



- Therefore, photosynthesis is significant to life on earth as a source of carbon in organic form & oxygen for respiration. Photosynthesis makes both carbon and energy available to living organisms and produces oxygen in the atmosphere, which is vital for all aerobic forms of life.

2) Location of photosynthesis

2.1 Leaf: Organ for photosynthesis

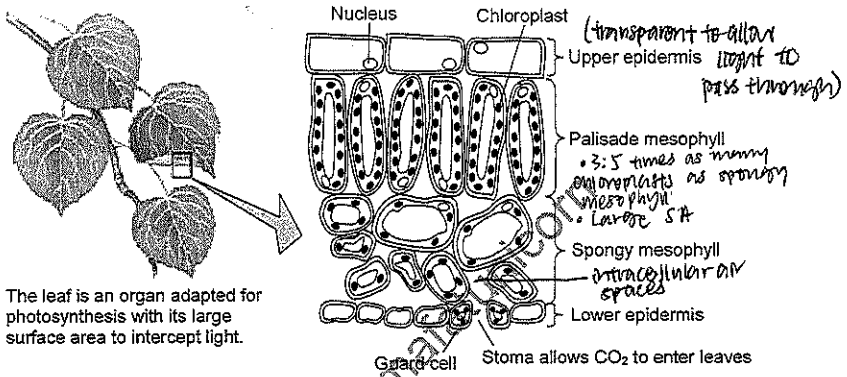


Figure 1: Tissue organization of a leaf

- Cells of the palisade mesophyll contain three to five times as many chloroplasts as those of the spongy mesophyll (Fig. 1). Palisade mesophyll cells are also closer to the upper epidermis where more sunlight can be received. These factors combine to allowing more efficient use of light for photosynthesis.
- Because the leaf is so thin, the carbon dioxide quickly diffuses through the intercellular air spaces of the spongy mesophyll layer into the palisade cells. The palisade cells have a large surface area, allowing for efficient gaseous exchange and hence efficient carbon fixation.
- The long axis of the palisade mesophyll is arranged perpendicular to the surface of the leaf to minimize the amount of light scattered and absorbed by the cell walls before it reaches the chloroplasts (Fig. 2).
- Chloroplasts in the peripheral cytoplasm can move via cytoplasmic streaming for optimal capture of light. This applies to both spongy as well as palisade mesophyils. (Cytoplasmic streaming is the circular flow of cytoplasm, involving the cytoskeleton, that speeds up the distribution of materials within cells.)

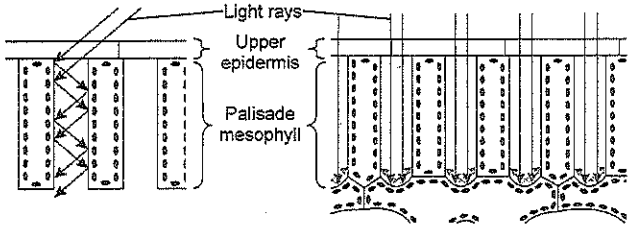


Figure 2: Light scattering in a leaf

2.2 Chloroplast: The site of photosynthesis

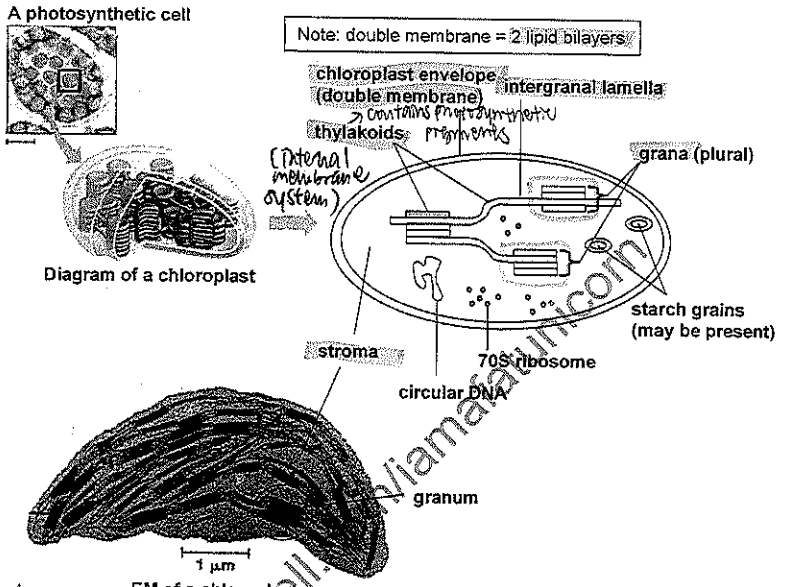


Figure 3: Structure of Chloroplast

- Chloroplast is a plastid containing **photosynthetic pigments.**
- **Chloroplast envelope** (double membrane) separates the inside of the chloroplast and the contents of the cell (cytoplasm).
- The inner membrane encloses the **stroma**, a concentrated solution of enzymes, including those required for carbohydrate synthesis.
- The stroma, in turn, surrounds a third membranous compartment, the **thylakoids**, which are fluid-filled membranous sacs.
- Photosynthetic pigments such as **chlorophyll** and **carotenoids** are located in the thylakoids.
- Stacks of thylakoid sacs form **grana** (singular = granum). Intergranal lamella link the grana. (plural)

3) Light-dependent reaction

3.1 The nature of sunlight

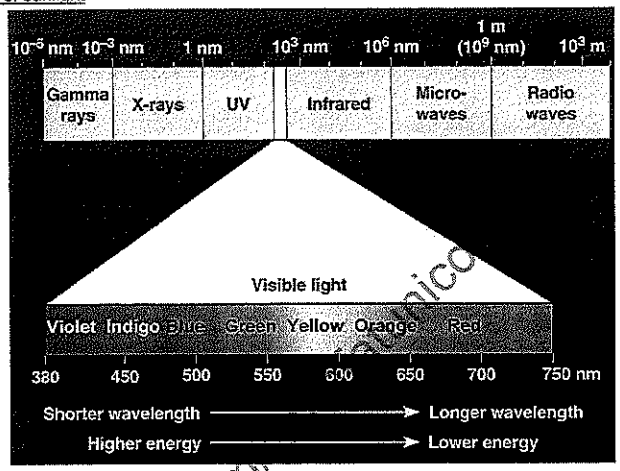


Figure 4: The electromagnetic spectrum

- The sun radiates the full spectrum of electromagnetic energy but the atmosphere acts like a selective window that allows visible light through but screens out most of the other radiation.
- As such, photosynthesis makes use of only a very narrow range of the electromagnetic spectrum – visible light. (Fig. 4)

3.2 Photosynthetic pigments: The light receptors

- When light meets matter, it may be reflected, transmitted or absorbed.
- Since leaves appear green, we can infer that not all the wavelengths of visible light are absorbed by the leaves. Leaves appear green because green light is transmitted or reflected off their surface.
- Photosynthetic pigments on the thylakoid membranes of chloroplasts can absorb light of certain wavelengths. Each type of pigment has a certain molecular structure such that it absorbs light strongly at specific wavelengths.
- There are several types of photosynthetic pigments but we will focus only on the following:
  1) Chlorophyll *a* is the most abundant photosynthetic pigment. They exist as several forms (e.g. P700 and P680) with different absorption peaks. They are the only pigments that participate directly in photosynthesis.
  2) Chlorophyll *b* is an **accessory pigment** that channels light energy absorbed to *chlorophyll a*. Accessory pigments play an indirect role in photosynthesis.

absorb light of other wavelengths

3) Carotenoids are a class of **accessory pigments** e.g. carotene and xanthophylls. Other than their indirect role in photosynthesis, they have a photoprotective role. They absorb and dissipate excess light energy that could damage chlorophyll or interact with oxygen forming reactive oxidative molecules that are dangerous to the cell. They also add colour to fruits and flowers which help in dispersal and pollination respectively.

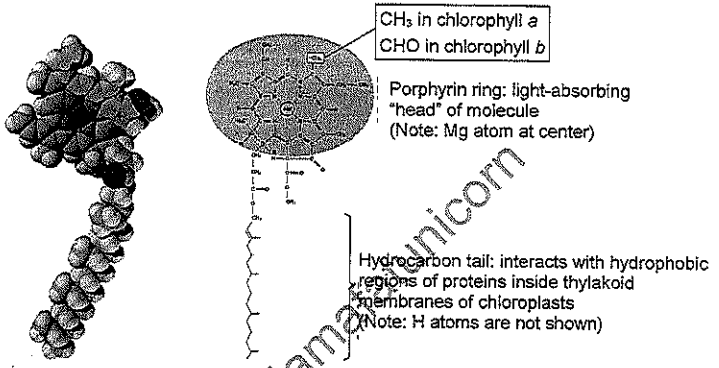
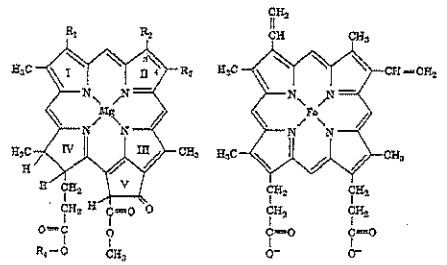


Figure 5: Structure of chlorophyll molecule

- The ability of a pigment to absorb various wavelengths of light can be measured by an instrument called a spectrophotometer. A spectrophotometer is a device that directs beams of light of different wavelengths through a solution of pigment and measures the fraction of the light transmitted at each wavelength.

(Additional information)

Chlorophyll's similarity to haemoglobin probably makes it easy for the body to use. Scientists have found chlorophyll to have anti-oxidant, anti-inflammatory and wound-healing potential.



Chlorophyll **Iron-protoporphyrin IX (Haem)**

How can we measure rate of photosynthesis? Light energy
- measure rate of O_2 produced
- measure rate of CO_2 consumed

$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$
substrate products

- A graph plotting a pigment's light absorption versus wavelength is called an **absorption spectrum**. (Fig. 6)
- An **action spectrum** (Fig.6) is a graph showing the effectiveness of different wavelengths of light in stimulating photosynthesis. Rate of photosynthesis can be used to determine the effectiveness of different wavelengths in stimulating photosynthesis.

(set-up)
inverted test tube
① - vol of water displaced = vol of O_2 released
② - using gas syringe

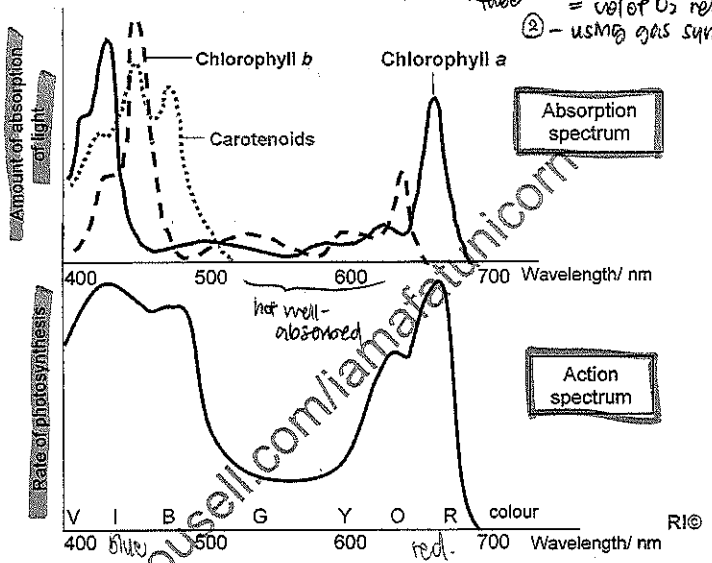


Figure 6: Comparing the absorption and action spectrums of photosynthetic pigments

Q. Observe the absorption spectrum in Figure 6 and note any significant observations.
- Each pigment has its own specific spectrum
- Each pigment has a different number, height and breadth of peaks
- The pigments do not absorb one green wavelengths of light

- Comparing the absorption spectrum of chlorophyll *a* (which is the only pigment that plays a direct role in photosynthesis) with the action spectrum, you should notice that the action spectrum is similar (wavelengths of peaks and trough) but does not exactly match the absorption spectrum for chlorophyll *a*.
- That is because chlorophyll *a* is not the only photosynthetically important pigment in photosynthesis. Chlorophyll *b* and carotenoids being accessory pigments, broadens the spectrum of wavelength over which photosynthesis can occur by channeling the energy absorbed to chlorophyll *a*.

3.3 Photosystems

- Chlorophyll molecules do not work in isolation. They cluster together to form a functional unit called a **photosystem**. Photosystems located on the thylakoid membrane of the chloroplast.

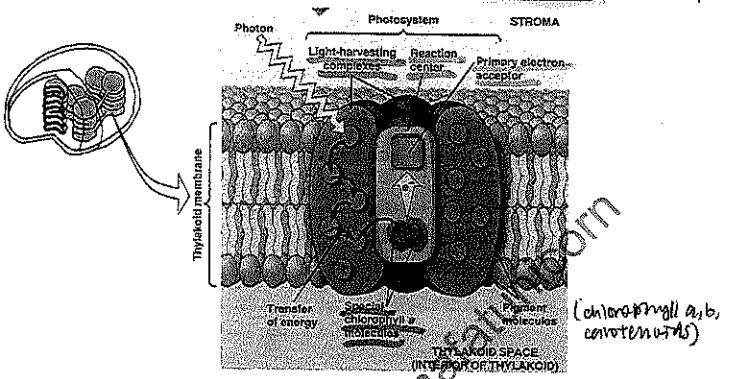


Figure 7: How a photosystem harvests light

- A photosystem is composed of a **reaction center** surrounded by a number of light-harvesting complexes. (Fig. 7)
  - The reaction center is a protein complex that includes two special **chlorophyll *a*** molecules and a molecule called the **primary electron acceptor**.
  - Each light harvesting complex consists of pigment molecules (chlorophyll *a*, chlorophyll *b*, carotenoids) bound to particular proteins. The variety of pigments allow light to be harvested over a wider range of wavelengths.
- The thylakoid membrane is populated by two types of photosystems, **photosystem II (PS II)** and **photosystem I (PS I)**, that cooperate in the **light-dependent reactions** of photosynthesis. (They are named in the order of their discovery.)
  - The two photosystems function sequentially with PS II functioning first.
- PS II contains **P680** – which is the special chlorophyll *a* molecule in PS II's reaction center. It is so named because it most effectively absorbs light of wavelength 680nm.
- PS I contains **P700** – which is the special chlorophyll *a* molecule in PS I's reaction center. It is so named because it most effectively absorbs light of wavelength 700nm.
- P700 and P680 are actually identical chlorophyll molecules. It is their association with different proteins in the thylakoid membrane that affects the electron distribution in the chlorophyll molecules and accounts for the slight differences in light-absorbing properties.
- There are two possible routes for the **light-dependent reaction**: non-cyclic and cyclic.
  - **Non-cyclic light-dependent reaction** is the *predominant* route.

3.4 Excitation of chlorophyll by light (i.e. photoactivation)

- When a chlorophyll molecule absorbs a **photon** of light (a photon is a discrete packet of energy that is transmitted in light as a subatomic particle), one of the pigment molecule's electrons is elevated from its ground state to its excited state (i.e. the photon boosts an electron to an orbital of higher potential energy). (Fig. 8)
  - Different pigments absorb only photons corresponding to specific wavelengths, which is why each pigment has a unique absorption spectrum.
- ① The excited state is unstable, and the electron cannot remain excited for long. When the excited electron quickly falls back to the ground state orbital, excess energy is released.
- The energy can then be relayed to another pigment via resonance transfer of energy.
- ② Alternatively, instead of falling to the unexcited state, electron is captured by the **primary electron acceptor** molecule in the reaction center of a **photosystem** (refer to section 3.3, Fig. 7)

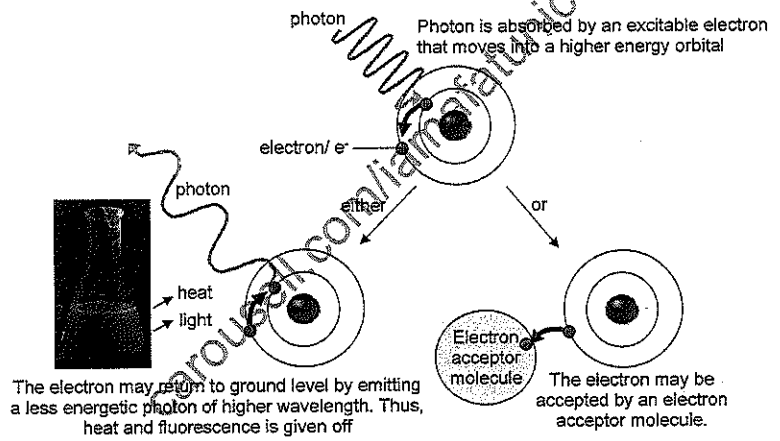


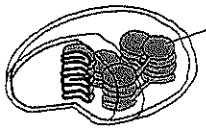
Figure 8: Interactions between light and atoms or molecules

Q: If an isolated solution of chlorophyll was exposed to the same UV light, fluorescence is seen. However, no fluorescence is observed for a chloroplast extract, containing a similar concentration of chlorophyll, when placed under the same conditions. Explain why.

- The excited electron is captured by the primary electron acceptor in the reaction center, which is only present in the chloroplast extract.
- The excited electron from chlorophyll a molecule is lost, and does not return to ground level
- Hence no fluorescence is observed.

3.5 Non-cyclic light-dependent reaction (Non-cyclic photophosphorylation)

- Location of reaction: thylakoid membrane (Fig. 9)
- In the non-cyclic light-dependent reaction, light drives the synthesis of:
  1) **NADPH** (also known as reduced $NADP^+$) — for use in Calvin cycle
     - $NADP^+$ stands for nicotinamide adenine dinucleotide phosphate.
     - It is a co-enzyme acting as a high energy electron carrier.
  2) **ATP** — for use in Calvin cycle
- The key to this energy transformation is the flow of electrons through the photosystems and other molecular components built into the thylakoid membrane.



Thylakoid membranes are the site of the light-dependent reactions of photosynthesis. Embedded in these membranes are
1) PS I and II
2) **electron transport chains**
3) enzymes e.g. **ATP synthase**

Figure 9: Site of light-dependent reaction in the chloroplast

- The non-cyclic light dependent reaction involves *both*:
  - **PS II**
  - **PS I**

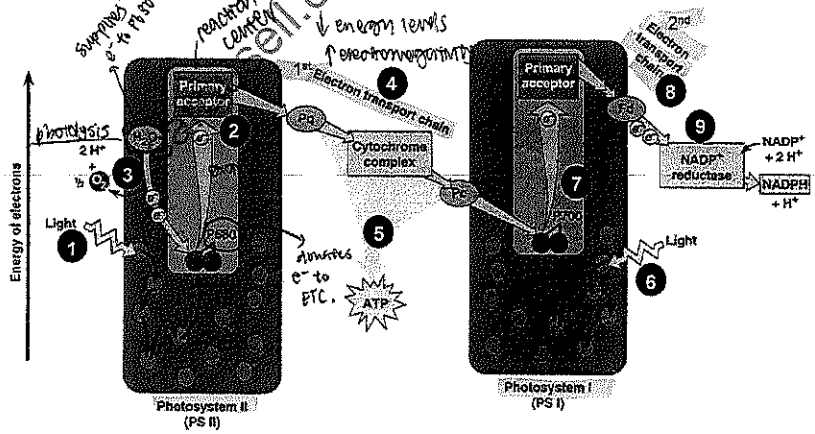


Figure 10: How non-cyclic light-dependent reaction generates ATP and NADPH

Note: *You need not know the components of the 2 electron transport chains*

***Photoactivation** at PS II* (Fig. 10)

1. A photon of light strikes a pigment molecule in a light harvesting complex and energy is relayed to other pigment molecules, via resonance transfer of energy until it reaches one of the two P680 chlorophyll *a* molecules in the PS II reaction center. It excites one of the P680 electrons to a higher energy state.

2. This excited electron is captured by the **primary electron acceptor** in the reaction center. The excited electron (in P680) needs to be replaced.

***Photolysis** of water* (Fig. 10)

3. An enzyme splits a water molecule into two electrons, two hydrogen ions, and an oxygen atom, in the presence of light.

$$H_2O \rightarrow 2e^- + 2H^+ + \tfrac{1}{2}O_2$$

The electrons are supplied one by one to the P680 molecules, each replacing an electron lost to the primary electron acceptor. The oxygen atom immediately combines with another oxygen atom, forming O_2 which is released as a by-product. The H^+ remains in the thylakoid space. (Also refer to Fig. 13)

1st Electron Transport from PS II to PS I (Fig. 10)

4. Each photoexcited electron passes from the primary electron acceptor of PS II to PS I via an **electron transport chain**. The electron transport chain between PS II and PS I is made up of a series of electron carriers with increasing electronegativity. A series of redox (reduction-oxidation) reactions take place as electrons are transferred down the chain from one electron carrier to the next.

5. As the excited electron travels down the electron transport chain, which consists of electron carriers of progressively lower energy levels, energy lost is coupled to the formation of ATP (refer to section 3.7 for details). This way of synthesizing ATP using light energy is called **photophosphorylation.**

↳ generating ATP from ADP & P_i.

Light harvesting at PS I (Fig. 10)

6. Meanwhile, light energy has been relayed via pigment molecules to the PS I reaction center, exciting an electron of one of the two P700 chlorophyll *a* molecules. This electron is captured by the primary electron acceptor in PS I, thus creating an electron "hole" in P700.

7. Those electrons from PS II (from 4) that have reached the end of the first electron transport chain will fill the electron "hole" left in P700.

Electron transport from PSI to NADP+ (Fig. 10)

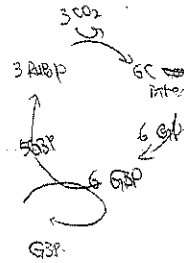
8. Photoexcited electrons are passed from PS I's primary electron acceptor down a second electron transport chain. (Note that this electron transport chain is made up of different components compared with the first electron transport chain and no ATP is formed here unlike the first.) → no photophosphorylation

9. The electrons are finally transferred to $NADP^+$. This reduction of $NADP^+$ to form **NADPH** is catalyzed by the enzyme $NADP^+$ reductase.

$$NADP^+ + 2e^- + H^+ \rightarrow NADPH$$

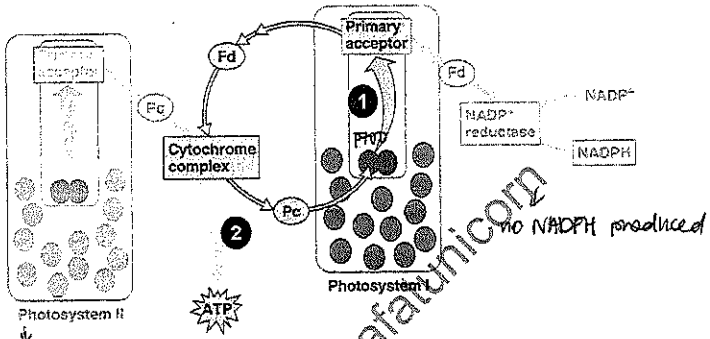
Hence, $NADP^+$ is the final electron acceptor. This reduction takes place in the stroma.

- Since the high energy electrons are stored in NADPH, it will provide the reducing power for the synthesis of sugar in the next step in photosynthesis, the **light-independent reaction** (i.e. **Calvin cycle**). (See Section 3.3, Fig. 8)



3.6 Cyclic light-dependent reaction (Cyclic photophosphorylation)

- Location of reaction: thylakoid membrane
- The cyclic light dependent reaction involves PSI only



no NADPH produced

no photolysis of water, no O_2 produced.

Figure 11: Cyclic electron flow

Note: *You need not know Fd or Pc*

1. The photoexcited electron from P700 is captured by the PS I's primary electron acceptor which then passes on the electron to the middle part of the first electron transport chain. An electron "hole" in P700 is created.

   ↳ does not get transported to ETC.

2. The loss of excitation energy is coupled to ATP production. This electron fills the electron "hole" left in P700, completing the cycle. → photophosphorylation

- No NADPH is produced, instead of passing the excited electrons to the second electron transport chain as in the non-cyclic light-dependent reaction, they are transferred to the first electron transport chain.
- No O_2 is produced as there is no photolysis of water.
- Electrons are recycled.
- While the non-cyclic light-dependent reaction produces ATP and NADPH in roughly equal quantities, **cyclic light-dependent reaction produces only ATP**. This is because, the *next stage* of photosynthesis, the **Calvin cycle**, makes use of more ATP than NADPH. Cyclic light-dependent reaction makes up the difference.

is demand equal for ATP & NADPH? no ☺ more demand for ATP

Q: In cyclic photophosphorylation, why doesn't the electron from PS I get passed to the 2nd ETC?

- High ATP requirements in the light independent reaction drive the plant to carry out more cyclic photophosphorylation.
- In addition, the electron from PSI gets passed to the 1st ETC, instead of the 2nd ETC, when the ratio of NADPH to NADP is too high. When the ratio is as such, there would be too little NADP available to accept electrons.

3.7 ATP synthesis occurs by photophosphorylation

- It was mentioned earlier that as the excited electron travel down the electron transport chain with electron acceptors of progressively lower energy levels. The energy lost is coupled to the formation of ATP.
- This process is called **photophosphorylation** and it involves the use of a proton concentration gradient to drive the synthesis of ATP from ADP and P_i (inorganic phosphate).
- Definition of **photophosphorylation:**
  The process of generating ATP from ADP and P_i by means of a **proton-motive force** generated across the thylakoid membrane of the chloroplast using light energy absorbed during the light-dependent reactions of photosynthesis.

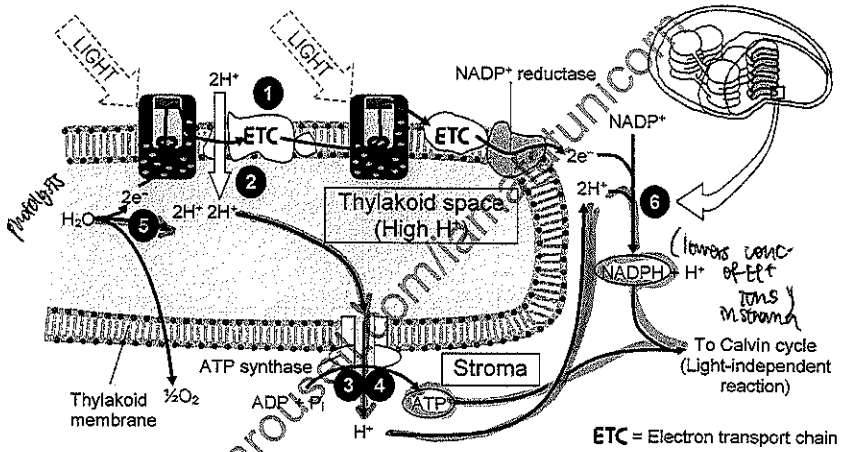


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Figure 12: Light-dependent reactions and chemiosmosis: The organization of the thylakoid membrane

1. As electrons travel down a series of electron carriers that are progressively more electronegative, certain **electron transport chain** proteins in the thylakoid membrane pumps H^+ **ions (protons)** across the membrane into the thylakoid space.
2. H^+ (protons) accumulates in the thylakoid space which serves as the H^+ (proton) reservoir. In this way, the electron transport chain transforms redox energy to a **proton-motive force.** (proton gradient)
3. **Chemiosmosis** occurs when H^+ (protons) diffuse down the concentration gradient (across the thylakoid membrane back into the stroma) via **ATP synthase** (also called stalked particles).
4. **ADP** is phosphorylated to **ATP** in the process.
5. Electrochemical **proton gradient** across the thylakoid membrane is further maintained by the accumulation of H^+ from the splitting of H_2O **(photolysis)** in the thylakoid space.

6. In addition, the uptake of H^+ ions (protons) from the stroma when NADP+ is reduced to NADPH by NADP+ reductase contributes to the proton gradient. The lowering of H^+ ions (protons) in the stroma coupled with the accumulation of H^+ ions in the thylakoid space ensures that the steepness of the gradient is maintained.

Q. What would happen to the pH of the thylakoid space and stroma if you shine light at chloroplasts? What would happen if the lights are turned off?

- Shine light: pH in thylakoid space drops and pH of stroma increases
- Turn off light: pH gradient is abolished.

Q: A poison / mechanical disruption disrupts the thylakoid membrane & renders it permeable to H^+ ions. Would ATP synthesis be affected?

- Yes. As the thylakoid membrane is disrupted, H^+ ions cannot accumulate in the thylakoid space. Thus, no electrochemical proton gradient would not build up across the thylakoid membrane
- As a result, there would be no ion flow of diffusion of H^+ ions through the ATP synthase → no / less ATP produced.

Q: If ATP can be generated through the light dependent reactions, why do plant cells require both chloroplasts and mitochondria?

- ATP produced by photophosphorylation in the chloroplasts is only sufficient for use in the Calvin cycle (to produce G3P) → G3P subsequently utilized to produce glucose & other organic compounds
- Thus, more ATP still needs to be produced through respiration in the mitochondria to fulfil other energy needs of the cells e.g. Translocation of sucrose.

Summary of the light-dependent reactions:

Type of reaction	Components and/or reactions involved	Outcome
Non-cyclic light-dependent reaction (**non-cyclic photophosphorylation**)	PS II, PS I and both electron transport chains Reaction catalysed by NADP+ reductase: $NADP^+ + 2e^- + H^+ \rightarrow NADPH$	• Generates ATP through chemiosmosis • Forms NADPH when electrons are finally transferred to $NADP^+$
	$H_2O \rightarrow 2e^- + 2H^+ + \frac{1}{2}O_2$ (Splitting of water during photolysis)	• Generates electrons to be used to fill the electron "hole" in the reaction centre in PS II • Produces O_2 as a byproduct • Generates H^+ (protons) to maintain high H^+ concentration in thylakoid space
Cyclic light-dependent reaction (**cyclic photophosphorylation**)	PS I and the 1st electron transport chain	• Generates ATP through chemiosmosis

3.8 Variegation

- Absence of chloroplast or pigments causes a sector of a leaf or stem to have white patches or have patches with different, often lighter, colours. This condition is termed variegation.
- The zones where chloroplasts are not present are zones where no photosynthesis occurs, hence a variegated leaf has a lower potential to fix carbon dioxide into sugars, and as a result, a variegated plant also tends to grow more slowly.

4) Light-independent reaction (Calvin cycle)

- Location of reaction: **stroma** of chloroplast (Fig. 13)

The **stroma** contains the enzymes that catalyse the reactions in **Calvin cycle** of photosynthesis. An example of enzyme involved in the Calvin cycle is **ribulose bisphosphate carboxylase/oxygenase (RuBisCO)**. Carbon fixation occurs here.

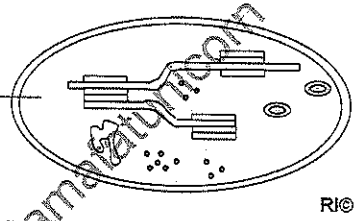

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Figure 13: Stroma: The site of light independent reaction

- Prerequisites for reaction to occur: products of light dependent reaction → NADPH and ATP
- Other requirement for reaction to occur: CO_2
- Calvin cycle is a pathway that reduces carbon dioxide to produce carbohydrates. Calvin, the scientist, used the isotope ^{14}C to trace the biochemical pathway of carbon fixation.

  Note: Radioisotopes are often used as radioactive tracers to trace the sequence of products formed in a pathway.

Q. Is the oxygen released during photosynthesis originated from carbon dioxide or water? Do you know how scientists found the answer?

- The Calvin cycle involves 3 major steps: Carbon fixation, reduction, and RuBP regeneration

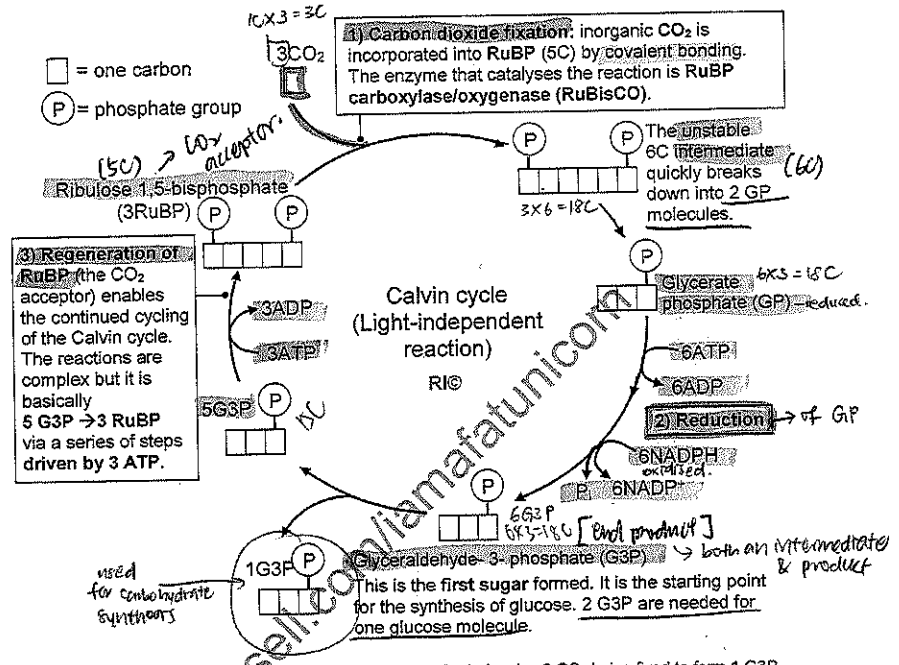


Figure 14: The Calvin cycle (light-independent reaction) showing 3 CO_2 being fixed to form 1 G3P

- **Step 1 (Carbon fixation)** (Fig. 14)
  - This step involves the fixing of carbon dioxide to **RuBP** (ribulose bisphosphate, a 5C sugar) and is catalyzed by **RuBP carboxylase/oxygenase (RuBisCO)**.
  - RuBP is the CO_2 acceptor. The product of this reaction is an unstable 6C intermediate that will immediately split to form 2 molecules of **glycerate phosphate** (for every one molecule of CO_2).
  - **Glycerate phosphate (GP)**, a 3C compound, is the first product of photosynthesis. This 3C-compound has 2 other names:
    1. phosphoglyceric acid (PGA)
    2. 3-phosphoglycerate (acids exists as salts in physiological systems and their names end with –ate)

- **Step 2 (Reduction of glycerate phosphate by NADPH)** (Fig. 14)
  - This reaction requires the reducing power of **NADPH** and energy of **ATP** (both are products of non-cyclic light-dependent reaction).

  - GP is reduced (gains electrons) to form another 3C compound, **glyceraldehyde-3-phosphate (G3P)**. G3P has 2 other names:
    1. phosphoglyceraldehyde (PGAL)
    2. triose phosphate (TP)
  - NADPH thus gets oxidized to $NADP^+$, while ATP is converted to ADP (P_i is also released).
  - The first sugar (3C sugar) formed in photosynthesis is G3P. It is also the end product of Calvin cycle. It is a basic sugar used to build up more complex carbohydrates such as glucose, sucrose or starch.

- **Step 3 (Regeneration of RuBP)** (Fig. 14)
  - G3P has to be used to regenerate **RuBP** which is the CO_2 acceptor.
    5 molecules of **G3P** (a 3C molecule; total 15C) used to regenerate 3 **RuBP** (a 5C molecule; total 15C)
  - **3 ATP** from the light-dependent reaction is also used in the regeneration step.

- **Fate of G3P**
  - For the net synthesis of 1 molecule of G3P for carbohydrate synthesis, 3 molecules of CO_2 have to be fixed.
  - G3P molecules are needed for the formation of glucose molecules.
  - Since G3P is used for RuBP regeneration and formation of glucose molecules, it is considered as both an intermediate and a product.
  - Depending on the type of glucose isomers formed: α-glucose polymerizes to form starch while β-glucose polymerizes to form cellulose.

Q: Thus, to produce 1 G3P (3C), determine how much of each reactant is needed?
CO_2: 3 *ATP:* 9 *NADPH:* 6

Q. Hence balance this cycle and determine how much of each reactant is needed to generate one glucose molecule (6C): Hint: Start with the regeneration of RuBP.
CO_2: 3x2=6 *ATP:* 9x2=18 *NADPH:* 6x2=12

Q. More ATP is used in total than NADPH. How does the cell cope with this?
By cyclic photophosphorylation.

Review Q. What is/are the roles of NADPH and ATP in the light-independent reaction?
- To produce G3P (by reducing GP) which is needed to form glucose.
- And ATP to also regenerate RuBP

In summary: (Fig. 15)

- Light-dependent reaction
  1. Light absorbed by chlorophyll drives a transfer of electrons and H^+ (protons) from water to $NADP^+$, which temporarily stores the energized electrons. $NADP^+$ is the final electron acceptor.
  2. Water is split (photolysis) in the process that gives off O_2 as a by-product.
  3. Generates ATP by chemiosmosis via the cyclic and non-cyclic photophosphorylation.
  4. Thus, light energy is converted to chemical energy in the form of NADPH which is a source of energized electrons ("reducing power") and ATP.
- Light-independent reaction
  1. Calvin cycle makes G3P from the products of light-dependent reaction, NADPH and ATP.
  2. Involves 3 stages: (1) carbon fixation (2) reduction and (3) regeneration of RuBP.
  3. Regenerates $NADP^+$ and ADP for utilization in the light dependent reaction.
- Neither reaction alone can make sugar from CO_2. The chloroplast integrates the two stages of photosynthesis.

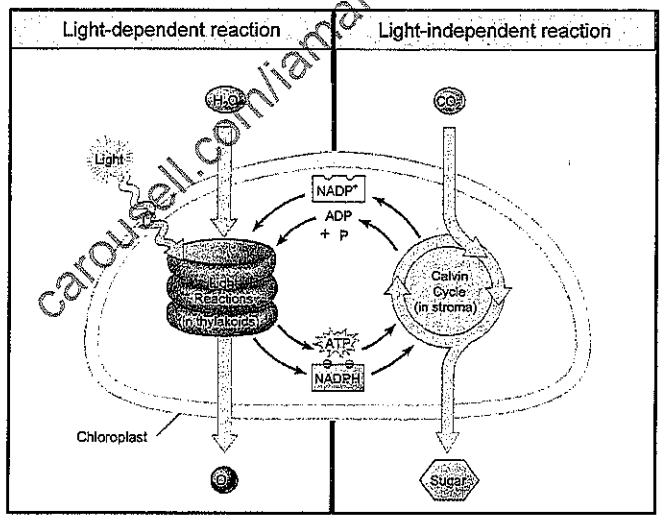


Figure 15: Summary of photosynthesis

Q. Compare light-dependent and light-independent reactions in photosynthesis.

	Photosynthesis	
Features	**Light-dependent reaction**	**Light-independent reaction**
Location of reaction in chloroplasts		
Reactions involved		
Reactants		
Useful products		
By-products		

5) Investigating the limiting factors of photosynthesis

5.1 Terminology

- **Limiting factor:** The principle of limiting factors state that when a chemical process is affected by more than one factor, its rate is limited by that factor which is nearest its minimum value. It is that limiting factor which directly affects a process if its magnitude is changed.

*(**Note: Rate limiting step:** The rate of a biochemical process which involves a series of reactions will be limited by the slowest reaction in the series.)*

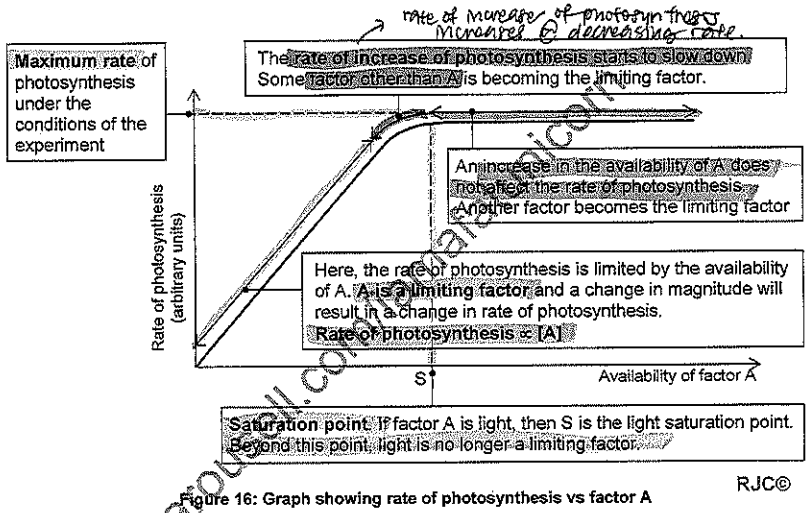


Figure 16: Graph showing rate of photosynthesis vs factor A

5.2 Limiting factors of photosynthesis

- **Light intensity**
  - In low light intensities, the rate of photosynthesis increases linearly with increasing light intensity. Gradually, the rate of increase falls as other factors become limiting.
  - Illumination on a clear summer's day is about 100 000 lux whereas the light saturation for photosynthesis is reached at about 10 000 lux (1 tenth).
  - Except for shaded plants, light intensity is not normally a major limiting factor. Very high intensities may bleach chlorophyll. Plants that live in areas of high light intensities protect themselves with thick cuticles and hairy leaves.
  - Apart from light intensity, light quality (wavelength) and light duration (photoperiod) also affect photosynthesis.

- **CO_2 concentration**
  - Carbon dioxide is a major limiting factor.
  - The atmospheric concentration of CO_2 is between 0.03% - 0.04%, which is far below the optimum concentration of between 0.1% - 0.5%.
- **Temperature**
  - Since both the light-dependent and to a larger extent, light independent reactions are enzyme-controlled, temperature will affect the rate of photosynthesis.
  - The rate of reaction doubles for every 10°C rise up to about 35°C. Beyond 40°C, the enzymes start to denature → rate of photosynthesis falls.
- **O_2 concentration (oxygenase function of RuBisCO)**
  - RuBisCO accepts O_2 as a competitive inhibitor when the CO_2:O_2 ratio is low.
  - This occurs on bright, hot, dry days. On such days, the stomata of the leaf close (Fig. 1) restricting water loss. This inevitably results in reduced CO_2 entering the leaf and CO_2 concentration plummets, while O_2 concentration builds up due to photosynthesis.
  - Oxygenase function of Rubisco causes RuBP to be split into a 3C (GP) and 2C (glycolate) compound. The 2C compound is exported to the peroxisomes and mitochondria where it is broken down into CO_2 *(i.e. **photorespiration**)* which generates no ATP).

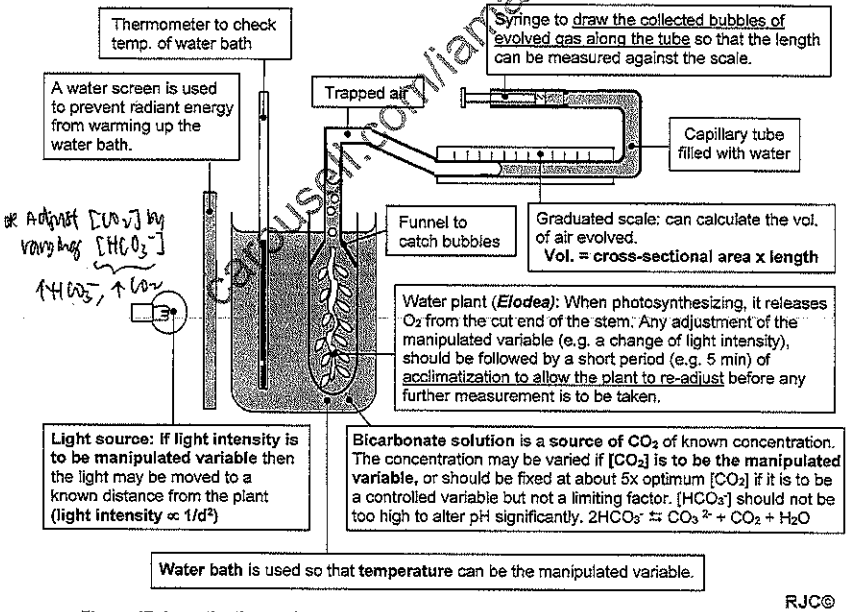


Figure 17: Investigation on how the 3 main external factors affect the rate of photosynthesis

Q. In the above investigation (Fig. 17), what is the measurable quantity and dependent variable?

↳ Rate of photosynthesis — volume of O_2 evolved

Rate of photosynthesis ∝ to the volume of gas evolved. The bubbles of evolved gas are collected over a fixed duration of time. Therefore,

$$\text{Rate of photosynthesis} = \frac{\text{collected volume/ mm}^3}{\text{time/ min}} = \text{___ mm}^3\ O_2 \text{ evolved/ min at a known temperature, t °C}$$

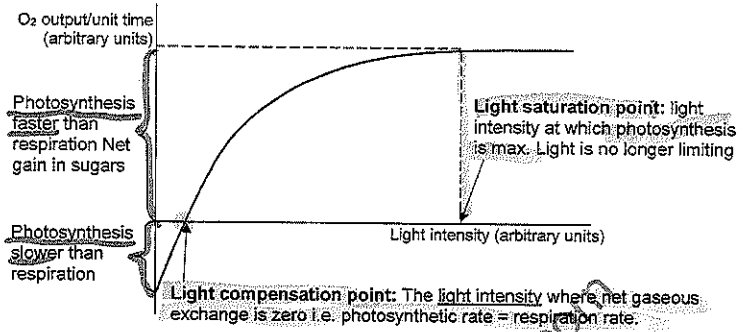
When carrying out an experiment on how a certain limiting factor affects the rate of photosynthesis,

- Explain in detail how the independent variable can affect the dependent variable (i.e. rate of photosynthesis) and relate this to the volume of gas evolved. Demonstrate that you have understood the biochemistry behind photosynthesis.
- Identify all the factors to be kept constant and optimal (controlled variables) and the factors to be manipulated. Explain how all these variables are kept constant.
- Describe how you measure the measurable quantity and how you carry out the experiment. Explain how the dependent variable is calculated.
- Include control(s)
- What graph would you present and what would you expect? (Fig. 18)

5.3 Compensation points

both processes can occur @ the same time.

- **Compensation point** is a point when photosynthetic rate equals to respiration rate, with no net change of O_2 and CO_2.
  - This is unique to plants as they photosynthesise (i.e. take in CO_2 and give out O_2), and respire (i.e. take in O_2 and give out CO_2) at the same time
- Photosynthesis can occur at a faster rate than respiration at some points in time and vice versa.



Compensation period: Time taken to reach compensation point.

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Figure 18: Light response curve for photosynthesis

6) Links

The topic of photosynthesis is relevant to the following topics and learning outcomes in the A level Biology syllabus.

	Topic	The links to photosynthesis (Teachers' comments)
1.	Cell Structure and Function	Structure-to-functional roles of the chloroplast in carrying out photosynthesis: The membrane of the chloroplast play two important roles: 1) they are sites where enzymes and electron carriers are located; 2) the membranes act as barriers to the movement of H^+ ions and only allow the flow of such ions through the ATP synthase. Endosymbiotic theory that proposes the existence of the double membrane organelles e.g. chloroplast and mitochondrion. Evidence supporting this theory that these organelles have prokaryotic origins are as follows: (1) contain circular DNA just like bacteria (a prokaryote) and thus can replicate independently of the nucleus (2) contain 70S ribosomes unlike the 80S ribosomes found in eukaryote cytoplasm and endoplasmic reticululum (3) some antibiotics e.g. chloramphenicol hinder protein synthesis of chloroplast and mitochondria but not that of the eukaryotic cell (4) their sizes are roughly the same as bacteria.
2.	Enzymes	Photosynthesis involves enzyme-catalyzed reactions. The concepts related to the action of enzymes and the effects of temperature, pH, enzyme concentration and substrate concentration should also apply to photosynthesis.

3.	Cellular Physiology and Biochemistry (Respiration)	Photosynthesis and respiration occurs at the same time during daytime in plants and they are reversible chemical reactions of each other. Thus, it is important to understand the connections between the two processes (i.e. the intermediate compounds that link photosynthesis and respiration as well as the gaseous exchange that occurs between the chloroplast and mitochondrion). Photosynthesis and respiration produce ATP. Think about what is the difference between ATP synthesis in these two processes.

7) Keywords

Chloroplast envelope
Stroma
Thylakoid space
Granum/grana
Photosynthetic pigments
Accessory pigments
Chlorophyll a
Chlorophyll b
Carotenoids
Action spectrum
Absorption spectrum

Photoactivation
Light dependent reaction
Photon
ATP
Photosystem
Primary electron acceptor
Reaction centre
Photolysis
NADPH/reduced $NADP^+$
Coenzyme
Electron Transport Chain
ATP synthase
chemiosmosis

H^+/protons
Proton gradient
Proton-motive force
Non-cyclic photophosphorylation
Cyclic photophosphorylation

Light independent reaction/Calvin cycle
Carbon fixation
Ribulose bisphosphate
RuBisCO
Glycerate phosphate (GP)/
Phosphoglycerate (PGA)
Glyceraldehyde-3-phosphate (G3P)/
Phosphoglyceraldehyde (PGAL)/
Triose phosphate (TP)
Reduction
Regeneration of RuBP

Limiting factor
Photorespiration
Compensation point

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NAME: CT GROUP:

CORE IDEA
(2) GENETICS AND INHERITANCE

GENETIC BASIS FOR VARIATION (I)

Content

- The passage of information from parent to offspring
- Genotypes and phenotypes
- Dihybrid crosses
- Linkage and crossing-over
- Interaction between loci
- The effect of genotype and environment on phenotype

Learning Outcomes
Candidates should be able to:

(u) explain the terms: *locus, allele, dominant, recessive, codominant , incomplete dominance, homozygous, heterozygous, phenotype, genotype* and *linkage.*
(v) explain how genes are inherited from one generation to the next via the germ cells or gametes.
(w) explain how genotype is linked to phenotype.
(x) use genetic diagrams to solve problems in dihybrid crosses, including those involving codominance, incomplete dominance, multiple alleles, sex linkage, autosomal linkage and epistasis.
(y) use genetic diagrams to solve problems involving test crosses.
(z) explain the meaning of the terms *linkage* and *crossing-over* and explain the effect of linkage and crossing-over on the phenotypic ratios from dihybrid crosses.
(bb) explain how the environment may affect the phenotype (including how diet affects the differentiation of honey bees and how temperature affects fur colour of Himalayan rabbits).

(Note: For epistasis and problem-solving involving epistasis, please refer to Part II of the notes.)
Use knowledge gained in this section in new situations or to solve related problems.

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*** This handout is the effort of several Biology teachers at RI(Yr 5-6). It has and will continue to be updated.**
****Any information given in a double-lined box is for your information only.**

TABLE OF CONTENTS

(A) COMMON TERMS AND LABELLING CONVENTIONS

LO2u Explain the terms, **locus, allele, dominant, recessive, codominant, incomplete dominance, homozygous, heterozygous, phenotype** and **genotype**.

1) Common terms used

Term	Explanation
Homologous chromosome	A diploid organism (2n) has 2 sets of chromosomes, one set from each parent. Homologous chromosomes are similar in size, shape, position of centromere and staining pattern. Homologous chromosomes have the same genes that determine a certain trait at corresponding loci. e.g. blood group, hair colour. However, the genes may be of different alleles which give rise to different observed trait (phenotype), e.g. blood group A, B, O and black/brunette hair colour (Note: Refer to Mitosis notes for more details.)
Locus	The **position of a gene on a chromosome**.
Gene	A gene is an ordered sequence of DNA nucleotides located at a particular locus on a particular chromosome that encodes a specific functional product i.e. a protein or RNA molecule.
Allele	An allele is one of a number of **alternative forms of a gene**, each possessing a unique nucleotide sequence, only one of which can occur at a given locus. The differences in the nucleotide sequences of each alleles, when translated may produce a different gene product which result in various observed traits. Alleles are usually represented by the same letter of the alphabet with upper case for dominant alleles and lower case for recessive alleles e.g. A or a.
Homozygous	The diploid condition in which the **alleles at a given locus** on a pair of homologous chromosomes are **identical**, AA or aa.
Heterozygous	The diploid condition in which the **alleles at a given locus** on a pair of homologous chromosomes are **different**, Aa.

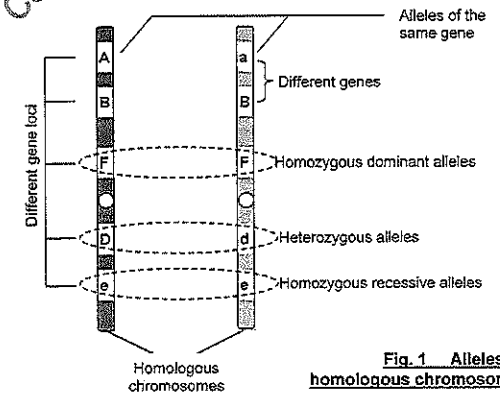


Fig. 1 Alleles on homologous chromosomes

Genotype	Genotype refers to the **combination of alleles**, situated on **homologous chromosomes at corresponding loci** (such as "Aa", "aa" or "AA"). The genotype determines a specific trait of an organism. The genetic characteristics are passed to offspring/inherited from parents via the alleles.
Phenotype	Phenotype refers to the **characteristic (trait)** of an organism that is expressed or observed. These characteristics (trait) arise from the **interaction between the genotype (i.e. the alleles on the homologous chromosomes) and the environment** in which the organism develops. The environment may alter the appearance of the organism. For example, while Himalayan rabbits can produce black fur, the black fur is only produced on parts of the body that are cool enough. Heat from other warmer parts of the body result in white fur (Page 6).
Dominant allele	The allele that shows its phenotype regardless of whether it is homozygous or heterozygous. In cases involving heterozygotes, it is said that the expression of the gene product of the dominant allele masks the effect of gene product expressed by the other (recessive) allele. E.g. black pigment vs colourless pigment → black pigment.
Recessive allele	The allele that shows its phenotype only in the presence of another identical recessive allele (homozygous recessive). In cases involving heterozygotes, the effect of the gene product expressed by the recessive allele is masked by the effect of the gene product expressed by the dominant allele.
Codominance	When two different dominant alleles of the same gene are **both expressed** and **influence the phenotype.** This results in a **heterozygote's** phenotype that is **different** from the **phenotypes of both homozygote** giving rise to **more than two possible phenotypes**. e.g. alleles I^A and I^B of the human ABO blood group system are codominant.
Incomplete dominance	The situation in which the phenotype of heterozygotes is intermediate between the phenotypes of individuals homozygous for either allele. (from Campbell)
Cross	The mating of two organisms.
F_1 generation (First filial)	The offspring of the crossing arising from the parental generation. Recessive alleles are not expressed in the F_1 generation if both parents are homozygous.
F_2 generation (Second filial)	The offspring produced by crossing two F_1 organisms. Homozygous recessive individuals can be expected to occur in the F_2 if the F_1 generation was heterozygous.
Self-fertilisation	Crossing of an organism with itself. This term is used for plants but not animals. Self-fertilisation over many generations can produce a pure line.
Pure breed / True breed	When an organism is **homozygous** for the traits concerned.
Wild type	The typical genotype found in a natural population of that species. While this term is much used by *Drosophila* geneticists, it is best avoided.
Test cross	Cross between an individual of unknown genotype or a heterozygote to a homozygous recessive individual (tester). Offspring ratios are used to deduce the genotype of the unknown, and/or the linkage relationships of its heterozygous loci.
Linkage	Two or more genes on same chromosome that do not assort independently in meiosis and are thus inherited together. Genes closer together less likely to cross-over and exchange alleles between homologous chromosomes;

2) How alleles should be labelled

(a) Alleles are usually represented with upper case for dominant allele and lower case letter for recessive allele.

The choice of the letter can be based on the mutant trait, e.g. **D** (tall) and **d** (dwarf). Or can also be chosen to represent the dominant allele, e.g. **R** (round seed) and **r'** (wrinkled seed).

An **apostrophe** is added to the lower case letter if the upper and lower cases of the letter look similar, e.g. **C** and **c'**, **W** and **w'**. Avoid upper and lower case versions which are difficult to distinguish when handwritten, e.g. c, o, s, u, v, w, x and y.

(b) When there are **multiple** alleles of the same gene, the gene should be allocated an uppercase letter and each allele an appropriate superscript.
For example:
Representation of the different alleles for human blood groups where:
I represents the gene
A, B and o represent alleles
The alleles are therefore indicated as I^A, I^B and I^o (note that the superscript 'o' is lowercase to denote it is a recessive allele for blood group O).

(c) For **sex-linked** (X-linkage), the sex chromosomes XX and XY should always be indicated and the dominant or recessive alleles of particular genes should be indicated by appropriate superscript of uppercase and lowercase letters.
For example:
In the transmission of haemophilia, X^HX^h designates a female carrier.
X^HY denotes a normal male and X^hY a haemophilic male.

(d) For genes with codominant alleles, a distinguishing letter of the alphabet will be added as a superscript.
For example:
ABO blood group system in humans where:
I^A and I^B are codominant.

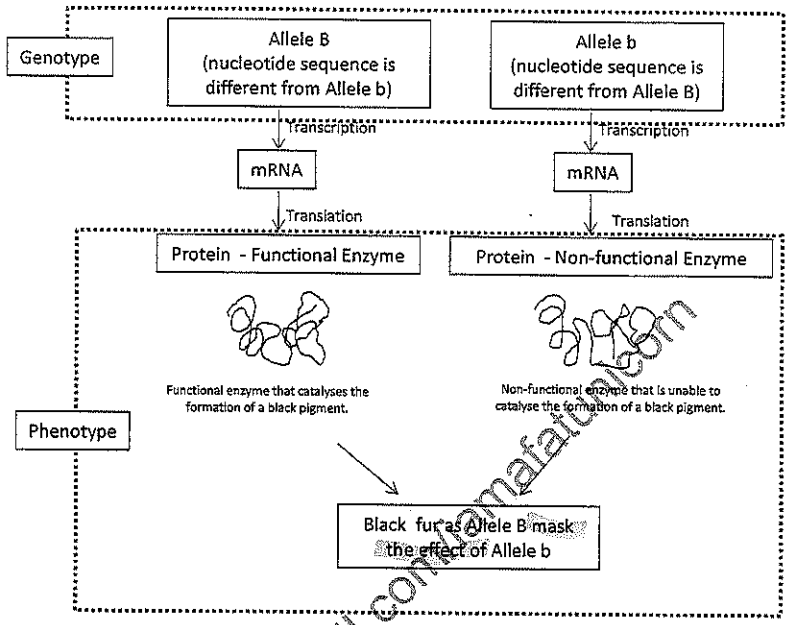
(B) FROM GENOTYPE TO PHENOTYPE

LO2v, w *Explain how genotype is linked to phenotype and how genes are inherited from one generation to the next via the germ cells or gametes.*

- The **genotype** of an organism is its **genetic makeup** which is the combination of alleles situated on corresponding loci on homologous chromosomes. The genotype determines a trait or phenotype observed.
- While the genotype is the genetic information, **phenotype** is the **expression** of that information that results in a **trait** that can be observed. For example, in the case of seed shape in pea plants, the genotypes RR and Rr will result in plants that produce round seeds (phenotype). The genotype rr will result in plants that produce wrinkled seeds (phenotype).
- As learnt previously (DNA and Genomics), nucleotide sequences determine gene products, such as proteins. The link between genotype and phenotype is closely tied to the Central Dogma.

For example, in the inheritance of coat colour in mice. The presence of the dominant allele (B) results in black fur while the presence of 2 copies of the recessive allele (b) results in white fur.

The diagram below shows how the genotype Bb determines the observed phenotype.



(C) GENES and ENVIRONMENT

LO2bb *Explain, with examples, how the environment may affect the phenotype.*

- The phenotype of an individual is determined by the combination of alleles (genotype)
- Phenotypic variation may result from the interaction of genotype and environment
- Environment may modify or limit the expression of gene(s) for e.g. a shortage or lack of food or necessary nutrients such as mineral salts or vitamins will lead to reduce growth in size or mass and possibly height of individuals. Lack of vitamins may also lead to serious health problems such as deficiency in vitamin A results in night blindness.

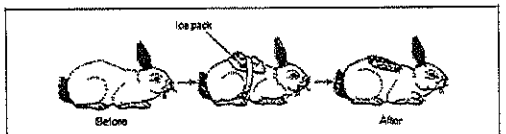
  E.g. **Phenylketonuria** (PKU) is an autosomal (non-sex linked) recessive genetic disorder characterised by a deficiency in the enzyme phenylalanine hydroxylase. This enzyme is necessary to metabolise the amino acid phenylalanine to the amino acid tyrosine. When phenylalanine hydroxylase is deficient, phenylalanine accumulates and is converted into a toxic substance.

  Left untreated, this condition causes problems with brain development, leading to progressive mental retardation and seizures. However, PKU is one of the few genetic diseases that can be controlled by diet. **A diet low in phenylalanine is very effective treatment** illustrating how an environmental factor such as diet can be manipulated to modify the resultant phenotype of a particular genotype.

- Environment may trigger or switch on certain genes which are always present in the genome:

(a) Temperature

E.g. temperature affects the **coat colour of Himalayan rabbits**. The Himalayan rabbit has a white body with black ears, nose, feet and tail. Only in parts of the body that are cool enough, eg. the extremities, does black fur grow. This is because heat (from the body) prevents the development of the black pigment. If the fur on the back is shaved and an ice-pack is fixed on the rabbit's back, left in position for weeks and kept cold, black hair begins to develop beneath the ice-pack.



The application of an ice pack to a region of shaved hair results in black hair growing back instead of the original white color.

Fig. 2 Effect of cold on Himalayan rabbit fur colour.

E.g. Nile crocodiles have **temperature-dependent sex determination**, which means the sex of their hatchlings is determined by the average temperature during the middle third of their incubation period. If the temperature inside the nest is below 31.7°C, or above 34.5°C, the offspring will be female. Males can only be born if the temperature is within that narrow range.

(b) UV light

E.g. The human skin contains melanocytes which are melanin-producing cells located in the bottom layer of the skin's epidermis. When exposed to UV light more melanins are produced. The color of the melanin is dark and it absorbs and blocks the UV-B light from passing the skin layer. More exposure to UV light results in darker colour skin.

(c) Photoperiod

E.g. Wavelength of light and duration of light exposure may affect flowering, growth and germination of seeds of the plants. Long-day plant flowers when the day length exceeds their critical photoperiod. These plants typically flower in the northern hemisphere during late spring or early summer as days are getting longer. Conversely, short-day plants flower when the day lengths are less than their critical photoperiod.

- Environment **effect** is usually **greater on polygenes**. Polygenic inheritance occurs when one characteristic is controlled by two or more genes. Examples of human polygenic inheritance are height, skin colour and weight. The frequency of the phenotypes of these traits generally follows a normal **continuous variation** distribution pattern.

- Environment may induce **mutation affecting phenotype** such as over exposure to UV light may cause chromosomal damage that triggers skin cancer. Chemicals such as asbestos and carcinogens in cigarette smoke may cause cancer cells formation.

(d) Food / nutrition

E.g. Larvae of honey bees fed with royal jelly become queen bees capable of reproducing and living a long time. But female larvae fed with worker jelly have slower development and become worker bees which are sterile and short-lived.

(D) MONOHYBRID CROSSES AND THE LAW OF SEGREGATION

- Gregor Mendel conducted **crosses** between **pure-breeding** plants which had different characteristics e.g. purple flowers vs white flowers.

1) Monohybrid crosses

Genetic crosses that studies the inheritance of **ONE** characteristic controlled by a single gene.

How Mendel made hybrids:
1. First he snipped off the anthers (male part of the flower with the pollen) while still immature to prevent "selfing".
2. Then he dusted the stigma with pollen taken from the desired male parent.
3. Finally, he tied bags over the flowers to keep out any stray pollen.
4. In this way, Mendel was able to control the parentage of each generation.

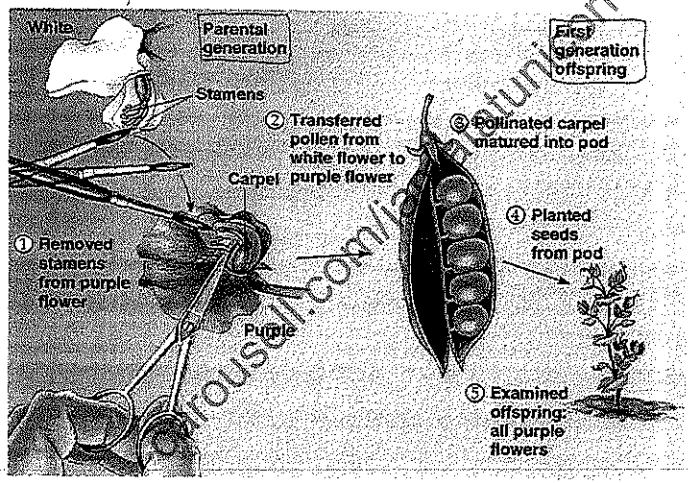


Fig. 3 Mendel's experiment

Crossing of two varieties = **hybridisation**.

- The example mentioned above, between pure-breeding plants with purple flowers vs white flowers, is a **monohybrid cross**, as it tracks the inheritance of a **single characteristic**, in this case, flower colour.
- The pure-breeding parents are the P **(for parental) generation**, their hybrid offspring are the F_1 **(for first filial) generation.**
- Allowing the F_1 hybrids to self-pollinate produces an F_2 **(second filial) generation.**

why choose peas?
- distinct characteristic
- easy to cultivate
- produces abundantly
- pure breeding
- cross-pollinated, crosses easily carried out
- short life cycle

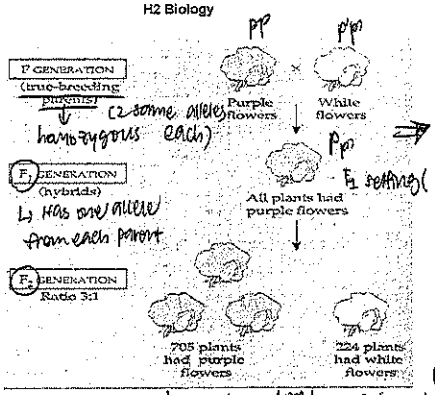


Fig. 4 Monohybrid cross: Mendel's flower-colour experiment

- Results (refer to Page 11 for the monohybrid genetic cross):
  - In F_1 generation, plants produced only purple flowers. (No white flowers.)
  - White flower characteristic = **recessive**, since the trait did not appear in the phenotype though the allele was present. (The F_1 plants are heterozygotes.)
  - In F_2 generation, ratio of **3 purple-flowered plants to 1 white-flowered plant**.
  - This **3:1** ratio is known as the **monohybrid ratio**.

In addition to flower colour, Mendel also studied 7 pairs of contrasting characteristics and the results of the experimental crosses are shown in the table below.

Characteristic	Parental appearance		F_2 appearance		Ratio
	(Dominant)	(Recessive)	(Dominant)	(Recessive)	
1. Length of stem	Tall	Dwarf	787	277	2.84 : 1
2. Shape of seed	Round	Wrinkled	5474	1850	2.91 : 1
3. Colour of seed	Yellow	Green	6022	2001	3.01 : 1
4. Shape of pod	Inflated	Constricted	882	299	2.95 : 1
5. Colour of pod	Green	Yellow	428	152	2.82 : 1
6. Colour of flower	Purple	White	705	224	3.15 : 1
7. Position of flower	Axial	Terminal	651	207	3.14 : 1
Total			**14949**	**5010**	**2.98 : 1**

In all cases, the ratio of the dominant to recessive characteristics in the F_2 generation was approximately **3:1**.

- Mendel's conclusions:
  - Parental stocks were true-breeding, so the purple variety must have two purple "factors", and the white variety, two white "factors" (the parental stocks are **homozygous** for the trait).
  - F_1 generation obtained **one** "factor" from each parent (the gamete that emerge from meiosis carried a single copy of a gene – since they are haploid).
  - **The separation of the pair of parental factors during meiosis, so that one "factor" is present in one gamete, is called Mendel's First law – Law of segregation.**

- We now call the "factors" determining characteristics on regions of chromosomes = **GENES**.
- Each pollen grain and egg has one flower colour gene, so the plant formed by fertilisation has two copies of the flower colour gene.
- This gene may be one of two distinct types of **ALLELES**. One allele, **P**, is for purple; the other one, **p'**, is for white.

- A diploid plant may have 2 of the same or 2 different alleles.
  An organism having a pair of identical alleles for a character is **homozygous** e.g. true-breeding pea plant for purple flowers (**PP**) and true-breeding pea plant with white flowers (**p'p'**).
  The purple F_1 hybrids with non-matching alleles (**Pp'**) are **heterozygous**.

- In a **heterozygote**, one allele may mask the presence of another.
  Therefore, the allele that shows itself in the phenotype is the **dominant allele**. The allele that is **masked** is the **recessive allele**.
  The allele **P** is dominant over **p'**. So a plant heterozygous for the flower colour gene with **Pp'** is purple.
  Allele **P** is expressed resulting in purple flower. Allele **p'** for white flower is masked.

- **Phenotype** = organisms' appearance e.g. purple flowers
- **Genotype** = genetic makeup e.g. **PP** or **Pp'** or **p'p'**

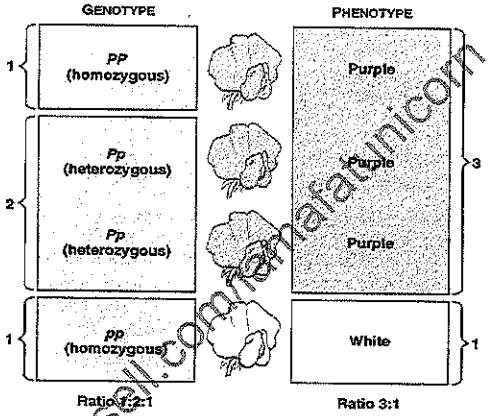


Fig. 5 Pictorial representation of the phenotypic and genotypic result of the cross between two flowers

2) Genetic representation of the monohybrid cross

The Genetic Diagram

Let: P represents the allele for purple flowers (dominant)
p' represents the allele for white (recessive)

Parental phenotype	Purple flowers	X	White flowers
Parental genotype (2n)	PP	X	p'p'
Meiosis			
Gametes (n)	P		p'
Fertilisation			
F_1 genotype	Pp'		
F_1 phenotype	Purple flowers		

F_1 selfing

F_1 phenotype	Purple flowers	X	Purple flowers
F_1 genotype (2n)	Pp'	X	Pp'
Meiosis			
Gametes (n)	P p'		P p'

Fertilisation

Gametes	P	p'
P	PP Purple	Pp' Purple
p'	Pp' Purple	p'p' White

F_2 genotypes	PP Pp' Pp'		p'p'
F_2 phenotypic ratio	3 purple flowers	:	1 white flower
F_2 phenotypic frequency	¾ purple	:	¼ white

To note:

- All gametes need to be circled
- Phenotypes always need to be linked to genotypes either in the Punnett square or in the ratio outcomes of the cross
- Important ratios in **complete dominance** monohybrid cross:
  - e.g. Pp X Pp → 3 : 1 (dominant phenotype : recessive phenotype)
  - Pp X pp → 1 : 1 see test cross below
  - PP X Pp/pp → All dominant phenotype

3) Mendel's first law – Law of Segregation

Gregor Mendel's Law of Segregation states that the two alleles for each trait separate during the formation of gametes. The Law of Segregation ensures that a parent with two copies of each gene can pass on either allele. (Note: This is not the same as the Law of Independent Assortment that you will learn later.)

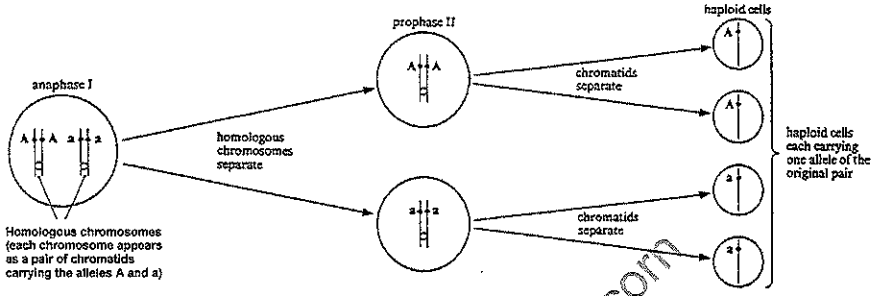


Fig. 6 Mendel's Law of Segregation of alleles A and a during the separation of homologous chromosome in meiosis

4) Test cross

- **Designed to reveal the genotype of an organism that exhibits the dominant trait** e.g. purple flowers.
- 2 possible genotypes for purple flowers:
  - PP (homozygous dominant) or
  - Pp' (heterozygous).
- Cross the organism with an individual expressing the **homozygous recessive trait**, e.g. the white-flowered parent, to ascertain the true genotype of the parent exhibiting the dominant trait.

Q. Parent plant with unknown genotype produces purple flowers. How would you determine if the parent is homozygous dominant or heterozygous. Show your answer in the form of a genetic diagram.

Two possible scenarios:

1) Between a homozygous dominant parent (purple flowers) and a homozygous recessive parent (white flowers)
2) Between a heterozygous parent (purple flowers) and a homozygous recessive parent (white flowers)

Scenario (1)

Let: **P** represents the allele for purple flowers (dominant)
p' represents the allele for white (recessive)

Parental phenotype	Purple flowers	X	White flowers
Parental genotype (2n)	PP	X	p'p'
Meiosis			
Gametes (n)	P		p'
Fertilisation			
F_1 Genotype	Pp'		
F_1 Phenotype	All plants with purple flowers		

Scenario (2)

Parental phenotype	Purple flowers	X	White flowers	
Parental genotype (2n)	Pp'	X	p'p'	
Meiosis				
Gametes (n)	P, p'		p'	
Fertilisation				
F_1 genotypes	Pp'		p'p'	
F_1 phenotypic ratio	1 purple	:	1 white	

Conclusion:
Parent producing purple flowers x parent producing white flowers,
- If F_1 phenotypic ratio is all plants with purple flowers, parent producing purple flowers is homozygous dominant.
- If F_1 phenotypic ratio is 1 plant producing purple flowers : 1 plant producing white flowers, parent producing purple flowers is heterozygous.

(E) DIHYBRID CROSSES & THE LAW OF INDEPENDENT ASSORTMENT

1) Mendel's second law – Law of independent assortment

LO2x Use genetic diagrams to solve problems in dihybrid crosses, including those involving codominance, incomplete dominance, multiple alleles, sex linkage, autosomal linkage and epistasis

- Mendel's Law of Independent Assortment states that the alleles of different genes on different pairs of chromosomes assort independently of each other. This is because:
  - Homologous pairs of chromosome align randomly on either side of the metaphase plate during metaphase I
  - Alignment of each homologous pair is independent of other homologous pairs
- This gives rise to "new" recombinant types (new combination of alleles in the gametes) as shown in the figure below.

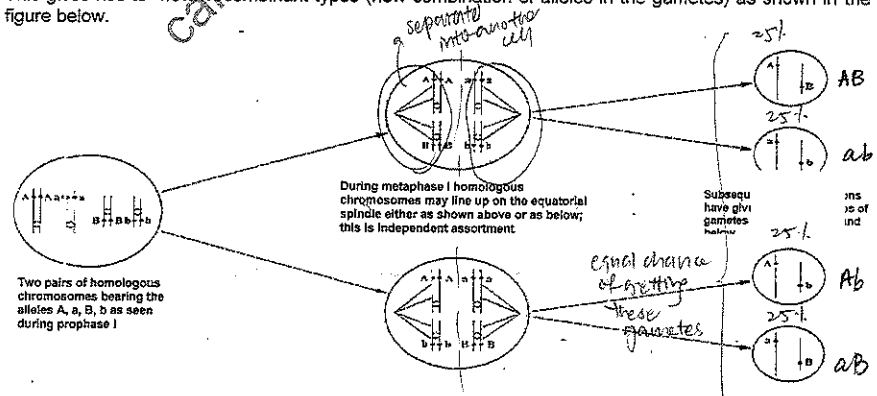


Fig. 7 Mendel's principle of independent assortment of alleles A, a, B and b described in terms of the separation of homologous chromosome which occurs during meiosis

2) Dihybrid inheritance

Genetic crosses that studies the inheritance of **TWO** different characteristics controlled by **TWO** different gene.
- Mendel also performed experiments with plants that differed in **two** characters – i.e. he performed **dihybrid crosses**. The purpose of this experiment was to see if the two traits were inherited independently.
- Mendel used pea shape (smooth or wrinkled) and pea cotyledon colour (yellow or green) as the characteristics.
- Yellow is dominant (**Y**) and green is recessive (**y'**).
- Round is dominant (**R**) and wrinkled is recessive (**r**).
- True breeding plants with yellow-round seeds (**YYRR**) were crossed with true breeding plant with green-wrinkled seeds (**y'y'rr**).

Q. Will seed colour and seed shape be transmitted from parents to offspring as a package, or are they inherited independently from each other?

Let: Y represents the allele for yellow seed (dominant)
y' represents the allele for green seed (recessive)
R represents the allele for round seed (dominant)
r represents the allele for wrinkled seed (recessive)

Parental phenotype	Yellow and round seed	X	Green and wrinkled seed
Parental genotype (2n)	YYRR	X	y'y'rr
Meiosis			
Gametes (n)	YR		y'r
Fertilisation			
F_1 genotype (2n)	Yy'Rr		
F_1 phenotype	Yellow and round seed		

F_1 selfing

F_1 phenotype	Yellow and round seed	X	Yellow and round seed
F_1 genotype (2n)	Yy'Rr	X	Yy'Rr
Meiosis			
Gametes (n)	YR, Yr, y'R, y'r		YR, Yr, y'R, y'r

Fertilisation

Gametes	YR	Yr	y'R	y'r
YR	YYRR Yellow-round	YYRr Yellow-round	Yy'RR Yellow-round	Yy'Rr Yellow-round
Yr	YYRr Yellow-round	YYrr Yellow-wrinkled	Yy'Rr Yellow-round	Yy'rr Yellow-wrinkled
y'R	Yy'RR Yellow-round	Yy'Rr Yellow-round	y'y'RR Green-round	y'y'Rr Green-round
y'r	Yy'Rr Yellow-round	Yy'rr Yellow-wrinkled	y'y'Rr Green-round	y'y'rr Green-wrinkled

F_2 genotypes	YYRR YYRr Yy'RR Yy'Rr	YYrr Yy'rr	y'y'RR y'y'Rr	y'y'rr
F_2 phenotypic ratio	9 Yellow-round	: 3 Yellow-wrinkled	: 3 Green-round	: 1 Green-wrinkled

Phenotypic frequency	
Yellow-round	9/16,
Yellow-wrinkled	3/16
Green-round	3/16
Green-wrinkled	1/16

indication that alleles inherit independently

Conclusion:
When a 9: 3: 3: 1 F_2 phenotypic ratio is obtained in a dihybrid cross, this indicates that the alleles of each character are inherited independently of one another. This means that the two alleles for seed colour **segregate independently** of the two alleles for seed shape i.e. whether **Y** or **y** is inherited in offspring is **independent** of whether **R** or **r** is inherited.

They are not found on the same chromosome.

Q. What is the F_2 phenotypic ratio, should the alleles for seed colour and seed shape, NOT segregate independently?

Let: **Y** represents the allele for yellow seed (dominant)
y' represents the allele for green seed (recessive)
R represents the allele for round seed (dominant)
r represents the allele for wrinkled seed (recessive)

Parental phenotype	Yellow and round seed	X	Green and wrinkled seed
Parental genotype (2n)	**YYRR**	X	**y'y'rr**
Meiosis			
Gametes (n)	(YR)		(y'r)
Fertilisation			
F_1 genotype (2n)	Yy'Rr		
F_1 phenotype	Yellow and round seed		

F_1 selfing

F_1 phenotype	Yellow and round seed	X	Yellow and round seed
F_1 genotype (2n)	Yy'Rr	X	Yy'Rr
Meiosis			
Gametes (n)	(YR) (y'r)		(YR) (y'r)

Fertilisation

Gametes	(YR)	(y'r)
(YR)	YYRR Yellow-round	Yy'Rr Yellow-round
(y'r)	Yy'Rr Yellow-round	y'y'rr Green-wrinkled

F_2 genotypes	YYRR Yy'Rr Yy'Rr		y'y'rr
F_2 phenotypic ratio	3 Yellow-round	:	1 Green- wrinkled
Phenotypic frequency	Yellow-round ¾ Green-wrinkled ¼		

If the alleles for seed colour and seed shape do NOT segregate independently, then F_2 phenotypic ratio of **3:1 is the same as that obtained in a monohybrid cross.** ⇒ When alleles are found on the same chromosome and are completely linked

Q. When do alleles not segregate independently from each other?
When alleles are found on the same chromosome & are completely linked

Q. Usually tomato plants have purple stems, but in some varieties the purple pigment is missing and the stem are green. Most tomato plants have 'cut' leaves, but some have so-called 'potato' leaves.

The table below shows the numbers and phenotypes of offspring, resulting from two separate crosses of tomato plants.

Parental phenotypes	*Number and phenotype of offspring*			
	Purple stem cut leaves	*Purple stem potato leaves*	*Green stem cut leaves*	*Green stem potato leaves*
(i) purple stem, cut leaves x green stem, potato leaves	**354**	**0**	**367**	**0**
(ii) purple stem, cut leaves x green stem, cut leaves	**693**	**241**	**0**	**0**

a. Which alleles are dominant? Explain.
b. Deduce the probable genotypes for the parents in crosses (i) and (ii).
Use : G/g for the dominant and recessive alleles respectively for stem colour
L/l for the dominant and recessive alleles respectively for leaf shape.

a.
Stem colour: dominant characteristic = PURPLE
Reason: cross (i): purple stem x green stem resulting all offspring in purple stems. Hence, the allele for green stem has been masked by the allele for the purple stem

Leaf shape: dominant characteristic = CUT
Reason: cross (i): cut leaves x potato leaves resulting all offspring w cut leaves. Hence, allele for potato leaves has been masked by the allele for cut leaves

Note:
- Analyse the phenotypic ratio of each character separately in a dihybrid cross. Use known monohybrid cross ratios to deduce.

b. Cross (i)

Offspring traits for stem colour	Purple	:	Green
Offspring ratio	1	:	1
Parental genotype	Gg	X	gg
Offspring trait for leaf type	All Cut		
Offspring ratio	All Cut		
Parental genotype	LL	X	ll

Purple stem cut leaves = GgLL
Green stem potato leaves = ggll

Cross (ii)

Offspring traits for leaf type	Cut	:	Potato
Offspring ratio	3	:	1
Parental genotype	Ll	X	Ll
Offspring trait for stem colour	All Purple		
Offspring ratio	All Purple		
Parental genotype	GG	X	gg

Parent with purple stem cut leaves = GGLl
Parent with green stem cut leaves = ggLl

Parents have 2 copies of same allele

Q. True-breeding flies that have long wings and dark bodies are mated to true-breeding flies with short wings and tan bodies. All the F_1 have long wings and tan bodies. The F_1 are allowed to mate and produce:

→ F_1 generation is heterozygous

88 tan, long	9
32 dark, long	3
28 tan, short	3
12 dark, short	1

Which alleles are dominant? Long wings and tan bodies
Show the ratios of the genotypes and phenotypes obtained in all the crosses.

Let: L represents the allele for long wing (dominant)
l represents the allele for short wing (recessive)
T represents the allele for tan bodies (dominant)
t represents the allele for dark bodies (recessive)

Parental phenotype	Long wing, dark body	X	Short wing, tan body
Parental genotype (2n)	LLtt	X	llTT

Meiosis

Gametes (n): (Lt) (lT)

Fertilisation

F_1 genotype	LlTt
F_1 phenotype	Long wing tan body

F_1 self

F_1 phenotype	Long wing, tan body	X	Long wing, tan body
F_1 genotype (2n)	LlTt	X	LlTt

Meiosis

Gametes (n): (LT) (Lt) (lT) (lt) (LT) (Lt) (lT) (lt)

Fertilisation

Gametes	LT	Lt	lT	lt
LT	LLTT Long wing, tan body	LLTt Long wing, tan body	LlTT Long wing, tan body	LlTt Long wing, tan body
Lt	LLTt Long wing, tan body	LLtt Long wing, dark body	LlTt Long wing, tan body	Lltt Long wing, dark body
lT	LlTT Long wing, tan body	LlTt Long wing, tan body	llTT Short wing, tan body	llTt Short wing, tan body
lt	LlTt Long wing, tan body	Lltt Long wing, dark body	llTt Short wing, tan body	lltt Short wing, dark body

F_2 genotypes	L_T_	L_tt	llT_	lltt
F_2 phenotype	Long wing, Tan body	Long wing, Dark body	Short wing, Tan body	Short wing, Dark body
F_2 phenotypic ratio	9 :	3 :	3 :	1

2 uses w homozygous recessive individual

Q. What is the result of the test cross of the flies with the genotype LlTt?

LO2y *Use genetic diagrams to solve problems involving test crosses.*
Test cross – Cross with an individual expressing the **homozygous recessive traits for both characters i.e. LlTt X lltt.**

Parental phenotype	Long wing, tan body	X	Short wing, dark body
Parental genotype (2n)	LlTt	X	lltt

Meiosis

Gametes (n): (LT) (Lt) (lT) (lt) (lt)

Fertilisation

	LT	Lt	lT	lt
lt	LlTt Long wing, tan body	Lltt Long wing, dark body	llTt short wing, tan body	lltt short wing, dark body

Phenotypic ratio 1 : 1 : 1 : 1

To note:
Important ratios in **complete dominance dihybrid cross**

- When both individuals are heterozygote for both genes are crossed
  e.g. LlTt X LlTt → offspring phenotypic ratio 9 : 3 : 3 : 1
- When an individual heterozygote for both genes is test crossed
  e.g. LlTt X lltt → offspring phenotypic ratio 1 : 1 : 1 : 1

(F) OTHER ALLELIC INTERACTION

1) Multiple alleles (LO2x)

- Condition where a single characteristic appears in several different forms as it is controlled by **three or more alleles**, of which any *two* may occupy the *same locus* on homologous chromosomes.

Example 1: Control of ABO blood group in humans

1. Inheritance of blood group in humans is controlled by **autosomal** gene.
2. Blood group with gene locus represented by the symbol **I** with 3 alleles: I^A, I^B and I^O.
3. I^A and I^B are dominant to I^O.
4. I^A and I^B are codominant (which means that both alleles are **equally expressed** in the phenotype).

Genotype	Phenotype	Genotype	Phenotype
I^OI^O	Type O	I^BI^O	Type B
I^AI^O	Type A	I^BI^B	Type B
I^AI^A	Type A	I^AI^B	Type AB

- Red blood cell (RBC) membranes contain surface **mucopolysaccharides** or **agglutinogens** which act as **antigens**. They react with antibodies called **agglutinins**.
- I^A and I^B are alleles for agglutinogen A and B respectively.
- I^O does not specify for any agglutinogen.
- A person with blood group A has agglutinogen A on RBC membranes but agglutinin B (anti-B) in plasma.

Blood group	
AB	A and B agglutinogens; no agglutinins
O	no agglutinogens; anti-A and anti-B
B	B agglutinogens; anti-A
A	A agglutinogens; anti-B

- Application to blood transfusion:
  The recipient's blood must not have agglutinins which will cause agglutination of the donor's RBC.
  It does not matter if donor's blood plasma has agglutinins which will react with recipient's agglutinogens because donor's plasma is diluted during transfusion into recipient so the agglutination activity is negligible.

Monohybrid & Dihybrid Ratios

Aa x Aa 3:1

Aa x aa (test cross) 1:1

AA x aa (test cross) All dominant trait

AaBb x AaBb 9:3:3:1

Example 2: Coat colour of rabbit

A multiple allele system determines coat colour in the rabbit. There are four alleles of the c gene – wild type ***C*** (brown), chinchilla c^{ch} (silvery grey), Himalayan c^h (white with black extremities such as ears, feet, nose and tail) and albino ***c***.

The wild type allele called agouti is completely dominant over the other three alleles; the chinchilla allele is partially dominant over Himalayan and albino alleles, and the himalayan allele is completely dominant over albino. The dominance relations can be summarized as:

$$C > c^{ch} > c^h > c$$

Pair of alleles can be combined with each other to make six different kinds of heterozygotes.

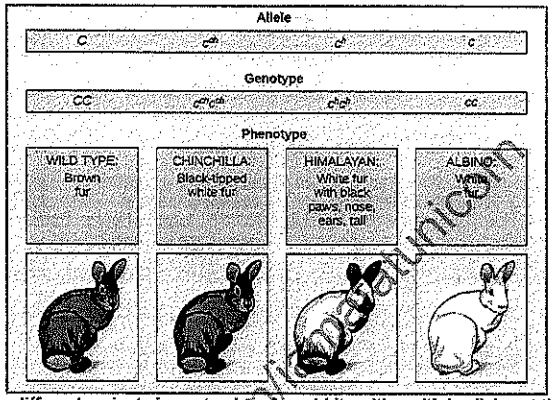


Fig. 8. Four different variants in coat colour for rabbits with multiple alleles at the c locus.

2) Incomplete dominance and Codominance (LO2x)

In Mendel's pea crosses, the F_1 offspring always looked like one of the two parents as one allele in the pair showed complete dominance over the other i.e. the gene product of the dominant allele masked the recessive allele. In such cases, the phenotypes of the heterozygote and the dominant homozygote are not distinguishable. In the case of incomplete dominance and codominance however, neither allele is completely dominant.

For **both** codominance and incomplete dominance, since neither allele is completely dominant, the characteristic (e.g. colour of petals) should be represented with a capital letter and the alleles indicated by the appropriate superscript capital letter (e.g. C^R for red petals and C^W for white petals).

Incomplete dominance

- In incomplete dominance, the heterozygote exhibits a phenotype that is intermediate between the two homozygous forms.
- Example: Flower colour in snapdragons (*Antirrhinum majus*)
- Since neither allele is completely dominant, instead of using uppercase and lowercase letters, the letter C with a superscript to indicate an allele for flower colour is used. C^R for red and C^W for white.
- Crossing homozygotes for red and white flowers yields F_1 heterozygous offspring with pink flowers. Selfing of the F_1 hybrids produces F_2 offspring with a phenotypic ratio of one red to two pink to one white (i.e. 1 red : 2 pink : 1 white).
- The intermediate pink phenotype, results from flowers of the heterozygotes having <u>less</u> red pigment than the red homozygotes.

Parental phenotypes	Pure-breeding red snapdragons	X	Pure-breeding white snapdragons
Parental genotypes	C^RC^R	X	C^WC^W
Meiosis Gametes	C^R		C^W
Fertilisation			
F_1 genotypes	C^RC^W		
F_1 phenotypes	All pink heterozygotes		
Selfing	C^RC^W	X	C^RC^W
Meiosis Gametes	C^R C^W		C^R C^W
Fertilisation			
F_2 genotypes	C^RC^R C^RC^W C^RC^W C^RC^W		
F_2 phenotypic ratio	1 Red : 2 Pink : 1 White		

Codominance

- In **codominance**, both alleles of a gene are **equally expressed** in the phenotype of a heterozygote. Hence, the two alleles affect the phenotype in separate and distinguishable ways. The lack of an intermediate phenotype distinguishes codominance from incomplete dominance.

  Example 1: The blood group system in human, where I^A and I^B is codominant.
  Four blood groups possible - A, B, AB and O

- Individuals with the Type AB blood group have two codominant alleles each coding for a specific molecule located on the surface of red blood cells, the A and B antigen. It is important to note that the AB phenotype is not intermediate between the A phenotype and the B phenotype. In fact individuals with the AB phenotype have both antigens on their surface. This distinguishes codominance from incomplete dominance.

Respective genotype -
People with I^AI^A and I^AI^O genotype are Type A.
People with I^BI^B and I^BI^O genotype are Type B.
People with I^AI^B are Type AB.
People with I^OI^O are Type O.

Parental phenotypes	Male with Type A blood group	X	Female with Type B blood group
Parental genotypes	I^AI^A	X	I^BI^B
Meiosis Gametes	I^A		I^B
Fertilisation			
F_1 genotypes	I^AI^B		
F_1 phenotypes	All progeny are type AB blood group with both A and B antigens		

To note:
- Codominance results in more than two traits for a particular gene
- The effects of both alleles can be detected in the phenotype of heterozygote
- Important ratio in **codominant** monohybrid cross:
  When heterozygote cross with heterozygote e.g. I^AI^B X $I^AI^B \rightarrow I^AI^A : 2\ I^AI^B : I^BI^B \rightarrow$ **1 : 2 : 1**

3) Lethal alleles

- An allele that causes death if organism is homozygous for that allele.
- Those carrying these alleles are disadvantaged through impaired biochemical or physical functioning.

Example: In chickens, Dominant allele (C) in chickens is responsible for profound developmental changes that result in aberrant forms called 'creepers'. These birds have short, crooked legs. Homozygous genotype (CC) is lethal.

Let **C** represent allele for creeper chicken.
Let **c'** represent allele for normal chicken

Parental phenotypes	Creeper	X	Creeper
Parental genotypes	Cc'	X	Cc'
Meiosis Gametes	C c'		C c'

Fertilisation

Gametes	C	c'
C	CC Dies	Cc' Creeper
c'	Cc' Creeper	c'c' Normal

Offspring genotypes	CC	Cc'	Cc'	c'c'
Offspring phenotypes	(Dies- no phenotype)	Creeper	Creeper	Normal
Offspring phenotypic ratio		2	:	1

The **2:1 ratio** replaces the 3:1 ratio because the CC homozygous embryos die.
To note:
Deviation from the standard Mendelian ratios indicates that the gene of interest may be a lethal gene.

Q. Wild mice have grey-colored fur, known as agouti. Some mice have yellow fur.
Crossing yellow mice produces offspring in the ratio of 2 yellow: 1 agouti

Show the cross between two yellow mice in the space below. Which is the dominant allele?
Yellow or agouti?

Let **A** represent allele for yellow fur (dominant)
Let **a** represent allele for agouti fur (recessive)

Parental phenotypes	Yellow fur	X	Yellow fur
Parental genotypes	Aa	X	Aa

Meiosis Gametes: A, a × A, a

Fertilisation

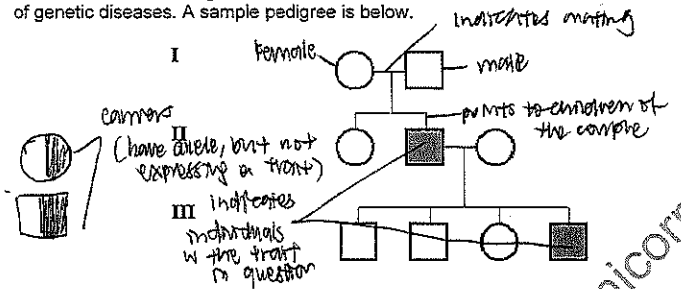
Gametes	A	a
A	AA Dies	Aa Yellow fur
a	Aa Yellow fur	aa Agouti fur

Offspring :			
Genotypes	AA	Aa	Aa	aa
Phenotypes	~~Dies no phenotype~~	Yellow fur	Yellow fur	Agouti fur
Phenotypic ratio		2	:	1

(G) PEDIGREE ANALYSIS

Note: You need to be able to interpret pedigree charts.

A pedigree is a diagram of family relationships that uses symbols to represent people and lines to represent genetic relationships. These diagrams make it easier to visualise relationships within families, particularly large extended families. Pedigrees are often used to determine the mode of inheritance (dominant, recessive, etc.) of genetic diseases. A sample pedigree is below.



In a pedigree, squares represent males and circles represent females. Horizontal lines connecting a male and female represent mating. Vertical lines extending downward from a couple represent their children. Subsequent generations are therefore written underneath the parental generations and the oldest individuals are found at the top of the pedigree.

If the purpose of a pedigree is to analyse the pattern of inheritance of a particular trait, it is customary to shade the symbol representing all individuals that possess this trait.

In the pedigree above, the grandparents had two children, a son and a daughter. The son had the trait in question. One of his four children (male) also had the trait.

found on non-sex chromosomes

(H) LINKED GENES

1) Autosomal linkage → genes on the same chromosomes

LO2z Explain the meaning of the terms linkage and crossing-over and explain the effect of linkage and crossing-over on the phenotypic ratios from dihybrid crosses.

e.g. (Allele A & Allele B)
hv to make reference to alleles in context of q

Linked genes are

- situated on the same chromosome
- located close enough together on a chromosome that the alleles of the **two genes tend to be inherited together.** However the two alleles from the two genes on the same chromosome are not always inherited together. **The closer the gene loci on the same chromosome, the higher the chance that the alleles of the genes will be inherited together as one linkage group.**

• parental phenotype occurs in much higher proportion • recombinant phenotypes are a result of crossing over which occurs by chance.

They **do not usually show independent assortment** (does NOT show 9:3:3:1 for dihybrid inheritance), and a variety of ratios are produced.

Example: In fruit fly, *Drosophila*, the genes for body colour and wing length are linked.
If we assume that the genes are **completely linked** (i.e. no crossing over takes place between the two genes as they are very close to one another), the following would occur:

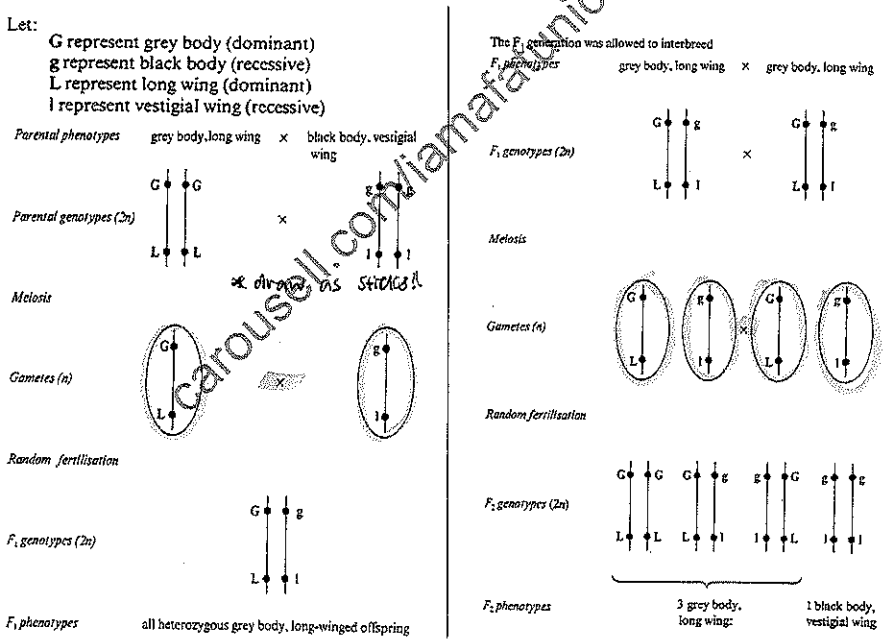


Fig. 9 Genetic representation to show autosomal linkage

- If there is **no crossing over** to separate linked genes, a 3:1 ratio (like that in a monohybrid cross) is obtained. However, in practice, the ratio of 3:1 obtained in the example above is rarely achieved as crossing over may occur.
- The new phenotypes that result from crossing over are called **recombinants**.

2) Crossing over

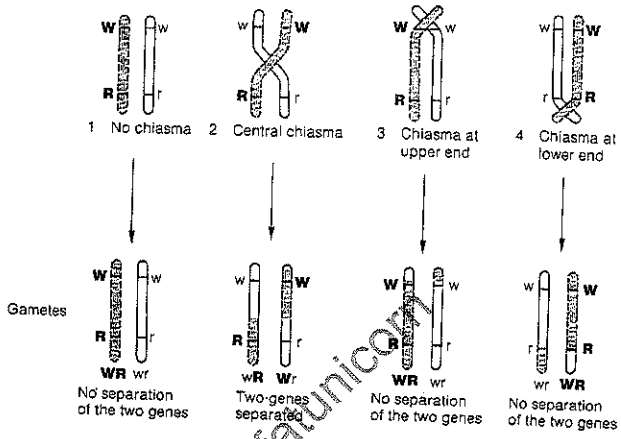


Fig. 10 Effect of different chiasma positions on the separation of two linked genes

- When **crossing over** occurs, **recombinants** may be formed.
- However, recombination does not always occur all the time and even if it does occur, the chiasma has to form at the precise location to disrupt the linkage group.
- Crossing over takes place quite regularly but as shown in the diagram above, the chiasma has to occur between the two genes to form recombinants. **The further the gene loci of the two genes on the chromosome, the greater the likelihood of crossing over occurring.**
- Genes that are close together in a chromosome tend to be inherited together as one linkage group.

Example: T. H. Morgan's experiment on *Drosophila*

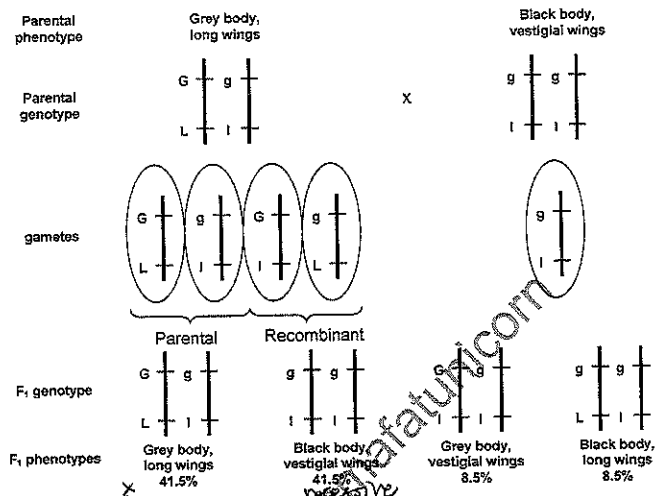
Parents: grey, long wings (heterozygous) x black, vestigial wings (homozygous)

If this is a dihybrid cross involving completely linked (no crossing over) genes, the expected offspring phenotypic ratio is 1 grey, long wings: 1 black, vestigial wings.

If crossing over occurs randomly by chance, expected offspring phenotypic ratio is 1 grey, long wings: 1 black, vestigial wings: 1 grey, vestigial wings: 1 black, long wings

Always produce parent gametes recombinant may sometimes be produced

However if the following occurs:
(where G – grey, g – black, L – normal wings, l – vestigial wings)

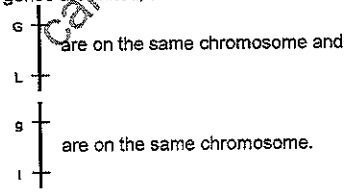


Experimental results obtained

Observed offspring phenotypes (out of 2,300)	965 grey, long wings (42%)
	944 black, vestigial wings (41%)
	206 grey, vestigial wings (9%)
	185 black, long wings (8%)

(larger proportion)
Parental phenotypes
Recombinant phenotypes

- The larger number of parental phenotypes to recombinant phenotypes indicate that parental genes are linked, therefore:

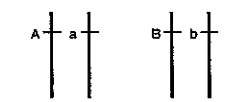
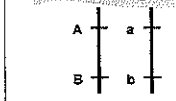
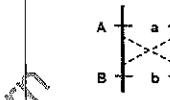
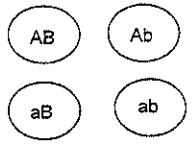
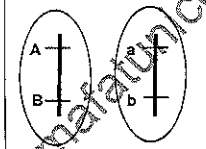
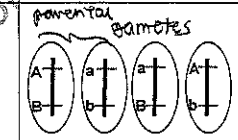


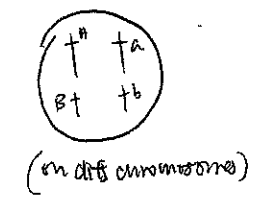
To note:
- For linked genes, draw 'stick' diagrams
- Gametes of 'stick' diagrams must also be circled

- As mentioned, most breeding experiments involving autosomal linkage produce larger numbers of parental phenotypes in comparison to recombinant phenotypes. The 2 parental phenotypes will be in approximately *equal numbers* (e.g. 965 grey, long wings and 944 black, vestigial wings – refer to example on Page 24) and the 2 recombinant phenotypes will be in approximately *equal numbers* (e.g. 206 grey, vestigial wings and 185 black, long wings) as well.
- The number of recombinants depends on the proximity of the linked genes.
- If two genes are close together on a chromosome, the chances of them being separated by a cross-over are smaller than if they were far apart.

To note:
- When the offspring phenotypic ratio of the **test cross** deviates from the usual Mendelian ratios (such as 1:1:1:1 in the wing length/body colour example on Page 17) and has **more parental types than recombinant types**, this indicates **autosomal linkage**.
- The further the distance between the two linked genes, the greater the chance of **crossing over** thus the higher the percentage of recombinant offspring.

Test your understanding:

	If genes are on separate chromosomes	Linked genes – no crossing over	Linked genes - recombinants formed, crossing over occurs
			
Gametes of F_1 individual with genotype AaBb			
F_2 (F_1 x F_1)	9A_B_ : 3A_bb : 3aaB_ : 1aabb	AABB : 2AaBb : aabb 3 : 1	A_B_ : aabb : A_bb : aaB_ Deviates from 9:3:3:1
Testcross (F_1 x aabb)	**1 : 1 : 1 : 1**	**1 : 1**	Deviates from 1:1:1:1


(on diff chromosome)


(on same chromosome)

3) Sex linkage (LO2x)

SEX DETERMINATION

- Human cells: 23 pairs of chromosomes → 22 pairs are described as **autosomal** (non-sex) chromosomes and 1 pair as **sex chromosomes.**
- Females: 2 identical sex chromosomes - XX **(homogametic)** - all eggs carry the X chromosome.
- Males: 1X and 1Y chromosome - XY **(heterogametic)** - 50% of sperms carry X chromosome and 50% carry Y chromosome.

- Function of Y chromosome varies with different organism.
  - In human, it controls the differentiation of testes from undifferentiated gonads of developing embryo. The Y chromosome carries very few genes - genetically inert.
  - The Y chromosome carries the SRY (sex-determining region Y) gene. This gene codes for a transcription factor, TDF (testis-determining factor), which induces the development of testes. The testes produce testosterone which then initiates the development of male sexual characteristics.

Sex Chromosome Abnormalities

Genotype	Gender	Syndrome	Physical Traits
XXY, XXYY, XXXY	Male	Klinefelter syndrome	Sterility, small testicles, breast enlargement
XYY	Male	XXY syndrome	Normal male traits
XO	Female	Turner syndrome	Sex organs do not mature at adolescence, sterility, short stature
XXX	Female	Trisomy X	Tall stature, learning disabilities, limited fertility

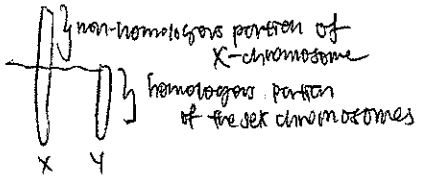
- In female mammals, one of the two X chromosomes is effectively 'shut down" very early in embryonic development - appears as a tightly coiled darkly staining body attached to the inside of the nuclear membrane - **Barr body**. (refer to Organisation and Control of Prokaryotic and Eukaryotic Genome II).

Sex determination of some other organisms:

Organism	Male	Female	
1. *Drosophila*	XY	XX	(similar to mammals)
2. Grasshoppers, cockroaches	XO	XX	(single copy of X in the male)
3. Birds (including poultry)	ZZ	ZW	

SEX LINKAGE

- Sex linkage refers to genes that are carried out the sex chromosomes that result in a characteristic being expressed mainly in one sex.
- In humans, sex linkage usually refers to genes carried on the X chromosome (X-linkage).
- The Y chromosome is smaller than the X chromosome.
- In the case of heterogametic chromosomes, there is a portion of the X chromosome for which there is no homologous region on the Y chromosome.



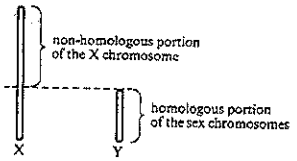


Fig. 11 Homologous and non-homologous regions of the sex chromosomes.

- Characteristics determined by genes carried on the non-homologous portion of the X chromosome therefore appear in males even if they are recessive (as there is no dominant allele to mask the effect of the recessive allele).
- Hence, these characteristics are **sex-linked.**
- Sex-linked characteristics can be detected by their unique pattern of inheritance.
  1. the male offspring will only obtain these features from their mothers
  2. daughters get their sex-linked features from both parents

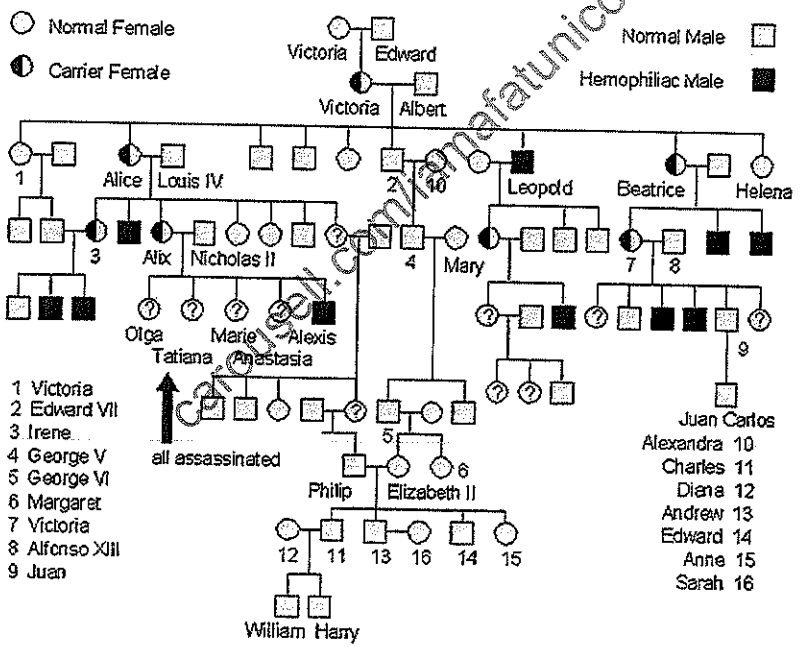


Fig.12 The family tree of Queen Victoria showing the inheritance of sex-linked disease haemophilia

Example 1: **Haemophilia – sex-linked, recessive** condition.
The following genotypes and phenotypes can occur:

Let: X^H represents the female chromosome carrying the normal allele for blood clotting (dominant)
X^h represents the female chromosome carrying the allele for haemophilia (recessive)
Y represents male chromosome

Genotype	Phenotype
X^HX^H	Normal female
X^HX^h	Normal female (carrier)
X^HY	Normal male
X^hY	Haemophilia male

Note:
A carrier refers to the heterozygous female with normal phenotype.

Parental phenotypes: normal female (carrier) X normal male
Parental genotypes (2n): X^HX^h X X^HY

Meiosis

Gametes (n): X^H, X^h, X^H, Y

Fertilisation

Gametes	X^H	Y
X^H	X^HX^H **normal female**	X^HY **Normal male**
X^h	X^HX^h **Normal female (carrier)**	X^hY **Haemophiliac male**

Offspring genotypes (2n)	X^HX^H	X^HY	X^HX^h	X^hY
Offspring phenotypes	normal female	normal male	normal female (carrier)	Haemophiliac male

Offspring phenotypic ratio: 1 : 1 : 1 : 1

Example 2: **Red-green color blindness – sex-linked, recessive condition**

Let: X^B represents the female chromosome carrying the normal allele for colour vision (dominant)
X^b represents the female chromosome carrying the allele for colour blindness (recessive)
Y represents male chromosome

Genotype	Phenotype
X^BX^B	Normal female
X^BX^b	Normal female (carrier)
X^BY	Normal male
X^bY	Colour-blind male

Cross 1

Parental phenotypes: normal female X colour-blind male
Parental genotypes (2n): X^BX^B X X^bY

Meiosis

Gametes (n): X^B, X^B, X^b, Y

Fertilisation

Gametes	X^b	Y
X^B	X^BX^b **Normal female**	X^BY **Normal male**
X^B	X^BX^b **Normal female (carrier)**	X^BY **Normal male**

Offspring genotypes (2n)	X^BX^b	X^BX^b	X^BY	X^BY
Offspring phenotypes	normal female (carrier)	normal female (carrier)	normal male	normal male

Offspring phenotypic ratio: 2 : 1 : 1

Cross 2

Parental phenotypes: normal female (carrier) X normal male
Parental genotypes (2n): X^BX^b X X^BY

Meiosis

Gametes (n): X^B, X^b, X^B, Y

Fertilisation

Gametes	X^B	Y
X^B	X^BX^B **normal female**	X^BY **Normal male**
X^b	X^BX^b **Normal female (carrier)**	X^bY **Colour blind male**

Offspring genotypes (2n)	X^BX^B	X^BY	X^BX^b	X^bY
Offspring phenotypes	normal female	normal male	normal female (carrier)	colour-blind male
Offspring phenotypic ratio	1 :	1 :	1 :	1

- The recessive gene causing colour blindness is exchanged from one sex to the other at each generation. This means that the father passes it to his daughters, who become carriers. The daughters may in turn pass it to their sons, who will be colour-blind.
- Colour blind females can only arise from a cross between a colour-blind carrier female and a colour blind male.

Note:

- **You need to know that haemophilia and colour blindness are sex-linked and recessive conditions.**
- **Male always inherit X chromosome from mother thus the X-linked disease since his Y chromosome is from his father.**

Q. A male and female fly, each with red eyes, are mated. All female progeny have red eyes, half of the males have red eyes, and half of the males have white eyes. What are the genotypes of the parents? Show the genetic diagram.

Hint: Determine if the female is homozygous or heterozygous.

Let R represent allele for red colour (dominant)
r represent allele for white eye colour (recessive)
XX represent female chromosomes
XY represent male chromosomes

Parental phenotypes	normal female (carrier)	X	normal male
Parental genotypes (2n)	X^RX^r	X	X^RY

Meiosis

Gametes (n): X^R X^r X^R Y

Fertilisation

		Gametes: X^R	Gametes: Y
Gametes	X^R	X^RX^R **Red Eye female**	X^RY **Red Eye male**
	X^r	X^RX^r **Red Eye female (carrier)**	X^rY **White eye male**

Offspring genotypes (2n)	X^RX^R	X^RY	X^RX^r	X^rY
Offspring phenotypes	red eye female	red eye male	red eye female (carrier)	white eye male
Offspring phenotypic ratio	1 :	1 :	1 :	1

Reciprocal cross

A reciprocal cross is used to determine if a particular characteristic is sex-linked. In a reciprocal cross, 2 separate crosses are conducted using the same characteristic but the sexes are reversed.

For example:

Cross 1 : A true-breeding red-eyed female crossed with white-eyed male
Cross 2 : A true-breeding white-eyed female crossed with red-eyed male

To illustrate this,

Let: X^R represents the female chromosome carrying the normal allele for red eye (dominant)
X^r represents the female chromosome carrying the allele for white eye (recessive)
Y represents male chromosome

Genotype	Phenotype
X^RX^R	Red-eyed female
X^RX^r	Red-eyed female
X^rX^r	White-eyed female
X^RY	Red-eyed male
X^rY	White-eyed male

Cross 1

Parental phenotypes	Red-eyed female	X	White-eyed male
Parental genotypes (2n)	X^RX^R	X	X^rY

Meiosis

Gametes (n): X^R X^R X^r Y

Fertilisation

		Gametes: X^r	Gametes: Y
Gametes	X^R	X^RX^r **Red-eyed female**	X^RY **Red-eyed male**
	X^R	X^RX^r **Red-eyed female**	X^RY **Red-eyed male**

Offspring genotypes (2n)	X^RX^r	X^RX^r	X^RY	X^RY
Offspring phenotypes	Red-eyed female	Red-eyed female	Red-eyed male	Red-Eyed male
Offspring phenotypic ratio	1	:	1	

Cross 2

Parental phenotypes: White-eyed female X Red-eyed male

Parental genotypes (2n): X^rX^r X X^RY

Meiosis

Gametes (n): X^r, X^r, X^R, Y

Fertilisation

		Gametes	
		X^R	Y
Gametes	X^r	X^RX^r Red-eyed female	X^rY White-eyed male
	X^r	X^RX^r Red-eyed female	X^rY White-eyed male

Offspring genotypes (2n)	X^RX^r	X^RX^r	X^rY	X^rY
Offspring phenotypes	Red-eyed female	Red-eyed female	White-eyed male	White-Eyed male
Offspring phenotypic ratio	1	:	1	

- ratio changes → sex-linked inheritance
- ratio does not change → autosomal inheritance

Analysing the phenotype of the offspring enables one to determine if the characteristic is sex-linked. In cross 1 where a true-breeding red-eyed female crossed with white-eyed male, all male and females are red-eyed. In cross 2 where true-breeding white-eyed female crossed with red-eyed male, all females are red-eyed whereas all males are white eyed.

The phenotypic frequencies shows that the eye colour is sex-linked as the phenotypic frequencies of the reciprocal crosses are different. If the characteristic is not sex-linked, the results of the reciprocal cross will be the same.

Single gene disorders

Autosomal dominant	Prevalence (approximate)	Description
Huntington's disease	1 in 15000	Huntington's Disease (HD) is a nervous degeneration. Onset of illness in early middle age and death within 10 years.
Achondroplasia (lethal gene)	1 in 26000	Is a form of dwarfism. All are genetically heterozygous as homozygous condition is lethal
Treacher Collins syndrome	1 in 50000	Characterized by craniofacial deformities and absence of cheekbones
Progenia (Hutchinson–Gilford syndrome)	1 in 8 million	Is an extremely rare genetic disease wherein symptoms resembling aspects of aging are manifested at an early age. Due to the mutation of gene LMNA results in producing an altered protein prelamin A. Thus making the nuclear envelope unstable and progressively damages the nucleus, making cells more likely to die prematurely. Live to mid-teens or early twenties.
Autosomal recessive	**Prevalence (approximate)**	**Description**
Sickle cell anemia	1 in 625	Distorted red blood cells block capillaries causing a cessation of blood supply results in severe haemolytic anaemia with onset in infancy.
Cystic fibrosis	1 in 2000	It is characterized by abnormal transport of chloride and sodium across an epithelium, leading to thick, viscous secretions. Results in poor growth frequent chest infections, and coughing or shortness of breath.
Tay-Sachs disease	1 in 3000	It causes a progressive deterioration of mental and physical abilities that commences around six months of age and usually results in death by the age of four.
Phenylketonaria	1 in 12000	A mutation in the gene for the hepatic enzyme phenylalanine hydroxylase (PAH), rendering it nonfunctional. When PAH activity is reduced, phenylalanine accumulates and is converted into phenylpyruvate. Untreated PKU can lead to mental retardation, seizures, and other serious medical problems.
Severe Combined Immunodeficiency (SCID)	1 in 500000	Is a genetic disorder in which both "arms" (B cells and T cells) of the immune system are impaired due to a defect in one of several possible genes. Victims are extremely vulnerable to infectious diseases and need to stay in a sterile environment e.g. the boy in the bubble.
Cockayne syndrome	1 in 100000	Is characterized by growth failure, impaired development of the nervous system abnormal sensitivity to sunlight (photosensitivity), and premature ageing. The underlying disorder is a defect in a DNA repair mechanism
X-linked recessive	**Prevalence (approximate)**	**Description**
Duchene Muscular Dystrophy	1 in 7000	A progressive neuromuscular disorder and is muscle weakness associated with muscle wasting with the voluntary muscles
Haemophilia	1 in 12000	Haemophilia impairs the body's ability to control blood clotting or coagulation, which is used to stop bleeding when a blood vessel is broken. Can lead to excessive blood lost and death.

Key words
dominant
recessive
homozygous
heterozygous
test cross
reciprocal cross
parental phenotypes
recombinant phenotypes
mask the effect of the recessive allele

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CORE IDEA
(2) GENETICS & INHERITANCE

GENETIC BASIS FOR VARIATION (II)

Content

- Interactions between loci

Learning Outcomes

Candidates should be able to:

(aa) describe the interaction between loci (epistasis) and predict phenotypic ratios in problems involving epistasis (knowledge of the expected ratio for various types of epistasis is not required; focus of this section is on problem solving)

(cc) explain the difference between genetic variation that is continuous (many, additive genes control a characteristic) and genetic variation that is discontinuous (one or a few genes control a characteristic)

(dd) use the chi-squared test to test the significance of differences between observed and expected results.

Use the knowledge gained in this section in new situations or to solve related problems.

References

- Campbell and Reece, BIOLOGY, 10th edition
- Hartl and Jones, GENETICS: ANALYSIS OF GENES AND GENOMES, 8th Edition
- Klug, Cummings and Spencer, ESSENTIALS OF GENETICS, 8th Edition
- Griffiths, Wessler, Lewontin and Carroll, INTRODUCTION TO GENETIC ANALYSIS, 9th Edition

*** This handout is the effort of several Biology teachers at RI (Yr 5-6). It has and will continue to be updated.**

****Any information given in a double-lined box is for your information only.**

Learning Outcome (LO) (cc)

(cc) explain the difference between genetic variation that is continuous (many, additive genes control a characteristic) and genetic variation that is discontinuous (one or a few genes control a characteristic)

A. Discontinuous and Continuous Variation

1. Discontinuous Variation

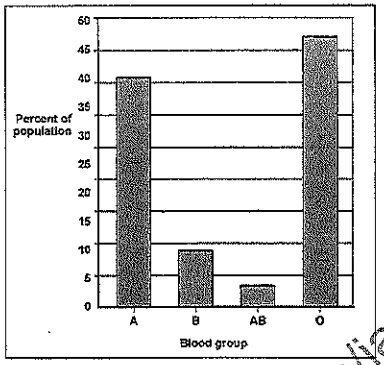


Figure 1
Discontinuous (discrete) variation showing the percentage of individuals in a population with four different (discrete) blood types

Features:

- There are distinct and discrete phenotypes.
- Generally caused by **different alleles** of a **single (few)** gene(s).
- Their phenotypic expression in many cases are **unaffected by environmental conditions**. e.g. blood groups in the ABO system - A, B, AB or O; green and yellow peas; wrinkled and smooth peas.

2. Continuous Variation

continuous variation
variation in the height of adult male humans; the results cluster quite closely around one value, and show a normal distribution (in this type of distribution the mean, median and mode values coincide); for convenience in plotting, the heights are grouped into 13 arbitrary groups, each covering a height range of 2 cm

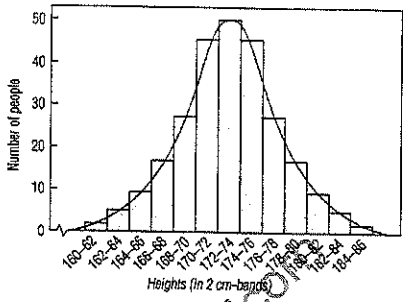


Figure 2. Example of continuous variation - height of adult male humans

Features:

- Differences are slight and **phenotypic differences vary along a continuum** in gradations. **No distinct groupings** are formed.
- There is a **range of phenotypes** for one characteristic e.g. human exhibit continuous variation in height, weight and eye colour.
- Continuous variation or **quantitative variation** usually indicates **polygenic inheritance**, where there is an **additive effect of two or more** (usually many) **genes** on a single phenotypic character.
- Polygenic inheritance will result in a **bell-shape normal distribution** curve (**unbroken range** of phenotypes) where **intermediate phenotypes are more common than extreme phenotypes.**
- **Phenotypes are affected by environmental factors.** For example, exposure to sun, also affects the skin-colour phenotype and make the graph smooth rather than a step-like histogram.

summarise

discontinuous variation	continuous variation
A few distinct & discrete phenotypes	Phenotypes vary along a continuum (no distinct groups)
caused by diff alleles of a single gene	Polygenic — involves 2 or more genes w an additive effect on phenotype
Generally unaffected by environment	influenced by environment, give rise to a normal distribution w more intermediate phenotypes v.s. extreme phenotypes

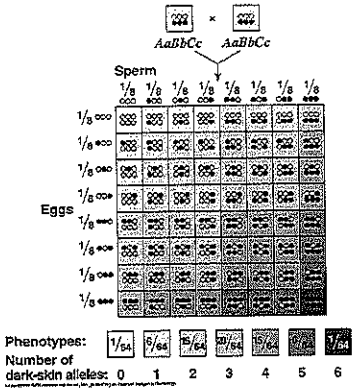


Figure 3.1

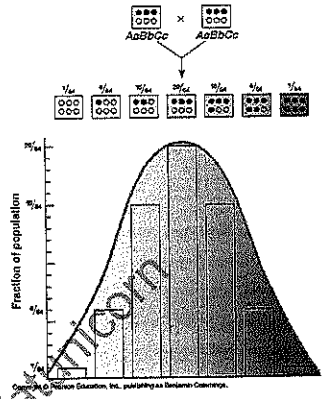


Figure 3.2

Figure 3.1

A simplified model for the polygenic inheritance of skin colour.

According to this model three separately inherited genes affect the darkness of skin.

The heterozygous individuals (**AaBbCc**) represented by the two rectangles at the top of these figures carry three dark-skin alleles (black circles, which represent **A**, **B**, or **C**) and three light-skin alleles (white circles which represent **a**, **b**, or **c**).

A dark-skin allele for each gene (**A**, **B**, or **C**) contributes on "unit" of darkness to the phenotype. An individual **AABBCC** would be very dark, while an aabbcc individual would be very light. An **AaBbCc** person would have intermediate shade.

Because alleles have a cumulative effect the genotype **AaBbCc** and **AABbcc** would make the same genetic contribution (three units) to skin darkness.

The Punnett square shows all the possible genetic combinations in gametes and in offspring of a large number of hypothetical matings between these heterozygotes. The results are summarized by the phenotype ratios under the Punnett square.

AaBbCc = AABbcc = AaBBcc = aaBbCC
3 3 3 3

Figure 3.2

Represents a bar chart of the results, with skin colour (number of dark-skin alleles) along the x-axis and fraction of offspring along the y-axis.

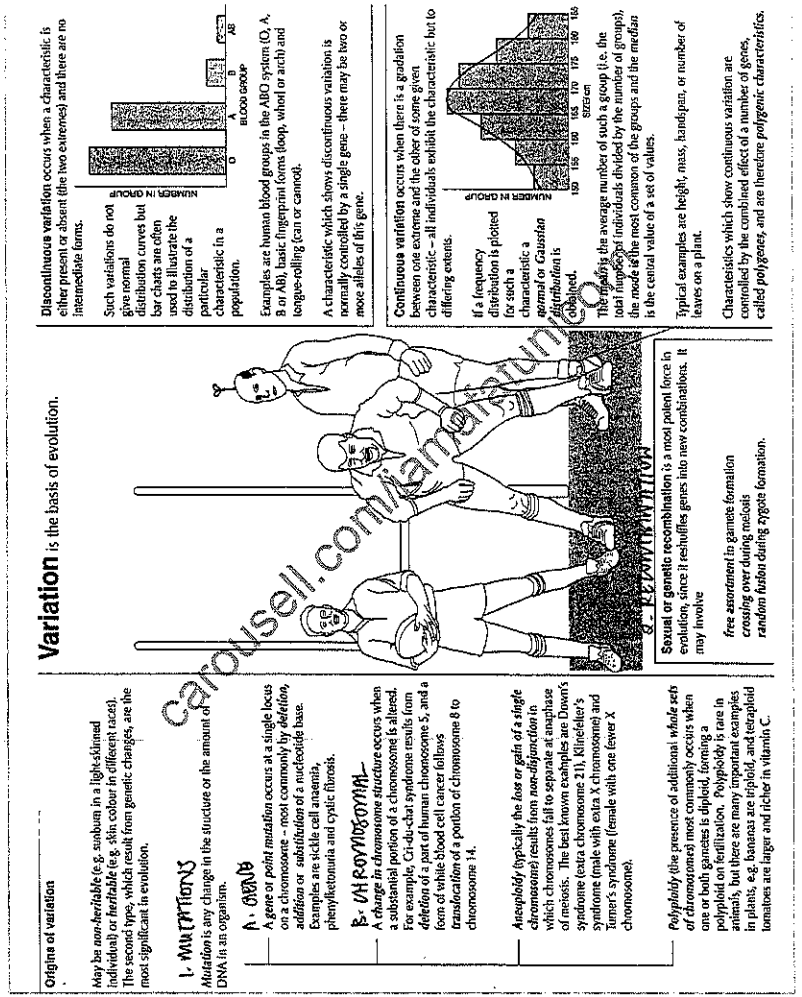


Figure 4. Summary on the concept of variation.

Learning Outcome (LO) (aa)

(aa) describe the interaction between loci (epistasis) and predict phenotypic ratios in problems involving epistasis (knowledge of the expected ratio for various types of epistasis is not required; focus of this section is on problem solving)

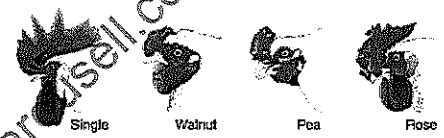
B. Epistasis

1. Definition of epistasis

Epistasis is a form of gene interaction in which **a gene at one locus** alters the phenotypic expression of a **gene at a second locus**.

- The gene whose phenotype is expressed is said to be **epistatic**, while the gene whose phenotype altered or suppressed is said to be **hypostatic**.
- Epistasis should not be confused with dominance, which is an interaction between alleles at the same gene locus.
- Epistatic alleles can be either dominant or recessive and each type gives a different modified dihybrid ratio.
  - A **recessive epistatic** allele hides the phenotypic effects of alleles of the other gene when the recessive epistatic allele is present in the **homozygous** condition.
  - A **dominant epistatic** allele hides the phenotypic effects of alleles of the other gene when it is present in the **homozygous** or **heterozygous** condition.
- In epistasis, we will observe **deviation from the 9:3:3:1 ratio** in different combination (e.g. 9:3:4, 9:7)

2. Example 1: Comb types of chicken (9:3:3:1 ratio) – *this is not the same as the usual dihybrid ratio!*



The first case of two different genes interacting to affect a single trait was discovered by William Bateson and Reginald Punnett in 1906 while they were investigating the inheritance of comb morphology in chickens. In their studies, Bateson crossed a Wyandotte breed having a **rose comb** with a Brahma breed having a **pea comb**. All F_1 offspring had **walnut comb**.

When these F_1 offspring were mated to each other, the F_2 generation consisted of chickens with four types of combs in the following phenotype ratio: **9 walnut : 3 rose : 3 pea : 1 single.**

The F_1 differed from both parents and two new phenotypes not seen in the parents appeared in the F_2. How can this result be explained? The first clue is the F_2 ratio. We have seen this ratio before when the F_1 from a dihybrid cross is selfed (or inter-mated). This observation suggests that two genes may control the phenotype of the comb.

Parental phenotype	rose comb	X	pea comb
Parental genotype	RRp'p'		rrPP
Gametes	Rp'		rP
F_1 genotype	RrPp'		
F_1 phenotype	All Walnut (new phenotype)		
Self-cross of F_1 generation	RrPp' X RrPp'		

F_2

Gametes	RP	Rp'	rP	rp'
RP	RRPP walnut	RRPp walnut	RrPP walnut	RrPp walnut
Rp'	RRPp walnut	RRp'p' rose	RrPp walnut	Rrp'p' rose
rP	RrPP walnut	RrPp walnut	rrPP pea	rrPp' pea
rp'	RrPp walnut	Rrp'p' rose	rrPp' pea	rrp'p' single

F_2 genotypes	9 R_P_ :	3 R_p'p' :	3 rrP_ :	1 rrp'p'
F_2 phenotypic ratio	9 walnut :	3 rose :	3 pea :	1 singe

A 9:3:3:1 ratio is obtained in the F_2 generation when the F_1 generation is heterozygous and these genes assort independently. However, an important difference here is that we have **four distinct categories** for a single trait. Based on the 9:3:3:1 ratio, Bateson and Punnett reasoned that a single trait (comb morphology) was determined by two genes.

R (rose comb) is dominant to **r**
P (pea comb) is dominant to **p'**
R and **P** (walnut comb) are codominant
rrp'p' produces a single comb

Phenotypes	Genotypes
Walnut	R_P_
Rose	R_pp
Pea	rrP_
Single	rrp'p'

IMPORTANT! This case study is a very special case of gene interaction. It should not be confused with the dihybrid ratio of 9:3:3:1 for a F_2 generation from the selfing of F_1 that you learnt in the earlier Mendelian Genetics I lectures.

In dihybrid ratios, we are referring to 2 genes, each controlling 1 trait whilst in Example 1 above, we are dealing with 2 genes controlling only 1 trait (e.g. comb-shape).

3. **Example 2: Flower colour in sweet pea (9:7 ratio)** → 3+3+1

Bateson and Punnett discovered another unexpected gene interaction. They crossed two different varieties of white-flowered plants. All the F_1 plants had purple flowers. When plants from the F_1 generation were allowed to self fertilise, the F_2 generation gave a phenotypic ratio of **9 purple flowers to 7 white flowers**. Explain the results.

Parental phenotype	White	X	White
Parental genotype	CCp'p'		c'c'PP
Meiosis	(Cp')		(c'P)
Gametes (n)			
F_1 genotype	Cc'Pp' (double heterozygote)		
F_1 phenotype	All purple		
Self-cross of F_1 generation	Cc'Pp'	X	Cc'Pp'
Fertilisation to produce F_2			

Gametes	(CP)	(Cp')	(c'P)	(c'p')
(CP)	CCPP Purple	CCPp' Purple	Cc'PP Purple	Cc'Pp' Purple
(Cp')	CCPp' Purple	CCp'p' White	Cc'Pp' Purple	Cc'p'p' White
(c'P)	Cc'PP Purple	Cc'Pp' Purple	c'c'PP White	c'c'Pp' White
(c'p')	Cc'Pp' Purple	Cc'p'p' White	c'c'Pp' White	c'c'p'p' White

F_2 genotypes	9 C_P_ :	3 C_p'p' :	3 c'c'P_ :	1 c'c'p'p'
F_2 phenotypic ratio	9 Purple	:	7 white	

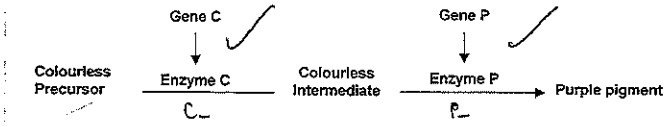
Biochemical pathway

Epistasis often occurs when two (or more) proteins are part of an enzymatic pathway. In this example, a colourless precursor molecule go through a pathway involving 2 different enzymes in order to produce the purple pigment.

Gene **C** codes for a **functional enzyme C**, which converts the colourless precursor into a colourless intermediate. The homozygous recessive alleles (**c'c'**) result in **absence** of functional enzyme C (either the enzyme is not produced or the enzyme produced is non-functional). Gene **P** codes for enzyme P, which converts the colourless intermediate into the purple pigment. Homozygous recessive allele (**p'p'**) results in **absence** of functional enzyme P.

If an individual is homozygous for either recessive allele (genotype **c'c'** or **p'p'**) it will not make any functional enzyme **C** or enzyme **P**, respectively. When either of these enzymes is missing, purple pigment cannot be made and the flower remains white.

This is shown in the diagram below: both genes must work, for pathway to work



Genotype	Flower Colour	Enzyme Activities
C_P_	purple pigment produced; flowers colored	functional enzymes C and P
C_p'p'	no purple pigment produced; flowers white	enzyme P non-functional
c'c'P_	no purple pigment produced; flowers white	enzyme C non-functional
c'c'p'p'	no purple pigment produced; flowers white	enzymes C and P non-functional

Type of Epistasis

Since genotype c'c' masks the phenotypic expression of P/p locus, we say that:
genotype c'c' is epistatic over PP or Pp', OR
genotype c'c' is epistatic over the P/p' locus.

Genotype p'p' also masks the phenotypic expression of C/c locus, we say that:
genotype p'p' is epistatic over CC or Cc', OR
genotype p'p' is epistatic over the C/c' locus.

This is an example of **recessive epistasis**.

recessive epistasis.
- C_p'p' has the same phenotype as c'c'p'p'
- → the phenotypic effects of the C allele is not seen
- → "masked" by the other gene (Gene P)
- → p'p' masks the phenotypic expression of the C/c' locus
- → p'p' is epistatic over C_ (CC/Cc')

recessive epistasis
- c'c'P_ has the same phenotype as c'c'p'p'
- → The phenotypic effects of the P allele is not seen.
- → "masked" by the other gene (Gene C)
- → c'c' masks the phenotypic expression of the P/p' locus
- → c'c' is epistatic over P_ (PP or Pp')

deviation from 9331 → may be epistasis

3+1

4. Example 3: Coat colour inheritance in Labrador retrievers. (9:3:4 ratio)

Coat colour inheritance in Labrador retrievers is determined by two genes. Two alleles **B** and **b**, of a **pigment gene**, determine (i) **black** and (ii) **brown**, respectively. Another gene, **C** allows **colour deposition** in the coat and **c'c'** prevents deposition, resulting in (iii) the **gold**/yellow phenotype

Pure bred black Labradors (**CCBB**) were mated with gold Labradors (**c'c'bb**). The offspring were all black. The black Labradors that are heterozygous for both genes (**Cc'Bb**) were mated. What will be the expected phenotype of the F_2 generation? Show your answer in a genetic diagram in the space below.

Parental phenotype	Black	X	Gold
Parental genotype	CCBB		c'c'bb
Meiosis			
Gametes (n)	CB		c'b
F_1 genotype	Cc'Bb		
F_1 phenotype	All black		
Self-cross of F_1 generation	Cc'Bb	X	Cc'Bb
Fertilisation to produce F_2			

Gametes	CB	Cb	c'B	c'b
CB	CCBB Black	CCBb Black	Cc'BB Black	Cc'Bb Black
Cb	CCBb Black	CCbb brown	Cc'Bb Black	Cc'bb Brown
c'B	Cc'BB Black	Cc'Bb Black	c'c'BB Gold	c'c'Bb Gold
c'b	Cc'Bb Black	Cc'bb Brown	c'c'Bb Gold	c'c'bb Gold

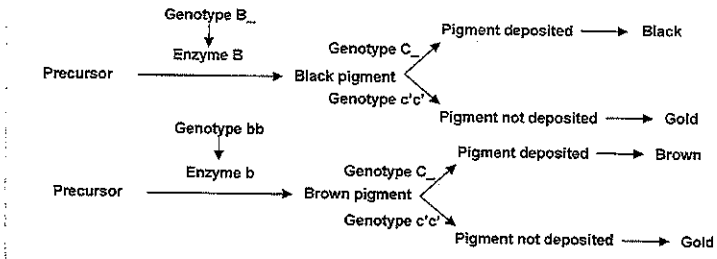
F_2 genotypes	9 C_B_ :	3 C_bb :	3 c'c'B_ :	1 c'c'bb
F_2 phenotypic ratio	9 Black :	3 Brown :	4 Golden	

Biochemical pathway

Allele **B** codes for enzyme **B** which produces **black** pigmentation. Allele **b** codes for enzyme **b** which produces **brown** pigmentation. A separate gene, **C** allows **colour deposition** in the coat and **c'c'** prevents deposition, resulting in the gold phenotype.

recessive epistasis

c'c'B_ has same phenotype as c'c'bb
→ phenotypic effects of the B allele is NOT seen
→ "masked" by the other gene (Gene C)
→ c'c' masks the phenotypic expression of the B/b locus
→ c'c' is epistatic over B/b locus



Genotype	Fur Color	Gene Actions
C_B_	Black	Presence of **C** allele allows pigment deposition and hence, allows the phenotypic expression of the **B** allele to result in a black fur coat.
C_bb	Brown	Presence of **C** allele allows pigment deposition and hence, allows for the phenotypic expression of the **b** allele to result in a brown fur coat.
c'c'B_	Gold	**c'c' genotype** does not allow pigment deposition and hence, prevents the phenotypic expression of the **B** allele.
c'c'bb	Gold	**c'c' genotype** does not allow pigment deposition and hence, prevents the phenotypic expression of the **b** allele.

Type of Epistasis

This case of epistasis is *not* caused by a block in a pathway leading to dark pigment. Gold/yellow dogs can make black or brown pigment, as can be seen in their nose and lips. The c'c' genotype does not allow for the deposition of pigment in hairs. In this case, the epistatic gene is *"developmentally downstream"*; the dominant allele **C** must be present before pigment can be deposited.

This is a case of recessive epistasis. The c'c' genotype is epistatic over the B/b locus.

9+3+3

5. Example 4: Kernel colour in wheat (15:1 ratio)

A pure line wheat plant with a coloured kernel (genotype = **AABB**) is crossed to plant with white kernels (genotype = **aabb**). All F_1 offspring had coloured kernel. When the resulting F_1 plants were selfed, the offspring gave a ratio of **15 coloured kernels: 1 white kernel**. Explain the results.

Parental phenotype	Coloured	X	White
Parental genotype	**AABB**		**aabb**
Meiosis	(AB)		(ab)
Gametes (n)			
F_1 genotype	**AaBb**		
F_1 phenotype	All coloured		
Self-cross of F_1 generation	**AaBb X AaBb**		

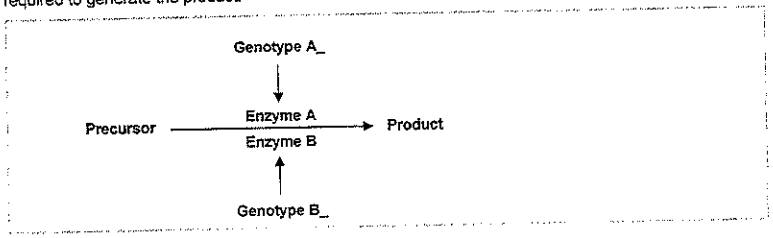
Fertilisation to produce F_2

Gametes	AB	Ab	aB	ab
AB	AABB Coloured	AABb Coloured	AaBB Coloured	AaBb Coloured
Ab	AABb Coloured	AAbb Coloured	AaBb Coloured	Aabb Coloured
aB	AaBB Coloured	AaBb Coloured	aaBB Coloured	aaBb Coloured
ab	AaBb Coloured	Aabb Coloured	aaBb Coloured	aabb white

F_2 genotypes	**9 A_B_ :**	**3 A_bb :**	**3 aaB_ :**	**1 aabb**
F_2 phenotypic ratio	15 Coloured			: 1 white

Biochemical pathway

For this type of pathway a **functional enzyme A or B** can produce a product from a common precursor. The product gives colour to the wheat kernel. Therefore, only one dominant allele at either of the two loci is required to generate the product.



Genotype	Kernel Phenotype	Enzymatic Activities
9 A_B_	coloured kernels	Functional enzymes A & B
3 A_bb	coloured kernels	Functional enzyme A only
3 aaB_	coloured kernels	Functional enzyme B only
1 aabb	white kernels	~~Partial~~ enzymes A & B non-functional

(15 coloured)

If we sum the three different genotypes that will produce a coloured kernel we can see that we can achieve a 15:1 ratio. Because either of the genes can provide the coloured phenotype; this interaction is also called *duplicate gene action.*

*In this case, it is **duplicate dominant epistasis**.*

The A_ genotype is epistatic over the B/b locus and the B_ genotype is epistatic over the A/a locus.

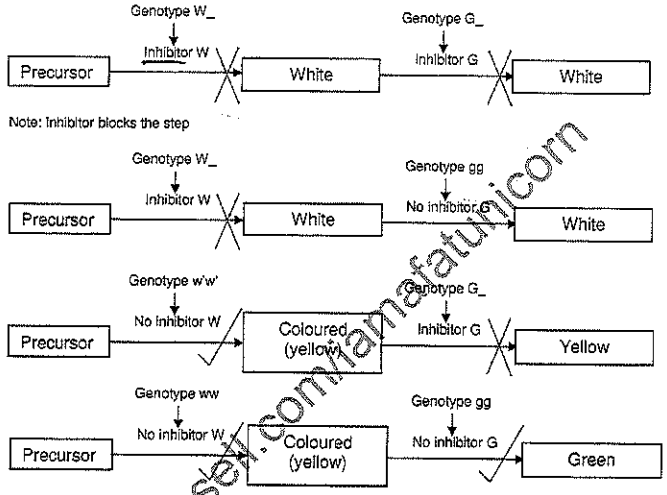
duplicate dominant epistasis

- A_bb & aaB_ has same phenotype as A_B_.
- phenotypic effects of ~~bb genotype~~ & aa genotype not seen
- A-/B_ masks phenotypic expression of the B/b locus / A/a locus respectively
- A_ is epistatic over B/b locus & B_ is epistatic over A/a locus

6. Example 5: Fruit colour in squash (12:3:1 ratio)

At the first gene locus, white is dominant to coloured, and the gene symbols are **W**=white and **w'**=coloured. This recessive allele must be expressed before the specific allele determining colour at a second locus is expressed. At the second gene locus, yellow is dominant to green, and the symbols used are **G**=yellow, **g**=green.

Biochemical Pathways



When 2 white squash plants with genotype Ww'Gg are selfed, a modification of the dihybrid 9:3:3:1 ratio will be produced. The following table provides a biochemical explanation for the 12:3:1 ratio.

Genotype	Fruit Color	Gene Actions
9 W_G_	White	Dominant W allele masks the phenotypic expression of the G/g locus (C/c G allele/ gg phenotype)
3 W_gg	White	
3 w'w'G_	Yellow	w'w' genotype allows for phenotypic expression of the yellow allele.
1 w'w'gg	Green	w'w' genotype allows for the phenotypic expression of the green allele.

(no yellow intermediate to be converted to green)

Type of Epistasis

Since W_ masks the expression of G/g locus, the W_ genotype is epistatic over the G/g locus

This is a case of dominant epistasis

7. Example 6: White Leghorn chicken.

*In the case of White Leghorn chickens (genotype **CCII**) and White Wyandotte chickens (genotype c'c'ii), both breeds have white feathers because the **C** allele is necessary for coloured feathers, but the **I** allele in White Leghorns is a dominant inhibitor of feather colouration. The F_1 generation of a dihybrid cross between these breeds has the genotype **Cc'Ii**, which is expressed as white feathers because of the inhibitory effects of the **I** allele. Predict the phenotypic ratio of the F_2 generation. Use a genetic diagram to explain your prediction.*

Parental Phenotype: White × White

Parental genotype: CCII × c'c'ii

Meiosis gametes (n): CI × c'i

F1 genotype: Cc'Ii

F1 phenotype: All White

F1 selfing: Cc'Ii × Cc'Ii

Punnett square

Gametes	CI	Ci	c'I	c'i
CI	CCII	CCIi	Cc'II	Cc'Ii
Ci	CcIi	Cc'i	Cc'Ii	Cc'ii
c'I	Cc'Ii	Cc'Ii	c'c'II	c'c'Ii
c'i	Cc'Ii	Cc'ii	c'c'Ii	c'c'ii

F2 genotypic ratio: 9 C_I_ : 3 C_ii : 3 c'c'I_ : 1 c'c'ii

F2 phenotypic ratio: 13 white : 3 coloured

Genotype	Feather	Explanation
C_I_	white	C allele allows for production of coloured feathers, but I allele produces an inhibitor for colouration.
C_ii	coloured	C allele allows for production of coloured feathers, ii genotype results in no functional inhibitor produced.
c'c'I_	white	c'c' genotype results in white feathers, I allele results in inhibitor for colouration
c'c'ii	white	c'c' genotype results in white feathers, ii genotype results in no functional inhibitor.

Figure 5. Overview of possible ratios for epistasis

9 A_B_				3A_bb		3 aaB_		aabb	
AABB	AABb	AaBB	AaBb	AAbb	Aabb	aaBB	aaBb	aabb	Unmodified ratio 9:3:3:1
1	2	2	4	1	2	1	2	1	

Possible ratios due to epistasis				Modified F_2 ratios
9	3	3	1	12:3:1
9	3	3	1	10:3:3
9	3	3	1	9:6:1
9	3	3	1	9:3:4
9	3	3	1	15:1
9	3	3	1	13:3
9	3	3	1	12:4
9	3	3	1	10:6
9	3	3	1	9:7

Each of the boxes represents a particular phenotype. For example, in the epistatic ratio of 12:3:1, there are two grey-shaded boxes representing 9 units and 3 units respectively (together for a total of 12) for a particular phenotype, one striped box with 3 units for another phenotype and finally one white box with 1 unit for the third phenotype.

Dihybrid ratios add up to **16**. A ratio of 9:3:3 = 15, is less than 16 and will likely indicate lethal alleles rather than epistasis.

⇒ dominant epistasis.

Learning Outcome (LO) (dd)

(dd) use the chi-squared test to test the significance of differences between observed and expected results.

C. Statistics

1. The Chi-square Test

The chi-square or "goodness of fit" test is used when a **sample result** needs to be **compared** to **expected results** or a **known ratio**.

- For example, if, according to Mendel's laws, you expect 100 of 200 offspring from a cross to be male but the actual observed number was 80 males, then you might want to know about the "goodness to fit" between the observed and expected results.
- Were the deviations (differences between observed and expected) the result of chance, or were they due to other factors?
- How much deviation can occur before you, the investigator, can conclude that **something other than chance** is at work, causing the observed to differ from the expected?

As a student, you must know how to;

- carry out a chi-square test, which involves 6 steps and
- determine whether the difference in results between the observed and expected results is due solely to chance.

You will be given the following formula and a chi-square table in the exams. You must know how to use the formula properly, i.e. carry out the correct mathematical operations denoted by the symbols in the formula. Therefore you need to know the meaning of the symbols!

chi-squared value → χ^2; observed value → O; expected value → E

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

This is the chi-square formula which you need to use to calculate the chi-square value (χ^2).

Σ = summation, O = observed value, E = expected value

$$df = n - 1$$

You will also need to calculate the degrees of freedom (*df*)

where *n* = the number of classes, usually phenotypes.

→ Compute $(O-E)^2/E$ for all phenotypic ratios.

STEPS

Step 1: **List the expected phenotypic ratio.**

Step 2: **State the null hypothesis (H_0).** $O = E$

Step 3: **Calculate the χ^2 value.**

Step 4: **Determine the degrees of freedom (*df*).**

Step 5: **Determine "p", using the *df* and χ^2 value to check against the chi-square table.**

Step 6: **Reject or do not reject H_0. Draw your conclusion.**

Example 1

A homozygous white flowered, long-stemmed tobacco plant, *Nicotiana affinis* was crossed with a homozygous pink flowered, short-stemmed plant. The F_1 generation had white flowers and long stems. When the F_1 generation was crossed with recessive tester stock, the following progeny were obtained:

51 white flowered, long-stemmed plants,

46 white flowered, short-stemmed plants,

49 pink flowered, long-stemmed plants,

44 pinked flowered, short-stemmed plants,

(a) State using suitable symbols,

(i) Genotype of F_1 generation

(ii) Genotypes of four phenotypes in the progeny of backcross

Let: W = white allele, w' = pink allele,

L = long-stemmed allele, l = short-stemmed allele

Genotype of F_1 generation: Ww'Ll

Genotypes of the four progeny of backcross (test cross):

White flowered, long-stemmed plants: Ww'Ll

White flowered, short-stemmed plants: Ww'll

Pink flowered, long-stemmed plants: w'w'Ll

Pink flowered, short-stemmed plants: w'w'll

(b) Do the results indicate that the inheritance of the genes for flower colour and stem length follow Mendel's laws? Explain, using χ^2 test to support your answer.

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

Where
O = observed value;
E = expected value;
Σ = sum of

Distribution of χ^2

degrees of freedom	*probability, p*				
	0.10	0.05	0.02	0.01	0.001
1	2.71	3.84	5.41	6.64	10.83
2	4.61	5.99	7.82	9.21	13.82
3	6.25	7.82	9.84	11.35	16.27
4	7.78	9.49	11.67	13.28	18.47

~~than~~ >5% ommu
↳ unobserved
things

if p > 0.10
↳ high probability that it is due to chance
↳ difference between expected & observed not significant.

Step 1: List the expected phenotypic ratio

The expected phenotype ratio of the F_1 generation test-crossed is:

1 White flowered, long-stemmed plants: 1 White flowered, short-stemmed plants: 1 Pink flowered, long-stemmed plants: 1 Pink flowered, short-stemmed plants

Step 2: State the null hypothesis (H_0)

H_0. There is no significant difference between the expected and observed values. Any difference is due to chance alone.

Step 3: Calculate the χ^2 value

Total number of offspring = 51 + 46 + 49 + 44 = 190

Expected frequency = 190÷ 4 = 47.5

(since we expect equal numbers of offspring for each class of phenotype)

F_1 phenotype	Observed frequency (O)	Expected frequency (E)	$(O-E)^2 / E$
white flowered, long-stemmed plants	51	47.5	$(51-47.5)^2 \div 47.5 = 0.258$
white flowered, short-stemmed plants	46	47.5	$(46-47.5)^2 \div 47.5 = 0.0474$
pink flowered, long-stemmed plants	49	47.5	$(49-47.5)^2 \div 47.5 = 0.0474$
pinked flowered, short-stemmed plants	44	47.5	$(44-47.5)^2 \div 47.5 = 0.258$

$\chi^2 = 0.611$ Σ — 0.61

Step 4: Determine the degrees of freedom (*df*)

df = n – 1 = 4 – 1 = 3 *(where n = number of classes)*

Step 5: Determine "p", using the *df* and χ^2 value to check against the chi-square table

The χ^2 value of 0.611 (which is less than 6.25)

Looking up the columns,

the 'p' value is more than 0.10

p > 0.10

0.80 < p < 0.90
as long as p > 0.5
↳ don't reject null hypothesis
(5% level of significance)

Step 6: Reject or do not reject H_0. Draw your conclusion.

Since p > 0.10 which is greater than p > 0.05 at 5% level of significance, we do not reject the null hypothesis

(The probability is very high that any difference is due to chance)

There is **no *significant* difference** between the observed and expected offspring. Any difference is due to **chance**.

(Note: Lack of evidence against a hypothesis is not evidence for it so you **do not accept** H_0. So you conclude that you do not have enough evidence to reject the null hypothesis).

What if at level of significance of 5%, $p < 0.05$?

- We would **reject the null hypothesis** and conclude the **difference between observed and expected results is not due to chance alone.**
- Then the results indicate that the inheritance of the genes for flower colour and stem length do not follow Mendel's laws. Some other factor could have contributed to the difference between the observed and expected values.

Example 2

The colour of wild mice is agouti while mice lacking the black band in each hair are yellow. In 1905, a geneticist reported that he had been unable to get homozygous yellow mice.

A number of geneticists then pooled their data on the results of crosses between yellow mice and found that out of a total of 1598 progeny, 1063 were yellow and 535 agouti. Individual litters tend to be smaller than normal.

↳ lethal genes

1. *What is the expected phenotypic ratio of the offspring for a cross involving two yellow parents?*

(Step 1: List the expected phenotype ratio)

~~1 white flowered, long-stemmed plants : 1 white flowered, short-stemmed plants :~~
~~1 pink flowered, long-stemmed plants : 1 pink flowered, short-stemmed plants.~~

2 yellow : 1 agouti

Step 2: State the null hypothesis (H_0)

H_0: There is no significant difference between the expected and observed ratios and any difference is due to chance alone.

Step 3: Calculate the χ^2 value

Fur colour	Observed (O)	Expected (E)	$(O-E)^2$	$(O-E)^2/E$
Yellow	1063	1198.5 $\frac{1598}{4} \times 3$	18 360.25	15.319
Agouti	535	399.5 $\frac{1598}{4} \times 1$	18 360.25	45.958

$\chi^2 = \sum \frac{15.319 + 45.958}{1598} = 61.277$

61-3

Step 4: Determine the degrees of freedom (*df*)

$df = n - 1 = 2 - 1 = 1$

Step 5: Determine "p", using the *df*, and χ^2 value to check against the chi-square table

$p < 0.001$

since $p \ll 0.05$, at 5% level of significance, we reject the null hypothesis

(the probability is very low that any difference is due to chance)

difference between the observed & expected is significant & not due to chance alone.

Step 6: Reject or do not reject H_0. Draw your conclusion.

Practice Question 3

Different inbred lines of chickens show pronounced differences in mortality following infection with equal doses of two different strains of the bacterium Salmonella typhimurium.

*In an investigation into the inheritance of resistance, a series of crosses was performed between two **inbred lines** of White Leghorn chickens, one line resistant to infection by the bacterium and one susceptible. The results of infecting newly hatched chicks of each inbred line, and of the cross between a susceptible male and a resistant female, are shown in Table 2.1.*

Table 2.1

Line of chickens	*Mortality / % chicks affected*	
	Strain of S. typhimurium	
	F98	Swindon
Resistant	40.0	0.0
Susceptible	100.0	35.7
Cross between susceptible male and resistant female (F_1 hybrid)	45.0	0.0

It is thought that resistance to S. typhimurium is controlled by a single gene.

a) i. *Using suitable symbols, draw a genetic diagram to show how resistance to S. typhimurium (Swindon strain) could be inherited in the cross shown in Table 2.1. [3]*

ii. *Predict the ratio of resistant to susceptible chicks resulting from crosses between the following birds.* [3]

Susceptible parent x F_1 hybrid

Resistant parent x F_1 hybrid

F_1 hybrid x F_1 hybrid

iii. *Suggest two reasons for the differences in the resistance of the chicks to the two different strains of S. typhimurium shown in Table 2.1.* [2]

When 20 chicks from crossing a susceptible male and a resistant female were infected with equal doses of strain F98 of S. typhimurium, 9 died, but when 19 chicks from the reciprocal cross of a resistant male and a susceptible female were infected with equal doses of the same strain of bacterium, only 3 died. In both cases, 8 chicks (40%) were expected to die.

b) i. *State two reasons why the observed results of such crosses may be different from expected results.* [2]

ii. *Use the chi-square (χ^2) test, and the table of probabilities, to find the probability of the results of the reciprocal cross (resistant male and susceptible female) departing significantly by chance from expectation. Show your working.*

χ^2 *test*

$$\chi^2 = \sum \frac{(O-E)^2}{E} \qquad df = n-1$$

df : degrees of freedom
n : number of classes
O : observed value
E : expected value

Distribution of χ^2

degrees of freedom	*probability, p*				
	0.10	0.05	0.02	0.01	0.001
1	2.71	3.84	5.41	6.64	10.83
2	4.61	5.99	7.82	9.21	13.82
3	6.25	7.82	9.84	11.35	16.27
4	7.78	9.49	11.67	13.28	18.47

χ^2 = _____ probability = _____ [4]

(Step 2)

(Step 3)

Fur colour	Observed (O)	Expected (E)	$(O-E)^2$	$(O-E)^2/E$
Survived				
Died				

(Step 4)

df =

(Step 5)

Probability

iii. *State the conclusion that can be drawn from your calculation of χ^2 (i).*

(Step 6)

The Chi-square Table

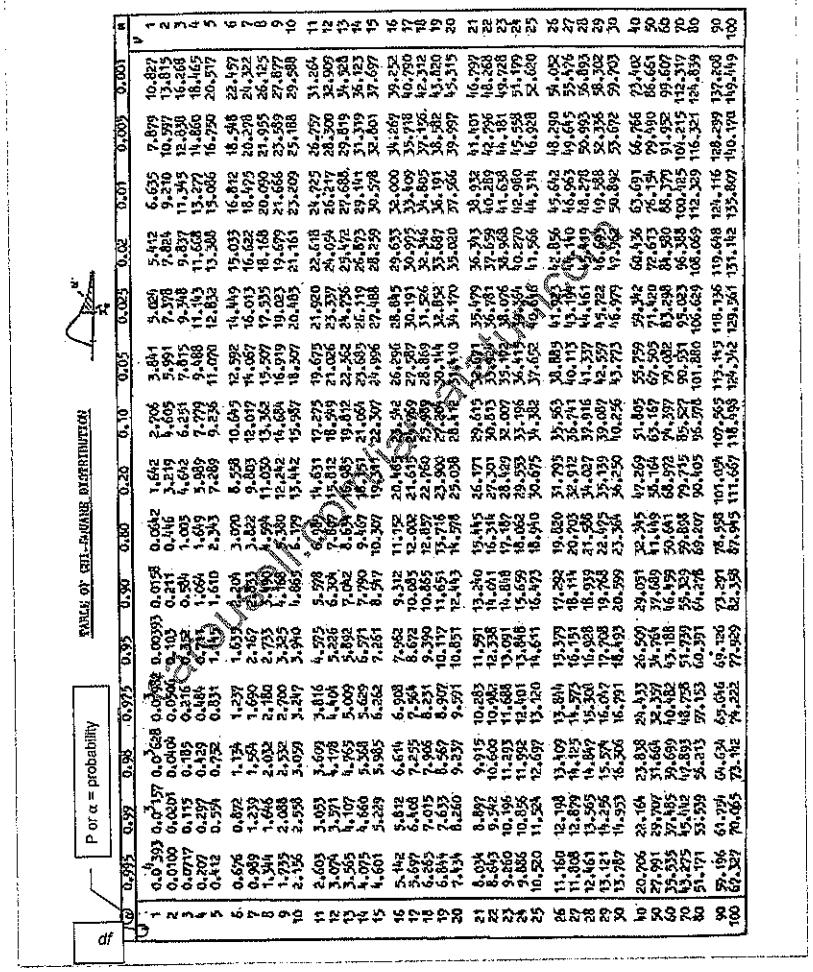


TABLE OF CHI-SQUARE DISTRIBUTION

P or α = probability

df = ν

ν	0.975	0.95	0.90	0.80	0.70	0.50	0.30	0.20	0.10	0.05	0.025	0.02	0.01	0.005	0.001
1	0.0^3 982	0.0^2 393	0.0158	0.0642	0.148	0.455	1.074	1.642	2.706	3.841	5.024	5.412	6.635	7.879	10.827
2	0.0506	0.103	0.211	0.446	0.713	1.386	2.408	3.219	4.605	5.991	7.378	7.824	9.210	10.597	13.815
3	0.216	0.352	0.584	1.005	1.424	2.366	3.665	4.642	6.251	7.815	9.348	9.837	11.345	12.838	16.268
4	0.484	0.711	1.064	1.649	2.195	3.357	4.878	5.989	7.779	9.488	11.143	11.668	13.277	14.860	18.465
5	0.831	1.145	1.610	2.343	3.000	4.351	6.064	7.289	9.236	11.070	12.832	13.388	15.086	16.750	20.517
6	1.237	1.635	2.204	3.070	3.828	5.348	7.231	8.558	10.645	12.592	14.449	15.033	16.812	18.548	22.457
7	1.690	2.167	2.833	3.822	4.671	6.346	8.383	9.803	12.017	14.067	16.013	16.622	18.475	20.278	24.322
8	2.180	2.733	3.490	4.594	5.527	7.344	9.524	11.030	13.362	15.507	17.535	18.168	20.090	21.955	26.125
9	2.700	3.325	4.168	5.380	6.393	8.343	10.656	12.242	14.684	16.919	19.023	19.679	21.666	23.589	27.877
10	3.247	3.940	4.865	6.179	7.267	9.342	11.781	13.442	15.987	18.307	20.483	21.161	23.209	25.188	29.588
11	3.816	4.575	5.578	6.989	8.148	10.341	12.899	14.631	17.275	19.675	21.920	22.618	24.725	26.757	31.264
12	4.404	5.226	6.304	7.807	9.034	11.340	14.011	15.812	18.549	21.026	23.337	24.054	26.217	28.300	32.909
13	5.009	5.892	7.042	8.634	9.926	12.340	15.119	16.985	19.812	22.362	24.736	25.472	27.688	29.819	34.528
14	5.629	6.571	7.790	9.467	10.821	13.339	16.222	18.151	21.064	23.685	26.119	26.873	29.141	31.319	36.123
15	6.262	7.261	8.547	10.307	11.721	14.339	17.322	19.311	22.307	24.996	27.488	28.259	30.578	32.801	37.697
16	6.908	7.962	9.312	11.152	12.624	15.338	18.418	20.465	23.542	26.296	28.845	29.633	32.000	34.267	39.252
17	7.564	8.672	10.085	12.002	13.531	16.338	19.511	21.615	24.769	27.587	30.191	30.995	33.409	35.718	40.790
18	8.231	9.390	10.865	12.857	14.440	17.338	20.601	22.760	25.989	28.869	31.526	32.346	34.805	37.156	42.312
19	8.907	10.117	11.651	13.716	15.352	18.338	21.689	23.900	27.204	30.144	32.852	33.687	36.191	38.582	43.820
20	9.591	10.851	12.443	14.578	16.266	19.337	22.775	25.038	28.412	31.410	34.170	35.020	37.566	39.997	45.315
21	10.283	11.591	13.240	15.445	17.182	20.337	23.858	26.171	29.615	32.671	35.479	36.343	38.932	41.401	46.797
22	10.982	12.338	14.041	16.314	18.101	21.337	24.939	27.301	30.813	33.924	36.781	37.659	40.289	42.796	48.268
23	11.689	13.091	14.848	17.187	19.021	22.337	26.018	28.429	32.007	35.172	38.076	38.968	41.638	44.181	49.728
24	12.401	13.848	15.659	18.062	19.943	23.337	27.096	29.553	33.196	36.415	39.364	40.270	42.980	45.559	51.179
25	13.120	14.611	16.473	18.940	20.867	24.337	28.172	30.675	34.382	37.652	40.646	41.566	44.314	46.928	52.620
26	13.844	15.379	17.292	19.820	21.792	25.336	29.246	31.795	35.563	38.885	41.923	42.856	45.642	48.290	54.052
27	14.573	16.151	18.114	20.703	22.719	26.336	30.319	32.912	36.741	40.113	43.194	44.140	46.963	49.645	55.476
28	15.308	16.928	18.939	21.588	23.647	27.336	31.391	34.027	37.916	41.337	44.461	45.419	48.278	50.993	56.893
29	16.047	17.708	19.768	22.475	24.577	28.336	32.461	35.139	39.087	42.557	45.722	46.693	49.588	52.336	58.302
30	16.791	18.493	20.599	23.364	25.508	29.336	33.530	36.250	40.256	43.773	46.979	47.962	50.892	53.672	59.703
40	24.433	26.509	29.051	32.345	34.872	39.335	44.165	47.269	51.805	55.759	59.342	60.436	63.691	66.766	73.402
50	32.357	34.764	37.689	41.449	43.989	49.335	54.723	58.164	63.167	67.505	71.420	72.613	76.154	79.490	86.661
60	40.482	43.188	46.459	50.641	53.809	59.335	65.227	68.972	74.397	79.082	83.298	84.580	88.379	91.952	99.607
70	48.758	51.739	55.329	59.898	63.346	69.334	75.689	79.715	85.527	90.531	95.023	96.388	100.425	104.215	112.317
80	57.153	60.391	64.278	69.207	72.915	79.334	86.120	90.405	96.578	101.880	106.629	108.069	112.329	116.321	124.839
90	65.646	69.126	73.291	78.558	82.511	89.334	96.524	101.054	107.565	113.145	118.136	119.648	124.116	128.299	137.208
100	74.222	77.929	82.358	87.945	92.129	99.334	106.906	111.667	118.498	124.342	129.561	131.142	135.807	140.169	149.449

paired sample
↳ 1 pop^n : take measurement before

2. The T Test → allows us to compare the avg values

***The T-test is NOT necessarily related to genetics, i.e. can come out for any question in your syllabus.
***You need to know how to interpret the formula and use it for calculations. There is no need to memorise the formula as it will be given to you.

- The T-test allows for comparison of mean between 2 unpaired samples
  i.e. 2 random, independent samples.

Null hypothesis
mean 1 = mean 2
If mean 1 ≠ mean 2

Characteristics of the T-test

- Suitable for small sample size
- Assumes samples follow normal distribution
  (i.e. where intermediates appear a lot more frequently than the extremes)

Formulae

standard deviation $s = \sqrt{\frac{\sum(x-\bar{x})^2}{n-1}}$

t-test $t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}}$ $\quad v = n_1 + n_2 - 2$

Symbols
x = observed value
$\bar{x}$ = mean
n = number of observations (i.e. number of values within a data set)

- Standard deviation is a measure of the variance / the spread of values within a set of data.
  e.g. When you compare the 2 sets of data below, you can see that set 1 has a larger spread of values than set 2

  Set 1: 1, 2, 5, 6, 7, 10
  Set 2: 2, 3, 5, 7, 8, 7

- The standard deviation, *s*, is used to calculate the t value. Since we are looking at 2 samples, we need to calculate 2 standard deviation values, s_1 and s_2.

Steps

Step 1: State the null hypothesis (H_0)
Step 2: Calculate $\bar{x}$
Step 3: Calculate $\sum(x-\bar{x})^2$
Step 4: Determine n_1 and n_2
Step 5: Calculate s_1 and s_2
Step 6: Calculate v (degrees of freedom)
Step 7: Calculate t value
Step 8: Determine "p", using the v and t value to check against the t distribution table
Step 9: Reject or do not reject Ho. Draw your conclusion

Example 1

The height of 4 males and 4 females were measured and tabulated below.
Find out if there is a significant difference in height between the males and females.

	Height of males/cm	Height of females/cm
1	169	160
2	171	164
3	172	166
4	182	172

Step 1:
State the H_0: There is no significant difference between the means of the 2 samples.

Step 2: Calculate $\bar{x}$ (the mean of the 2 heights)

	Height of males/cm	Height of females/cm
1	169	160
2	171	164
3	172	166
4	182	172
Mean	$\bar{x}$ = 173.5	$\bar{x}$ = 165.5

Step 3: Calculate $\sum(x-\bar{x})^2$ (for males and females)

	Height of males/cm	Height of females/cm	$(x-\bar{x})^2$ for males	$(x-\bar{x})^2$ for females
1	169	160	$(169-173.5)^2$	$(160-165.5)^2$
2	171	164	$(171-173.5)^2$	$(164-165.5)^2$
3	172	166	$(172-173.5)^2$	$(166-165.5)^2$
4	182	172	$(182-173.5)^2$	$(172-165.5)^2$
Mean	173.5	165.5	$\sum$ = 101	$\sum$ = 75

Step 4: Determine n_1 and n_2 (number of observations) (for males and females)

For males: $n_1 = 4$
For females: $n_2 = 4$

Step 5: Calculate s_1 and s_2. (the standard deviation for males and females)

s_1 for males = $\sqrt{\frac{101}{3}} = 5.80$ s_2 for females = $\sqrt{\frac{75}{3}} = 5$

Step 6: Calculate v (the degree of freedom)

$v = n_1 + n_2 - 2$
$= 4 + 4 - 2$
$= 6$

Step 7: Calculate t value

$$t = \frac{|173.5-165.5|}{\sqrt{\frac{5.80^2}{4}+\frac{5^2}{4}}} = 2.09$$

Step 8: Determine "p", using the v and t value to check against the t distribution table

TABLE B *t* Distribution Critical Values

	TAIL PROBABILITY *P*											
df	.25	.20	.15	.10	.05	.025	.02	.01	.005	.0025	.001	.0005
1	1.000	1.376	1.963	3.078	6.314	12.71	15.89	31.82	63.66	127.3	318.3	636.6
2	.816	1.061	1.386	1.886	2.920	4.303	4.849	6.965	9.925	14.09	22.33	31.60
3	.765	.978	1.250	1.638	2.353	3.182	3.482	4.541	5.841	7.453	10.21	12.92
4	.741	.941	1.190	1.533	2.132	2.776	2.999	3.747	4.604	5.598	7.173	8.610
5	.727	.920	1.156	1.476	2.015	2.571	2.757	3.365	4.032	4.773	5.893	6.869
6	.718	.906	1.134	1.440	1.943	2.447	2.612	3.143	3.707	4.317	5.208	5.959
7	.711	.896	1.119	1.415	1.895	2.365	2.517	2.998	3.499	4.029	4.785	5.408
8	.706	.889	1.108	1.397	1.860	2.306	2.449	2.896	3.355	3.833	4.501	5.041
9	.703	.883	1.100	1.383	1.833	2.262	2.398	2.821	3.250	3.690	4.297	4.781
10	.700	.879	1.093	1.372	1.812	2.228	2.359	2.764	3.169	3.581	4.144	4.587

Step 9: Reject or do not reject Ho. Draw your conclusion

1. Since 0.025 < p < 0.05 where **p < 0.05, at 5% level of significance**, therefore we reject the null hypothesis *(The probability is very LOW that any difference is due to chance)*
2. We can conclude that the **difference in means of heights between the males and females is significant** and not due to chance alone.

Comparison between t-test and chi-squared (χ^2) - test

Steps	t-test	χ^2-test
List the expected phenotypic ratio	✗ (because we are just comparing 2 means)	✓
State the null hypothesis (H_0)	No sig. diff. between means of 2 samples	No. sig. diff between observed & expected
Calculate the statistic	Calculate s for both samples & compute t-statistic	Calculate χ^2 value
Determine df	df = $n_1 + n_2 - 2$, n = no. of observations in a sample (i.e. how many times you take the measurement to obtain the mean value)	df = n – 1, n = no. of phenotypic groups (i.e. no. of categories of phenotypes)
Determine "p"	t-distribution table	χ^2 distribution table
Reject (or do not reject) H_0	(At 5% level of significance) p>0.05 → difference not significant → do not reject H_0 p<0.05 → difference is significant → reject H_0	

CORE IDEA
(2) Genetics & Inheritance

MOLECULAR BIOLOGY TECHNIQUES

Content
- DNA Analysis and Genomics

Learning Outcomes
Candidates should be able to:

(k) Describe the principles and procedures of these molecular techniques:
- i. polymerase chain reaction (including its advantages and limitations);
- ii. gel electrophoresis; and
- iii. Southern blotting and nucleic acid hybridisation.

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This handout is the effort of several Biology teachers at RI. It has and will continue to be updated.

TABLE OF CONTENTS

A. Molecular Techniques

1. Polymerase Chain Reaction (PCR)

What is PCR?

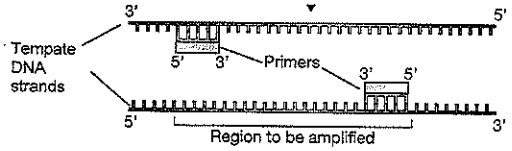
Polymerase Chain Reaction (PCR) is a technique that allows **amplification** (making many copies) of a specified segment of DNA *in vitro* (in a test tube).
- Amplifying DNA through PCR has extensive applications such as in basic research, human genetics testing and forensics.

Until the mid-1980s, the only way to make many copies of DNA was to insert the DNA pieces into bacteria and select the desired one from many different colonies growing on a plate (this takes several weeks!).
In 1985, Kary Mullis (shown right) invented a precise and radical new method of selecting and amplifying a section of DNA – the PCR which takes less than a few hours to complete. The revelation came to this eccentric character during a late night drive with his girlfriend.

Prerequisite knowledge

1. Structure of DNA:
   - DNA strands unwind and separate (**denature**) at high temperatures as **hydrogen bonds** between the bases are broken.
   - DNA strands **anneal** through **complementary base-pairing** upon cooling.
2. DNA replication:
   - **DNA primers** (not RNA primers!) are needed when DNA is replicated during PCR as the primer provides an existing **free 3' hydroxyl group** required by DNA polymerase for chain extension to take place.

Components of reaction mixture for PCR

PCR component	Details
Template DNA	▪ DNA containing the segment to be amplified. This can usually be genomic DNA, plasmid, or fragmented DNA.
Oligonucleotide primers (oligo = short)	▪ Synthetic single-stranded DNA primers are typically 20-30 nucleotides long and are needed to **initiate DNA synthesis.** ▪ Primers are synthesised to be complementary to the sequence at 3' ends of both strands of target DNA sequence. **Two different primers** are needed during PCR, each one complementary and able to bind to the 3' ends of each template strand of DNA double helix.  Knowledge of the sequences flanking / at the ends of the DNA segment of interest is thus crucial to artificially synthesising the primers. ▪ Primers are present in large excess in reaction mixture to increase likelihood of primers binding to target DNA (relative to template strands reannealing to each other again). ▪ The primers become part of the amplified segments.

Taq polymerase	▪ A thermostable (resistant to denaturation at high temperatures) **DNA polymerase** isolated from a thermophilic prokaryote living in hot springs, *Thermus aquaticus* (*Taq*). ▪ Enzyme is stable at 95°C and works optimally at 72°C.
Deoxyribonucleoside triphosphates (dNTPs)	▪ dATP, dTTP, dCTP and dGTP are the substrates for DNA replication.
Buffer containing Mg^{2+}	▪ Mg^{2+} is a cofactor[1] for proper DNA polymerase function.

Protocol:

1. Place all the components into a PCR tube, and place the tube into the thermocycler (Fig. 1). Run a standard programme that involves heating the tube to different temperatures for different periods of time. Entire process is now fully automated.

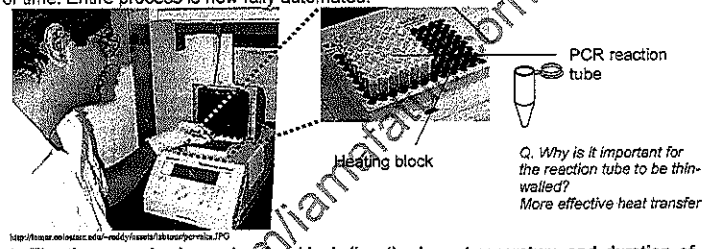


Figure 1: The thermocycler has a heating block (inset) whose temperature and duration of heating can be programmed precisely. The many wells in the heating block enable several reactions to run simultaneously.

2. Steps during PCR:
The following three steps /stages constitute 1 cycle. The cycle is then repeated (Figs. 2, 3).

Step / Stages	Details
1. Denaturation	A brief heat treatment (up to **95°C**) to **denature** and separate the two strands of the DNA double helix. This exposes the bases for complementary base pairing required in steps 2 and 3.
2. Primer annealing	Cooling of the DNA (e.g. **64°C**) in presence of a **large excess of DNA primers** allows their specific **annealing** to complementary sequences at the 3' ends of each of the template DNA strand.
3. Extension	*Taq* polymerase **synthesises** the complementary DNA strand by catalysing the formation of phosphodiester bonds between dNTPs at an optimum **72°C**. Chain extension occurs from 3' end of DNA primer which provides free 3' OH group required by *Taq* polymerase.

[1] Cofactors are additional non-protein substances required by some enzymes for catalytic activity. Cofactors can be 1) inorganic ions that are metal ions such as Ca^{2+}, Mg^{2+}; 2) Organic coenzymes such as NAD or 3) prosthetic groups such as haem in cytochrome oxidase or haemoglobin

3. Each cycle results in a **doubling in number of DNA** molecules being replicated. *n* cycles will yield 2^n molecules of target DNA. The amount of desired sequence hence **increases exponentially**.
Usually 25-30 cycles are run, resulting in the eventual synthesis of millions of copies of the desired fragment.

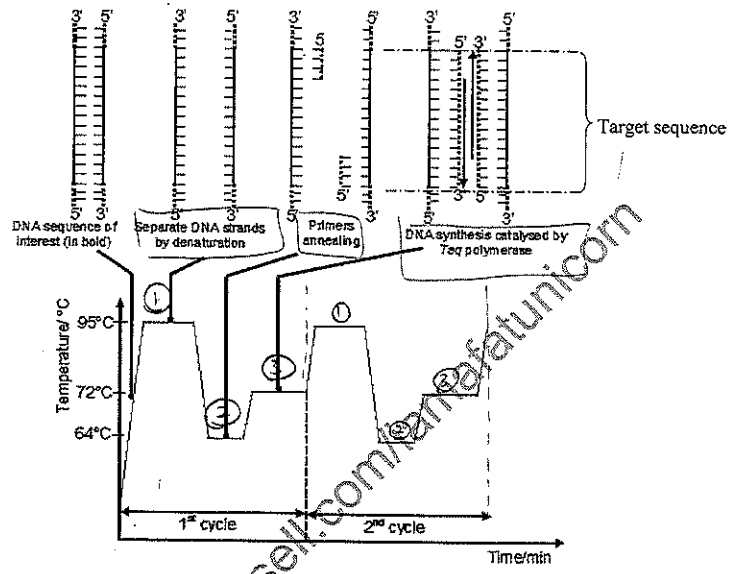


Figure 2: Principle behind Polymerase Chain Reaction (PCR)

Q: Why is it usually recommended to run about 25 to 30 cycles for PCR?

Fewer cycles → not enough DNA for analysis
Too many cycles → too many mistakes in replication (no proofreading mechanism) → Taq polymerase

Q: Why is the discovery of Taq polymerase a breakthrough? What would you do if you have only normal DNA polymerase?
*When first developed, PCR was slow and laborious because a crucial enzyme for the process, DNA polymerase, was denatured at high temperature and had to be replaced after each cycle. However the introduction of the **thermostable enzyme, Taq DNA polymerase** revolutionized PCR so that the technique could be **fully automated**.*

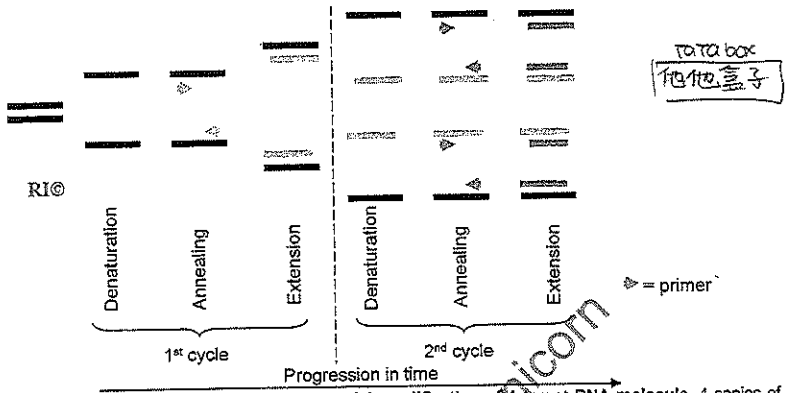


Figure 3: Process of PCR showing exponential amplification of 1 target DNA molecule. 4 copies of target DNA molecules are produced at the end of 2 PCR cycles.

Number of copies of target DNA molecules = 2^n
(n = number of cycles)

Advantages of PCR

As an analytical tool, PCR has several advantages.

(a) **sensitivity** – only a **minute amount of source DNA** is required. The number of copies of target DNA is doubled following each cycle of PCR. Therefore, the amount of desired sequence increases exponentially even from very low copies of starting material.

(b) **speed** – only a few hours are required to generate enough amplified DNA compared to the multiple steps of cloning which need at least a week. The technique is **fully automated** within the thermocycler. PCR is a convenient way to **amplify DNA** in a fast and efficient manner.

Applications of PCR

Field	Use	Details
Clinical diagnosis	Pre-natal screening for certain genetic diseases	Fetal cells can be removed from amniotic fluid and DNA obtained from the cells is subjected to PCR even before the phenotype is expressed. A gene of interest can be amplified through PCR and subsequently sequenced to detect disease-causing mutations. Such genetic screening enables couples to assess risks of their unborn children, eg. cystic fibrosis.
	Early detection of infection	PCR can detect presence of the HIV genome within a patient at very early stages of infection, before symptoms appear, when only a few thousand blood cells in a patient are infected with the virus. This provides physicians a head-start in treatment of the disease.
Forensic analysis	Identification of suspects	Minute traces of DNA in organic matter e.g. hair, semen, blood, can be amplified to sufficiently large amounts to be analysed. This helps to identify suspects by matching with DNA found on crime scenes.
Evolution	Study evolutionary relatedness	Fragments of DNA found in prehistoric specimens can be amplified for further study. DNA sequences from both living and extinct organisms can be compared to determine evolutionary relationships.

Limitations of PCR

Feature	Limitations
Taq polymerase **lacks 3' to 5' proofreading ability**	*Taq* polymerase cannot check and/or remove an incorrectly incorporated base. **Errors** occurring early in the PCR reaction will get compounded with each replication cycle and all daughter molecules resulting from this early error will be exponentially affected.
Synthesis of PCR primers depends on **sequence information** from target region	Success of PCR **requires knowledge of sequences** flanking target region to be amplified. If the flanking sequences of a gene of interest are unknown, no proper primers can be synthesised to amplify the target DNA sequence. If primers are designed incorrectly, no amplification occurs / wrong DNA fragment(s) may be amplified.
Limit to size of DNA fragment to be amplified	DNA fragments to be amplified are **limited to about 3 kb**[2]. Further increase in length of target sequence decreases efficiency of amplification. This is because the polymerase tends to 'fall off' the DNA template before chain extension is complete.
Exponential amplification of **contaminant DNA**	It is possible to contaminate a fresh PCR reaction with **minute amounts** of contaminant DNA due to poor laboratory skills. Such unwanted DNA sequences may be amplified to significant amounts, alongside the target DNA sequences.

Q: Why is proof-reading activity from 3' to 5' and not 5' to 3'?
It takes place only after the nucleotide has been added in the 3' end of the extending chain. Correction (removal of wrongly-incorporated nucleotide) can take place in the reverse orientation.

Q: Would an error occurring earlier or later during PCR be worse?
Early. Errors occurring early will get compounded with each replication cycle and all daughter molecules resulting from this early error will be affected.

Q: Is it possible to amplify an entire eukaryote or even prokaryotic genome using PCR?
No. Genomic DNA is too large to be amplified entirely by PCR.

[2] Kb or kilobase is a measurement of the length of a DNA/RNA molecule. 1kb is the length of 1,000 nucleotides.

2. Gel Electrophoresis

What is gel electrophoresis?

Gel electrophoresis is a technique that separates mixtures of DNA, RNA or proteins according to their molecular size.

Agarose gel electrophoresis :

Agarose gel electrophoresis **separates DNA based on size.**

Principle (Fig. 4):

1. Electrophoresis is the movement of charged molecules in an electric field.
2. Biological molecules such as DNA, RNA and proteins exist in solution as electrically-charged particles at a given pH. For example, DNA is negatively-charged due to phosphate groups of sugar-phosphate backbone.
3. When placed in an electric field, **negatively-charged** DNA molecules will move away from the negative electrode (cathode) and towards the positive electrode (anode).
4. **Agarose** is a polysaccharide derived from seaweed that forms a gel when heated and dissolved in aqueous solution.
5. Meshwork of polymer fibers that makes up agarose gel impedes movement of the DNA fragments, **impeding the longer fragments more than shorter ones.** Therefore, shorter DNA fragments will move **towards the positive electrode at a higher rate** than longer fragments.
6. DNA fragments of different sizes are separated out based on their different rates of migration.

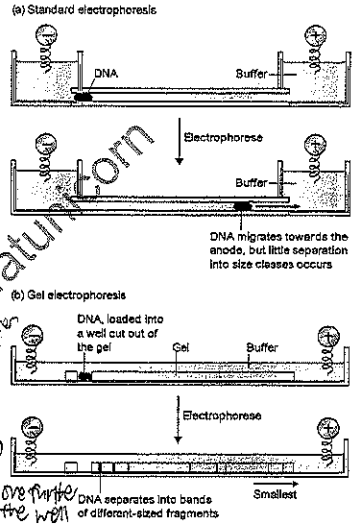


Figure 4: Principles of agarose gel electophoresis. (a) When placed in a electric field, negatively-charged DNA molecules move towards the anode. (b) The meshwork of polymer fibers of the agarose gel impedes this movement, affecting larger fragments more than shorter ones. This results in the separation of DNA fragments based on size.

Protocol:

1. A slab of agarose gel is placed in a buffer solution which allows conduction of electricity to generate an electric field (Figs. 4, 5). The gel has been pre-cast with little indentations called **wells** at one end of the gel slab, using an apparatus called a comb. DNA samples are loaded into these wells. Each well corresponds to one lane.
2. Prior to loading, the DNA sample is first mixed with a dense **loading dye** which helps the **DNA sample sink** to the bottom of the well. The loading dye contains 2 different coloured loading dyes which also travel along in the gel just like the DNA sample does during electrophoresis.
3. As DNA is invisible to the naked eye, the 2 loading dyes help to monitor progress of electrophoresis (Fig. 6). Usually, the dark blue dye corresponds to 100 bp, while the light blue dye corresponds to 1100 bp.

Q: If I was running a gel containing fragments of size 110 bp, when do I stop electrophoresis?
Stop it before the dark blue band runs out of the gel.

Q: What is the use of the light blue band then?
It is for you to gauge the positions of larger fragments and allow you to estimate when to stop electrophoresis.

4. Usually a DNA ladder is loaded into the well of first or the last lane of the gel. The DNA ladder is a mixture of DNA fragments of known sizes which act as a standard that can be compared with fragment of unknown sizes in the sample.
5. When the current is turned on, the negatively-charged DNA fragments migrate out of the well into the agarose. DNA is **attracted towards the positive electrode (anode)** due to negative charges on its sugar phosphate backbone.
6. The **meshwork of polymer fibers** that makes up agarose gel **impedes movement of the longer DNA fragments more than shorter ones.** Therefore, **shorter DNA fragments will migrate towards the positive electrode at a higher rate** than longer fragments.
7. The fragments of different sizes in the DNA sample separate out in the gel based on their different rates of migration. If the **amount** of the fragments is high enough, these are seen as **discrete bands** on the gel after staining. Each band consists of DNA molecules of the same length.
8. Before loading dye reaches end of the gel, the current is turned off.
9. To visualise the bands, gel slab is next stained with a DNA-binding dye, usually **ethidium bromide** (a carcinogen). When the gel is placed under UV light afterwards, DNA bands will be revealed as the dye bound to the DNA fluoresces.
10. It is possible to alter the resolution of the gel by varying the concentration of agarose used. The higher the concentration, the better the resolution i.e. even fragments with very small differences in size can be separated.

Electrophoresis can help to estimate:

(a) **fragment size** (by comparing position of the band relative to bands of the DNA ladder)

(b) **amount of DNA** (Note: not an accurate measure. Amount of DNA may be reflected through the intensity in which the band fluoresces, and may be translated to thickness of band on 2D gel diagrams. e.g. on Fig. 7, the 1857 bp band on the ladder fluoresces more in comparison to the band on lane 1. Thus, one can infer that there is a greater amount of DNA on the ladder than on lane 1.)

Applications of gel electrophoresis

Results from gel electrophoresis can be used to analyse and verify DNA fragments such as PCR products and restriction fragments (Fig. 7).

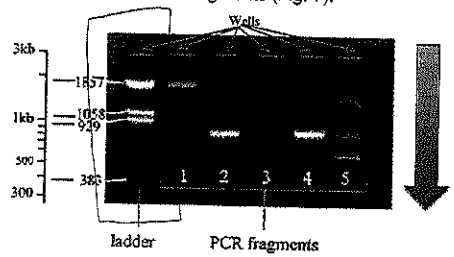


Figure 7: Verification of DNA fragments (in this case, PCR products) on agarose gel. DNA fragments always move away from the wells with the larger fragments moving the slowest and hence, found nearest the well. The smallest fragments move fastest and are hence found furthest from the well.

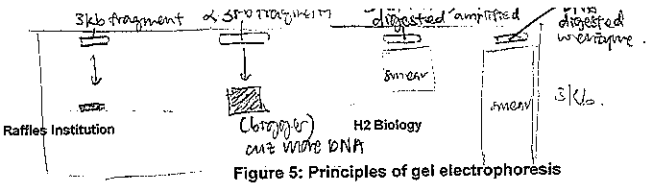


Figure 5: Principles of gel electrophoresis

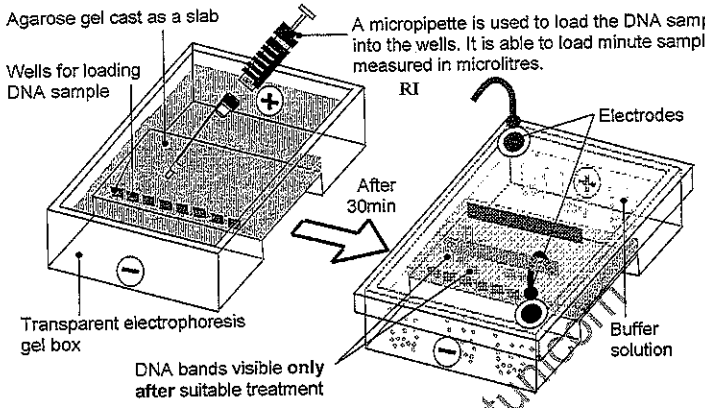
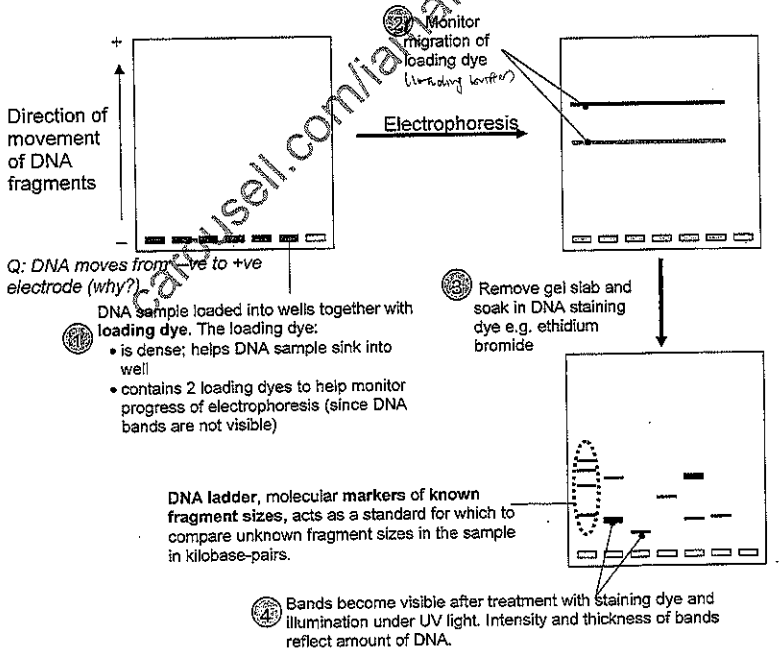


Figure 6: Patterns on gel slab during and after electrophoresis



3. Southern Blotting – a nucleic acid hybridisation technique used in RFLP analysis

Southern blotting, which is typically carried out after gel electrophoresis, is a technique used to **detect** fragments containing **specific nucleotide sequences** within a sample of DNA.

Protocol:

1. After the DNA sample is amplified by PCR or cut by restriction enzymes, the DNA fragments are separated by electrophoresis.
2. The gel slab containing the DNA fragments is placed on top of an absorbent sponge in a tray of **alkaline solution**. A **nitrocellulose membrane** and a stack of paper towels are placed on top of the gel slab. A heavy weight is stacked right on top of the paper towels. (Fig. 8)
3. The absorbent paper towels draw the alkaline solution towards themselves. This capillary action draws the alkaline solution upwards through the gel. The **alkaline solution denatures** the double-stranded DNA fragments in the gel into single strands. The **single-stranded DNA** in the gel is then drawn upwards **onto nitrocellulose membrane and binds to the membrane.** The DNA binds to the membrane in exactly the same position as they were in the gel.
4. The nitrocellulose membrane is removed and incubated with a **radioactive single-stranded DNA probe** which is **complementary in sequence** to part of the target sequence. DNA fragments containing this part of the target sequence will **hybridise to the probe by complementary base-pairing** (Fig. 9).
5. After hybridisation, membrane is washed to remove any unhybridised probes.
6. **Autoradiography** is performed by placing an **X-ray film** over the membrane. The radioactivity of the bound probes exposes the film forming an image corresponding to the bands that have hybridised to the probe.

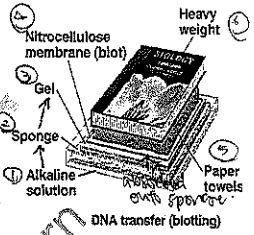


Figure 8: Transfer to DNA fragments from gel slab to nitrocellulose membrane (protocol, step 2).

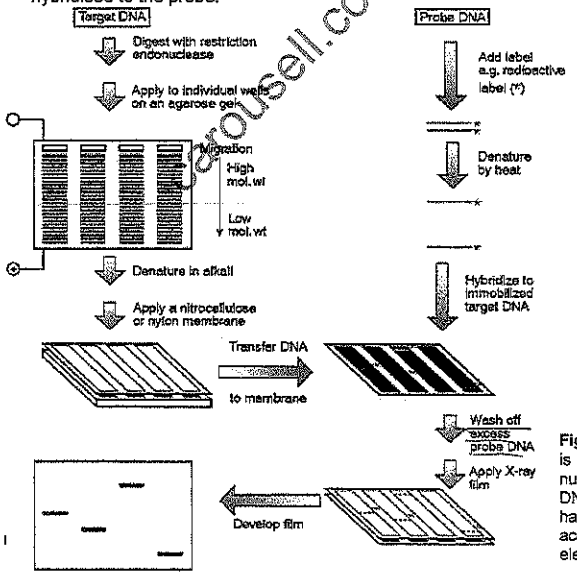


Figure 9: Southern blotting is used to detect specific nucleotide sequences in DNA fragments with that have been separated according to size by gel electrophoresis.

B. Restriction Enzymes

1. Origin of restriction enzymes

- In nature, **restriction enzymes** are produced by **bacteria**.
- Each enzyme is named after the bacterium from which it originates. e.g. The enzyme *Eco*RI comes from *E. coli*.

2. Restriction enzymes as tools for cutting DNA

- Restriction enzymes / restriction endonucleases belong to a group of enzymes called nucleases which **break phosphodiester bonds** that link adjacent nucleotides in DNA.
- Restriction enzymes recognise and bind to specific nucleotide sequences known as **restriction sites** in double-stranded DNA, and **cleave the phosphodiester bond on both strands** of the DNA.
- Most restriction sites are about 4 or 6 bases long and are **palindromic**, i.e. the sequences when read from 5' to 3' on both strands are the same.

Example of a restriction site (fill in the blanks):

5' –	G	A	T	A	T	C	3'
3' –	C	T	A	T	A	G	– 5'

- Some restriction enzymes produce **sticky ends** while others produce **blunt ends** (Fig. 10).
  - Sticky ends:
    - Produced when restriction enzymes leave a **staggered cut** resulting in single stranded overhangs / sticky ends.
    - These short overhangs will form **hydrogen bonds** and **anneal** by **complementary base pairing** with complementary single-stranded stretches on other DNA molecules cleaved with the **same** restriction enzyme.
  - Blunt ends:
    - Produced when restriction enzymes make a simple **cut across both strands** at a single point.

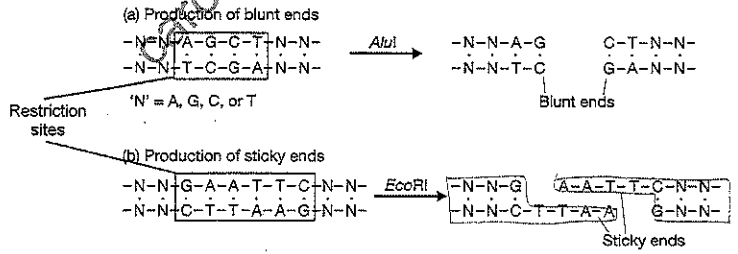


Figure 10: Ends produced by cuts with different restriction enzyme
(a) *Alu*I producing blunt ends (b) *Eco*RI producing sticky ends

3. Natural Function of Restriction Enzymes

- Bacteria produce restriction enzymes as a **defense mechanism** – they **cleave foreign DNA** molecules (from sources such as bacteriophage) into **non-infective** fragments.
- When a bacteriophage infects a bacterium, it first injects its DNA into the bacterium. Cleaving the phage DNA prevents the phage from replicating, conferring the bacterium with resistance to bacteriophage infection.
- The bacterium's own DNA is not cleaved because it is **methylated** at the restriction sites that are recognised by its own restriction enzyme. (Fig. 11)
  - Methylation:
    Methylase **adds methyl groups** ($-CH_3$) to adenine or cytosine residues in the DNA sequence that constitutes the **restriction site**. This changes the conformation of the DNA at the restriction site such that it is **no longer complementary in shape and charge** to the enzyme's **active site**. Hence, the restriction enzyme will be unable to recognise and to cleave the DNA.
    Therefore, methylation protects bacterial DNA from cleavage by its own restriction enzymes.

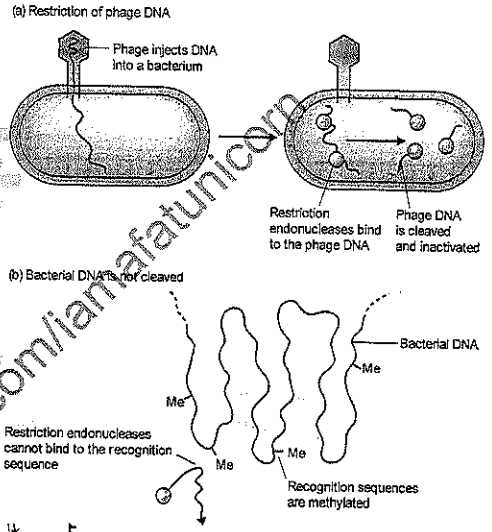


Figure 11: Function of restriction enzymes in a bacterium
(a) Phage DNA is cleaved (b) Bacterial DNA protected due to methylation of restriction sites.

C. Restriction Fragment Length Polymorphism

1. Introduction

- **DNA polymorphism** refers to **differences in DNA sequences** in homologous regions among different individuals.
- In general, DNA polymorphisms at a particular chromosomal locus differ either in **nucleotide sequence** or in the **number of tandemly repeated nucleotide units**.
- When genomic DNA is digested by restriction enzymes and separated by gel electrophoresis,

  1. the presence of a **single nucleotide polymorphisms** (SNPs, pronounced 'snips') which is due to a **difference in single base-pair** in the homologous regions of different individuals
     → results in the **variation in number and/or location of restriction sites** and produces fragments of **different lengths** from different individuals.

  2. the presence of **variable number tandem repeats** (VNTRs) in the homologous regions of different individuals
     → produces fragments of **different lengths** from different individuals

- **Restriction Fragment Length Polymorphism (RFLP)** hence refers to the unique banding pattern among individuals when their genomic DNA is digested by restriction enzymes and separated by gel electrophoresis.

2. Applications of RFLP Analysis

a) RFLP analysis in disease detection – sickle-cell anaemia

Detecting Single Nucleotide Polymorphisms (SNPs)

1. The vast majority of SNPs occurs in non-coding regions of the genome, but some **occur in coding regions** as well, which can lead to genetic diseases in humans.
   e.g. sickle-cell anaemia is due to point mutation

2. These single nucleotide mutations **alter the DNA sequence recognised by a restriction enzyme.**
   Single nucleotide polymorphisms (SNPs) can hence lead to different number of restriction fragments in different individuals.

3. RFLP analysis can thus be used to distinguish between SNPs and detect diseases directly.
   - The single nucleotide substitution is the disease-causing mutation and occurs within the coding region.
     e.g. in **sickle-cell anaemia**, the disease-causing mutation occurs at restriction site for *Mst*II within the β-globin gene. (Fig. 12)
     - In the disease allele, this *Mst*II restriction site is eliminated.
     - In the normal allele, this *Mst*II restriction site is retained.

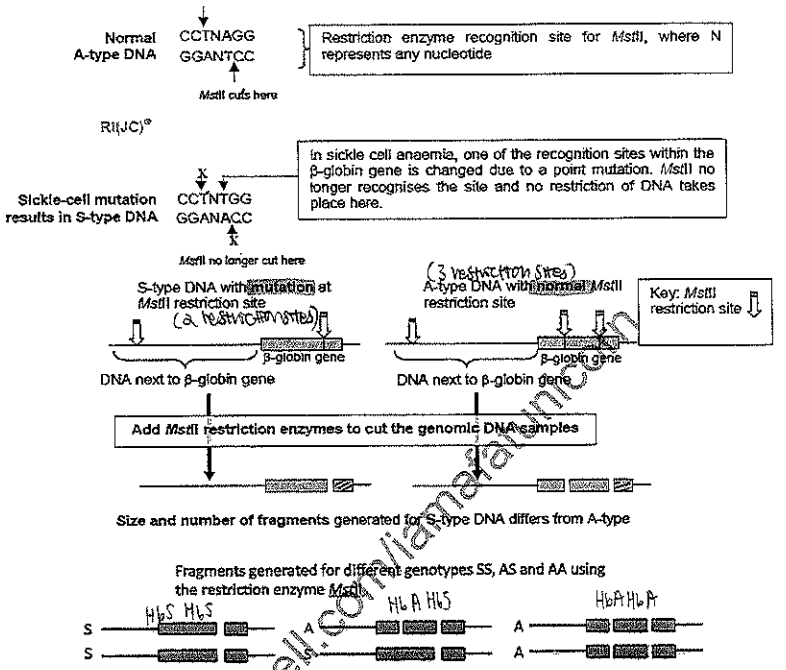


Figure 12: The basis of using RFLP to detect the sickle cell anemia allele.

Protocol:

Method 1 (Fig. 13a)	Method 2 (Fig. 13b)
Genomic DNA from an individual with sickle cell anaemia and a normal individual are isolated.	
1. Digest with **restriction enzyme**, *Mst*II Different-sized restriction fragments are produced	1. Conduct **PCR** By designing primers that flank the boundaries of the β-globin gene
2. Run **gel electrophoresis** to separate DNA fragments	2. Digest with **restriction enzyme**, *Mst*II Different-sized restriction fragments are produced
3. **Southern blot** and **nucleic acid hybridisation** using a specific radioactive single-stranded **probe** that complementary to part of the β-globin gene. Visualise bands via **autoradiography**.	3. Run **gel electrophoresis** Visualise bands by adding ethidium bromide, and view under UV light.
4. Analysis of results (banding patterns)	

Note: Both methods are similar, the 1st method uses specific radioactive probe (complementary to β-globin gene) to detect the relevant bands, whereas in the 2nd method, probes are not needed, as PCR primer allows only for the target sequence (β-globin gene) to be amplified.

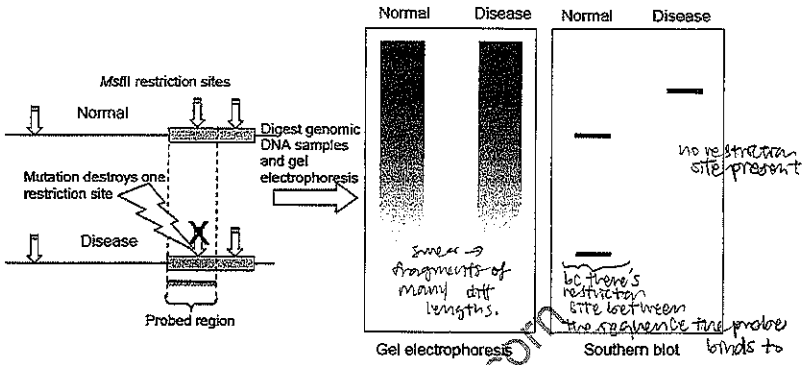


Figure 13a) Method 1 - RFLP can be used for detection of sickle-cell anaemia. In order to detect discrete bands in the smear, Southern blotting must be carried out.

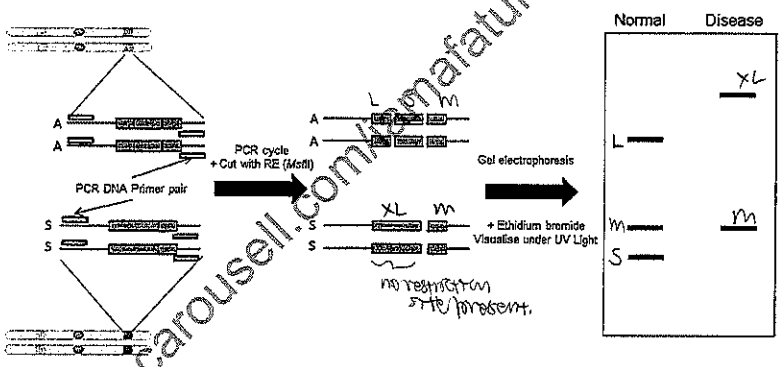


Figure 13b) Method 2 - RFLP can be used for detection of sickle-cell anaemia. Using PCR primers to zoom in on the desired locus (β-globin gene).

b) RFLP in DNA fingerprinting

Detecting varying number of tandem repeats

1. No two humans (except identical twins) have exactly the same genome.
2. Discovery of DNA polymorphisms has led to the development of **DNA fingerprinting** (a.k.a. DNA typing / profiling) techniques which have major applications in:
   - **forensic science** and
   - **paternity testing.**
3. Tandem repeats of a particular DNA sequence occur in **varying numbers** in different individuals – discriminating enough to generate a **unique genetic profile**/ DNA fingerprint for each individual (Fig 14, Fig 15).

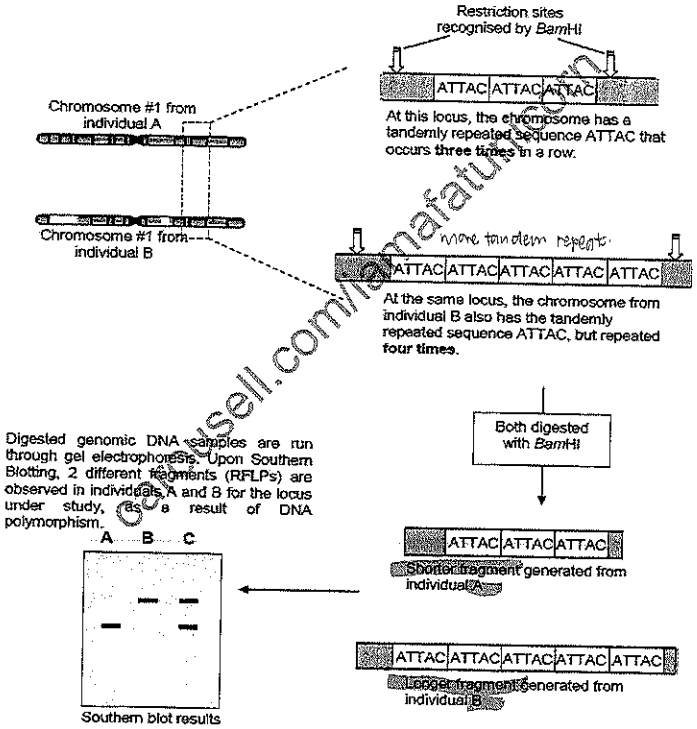


Figure 14: DNA polymorphism in terms of tandem repeats can result in different restriction fragments. Genomes from 2 individuals A and B, are cut using *Bam*HI. The 2 individuals differ in the number of tandem repeats (of the sequence ATTAC) at a particular locus on chromosome #1. As a result of the larger number of tandem repeats present, individual B generates a longer restriction fragment which travels a shorter distance down the gel during electrophoresis. The 2 different-sized fragments are detected using the same probe which has a sequence complementary to segments of both fragments e.g. ATTAC.

4. Since the number of repeats of a particular sequence is heritable, if the banding patterns of two DNA samples are very similar, it means that they are from two very closely-related individuals.
   - The probability of 2 individuals having the exact same banding pattern is infinitesimally small; there is a calculated probability of 1/ 575 trillion that two persons will have the same DNA fingerprint.

Protocol:

(Note: Method is similar to the 2 methods mentioned in pg 14)

Method 1 :
- Digest with restriction enzyme(s) that **cut on either side of a tandem repeat loci**.
- Separation of restriction fragments by gel electrophoresis.
- Followed by incubating with **radioactive probes** for **tandem repeats** (Fig. 15),
- visualise via autoradiography.

OR

Method 2 :
- Design **multiple PCR primer** pairs which anneal to the flanking sequence of **different tandem repeats** loci.
- Amplification of multiple tandem repeat loci.
- Separation of products by gel electrophoresis.
- Stain with ethidium bromide and visualise under UV light.

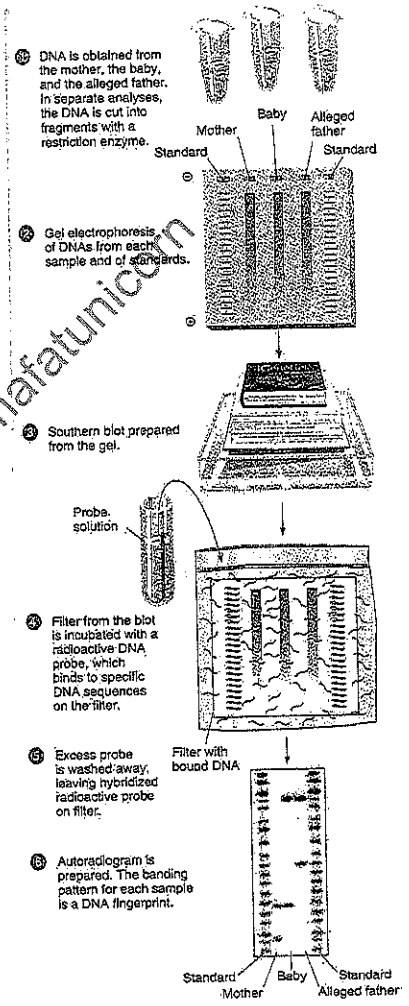


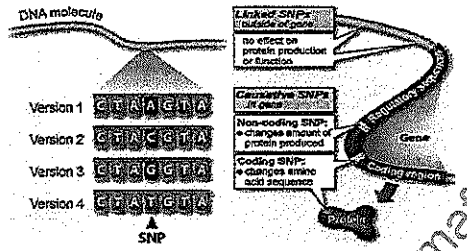
Figure 15: RFLP used in paternity testing. A particular locus on the chromosome is targeted for RFLP analysis.

KEYWORDS

Taq polymerase	Restriction enzyme
Anneal	Restriction sites
Complementary base pairing	Palindromic
Negatively-charged DNA	Sticky ends
Radioactive probe	Phosphodiester bonds
Autoradiography	

Appendix

Additional Information:
More about SNPs (not in syllabus)



SNP (pronounced "snip") stands for Single Nucleotide Polymorphism. SNPs are single-nucleotide substitutions of one base for another. Each SNP location in the genome can have up to four versions: one for each nucleotide, A, C, G and T. Not all single-nucleotide changes are SNPs, though. To be classified as a SNP, two or more versions of a sequence must each be present in at least one percent (1%) of the general population.

SNPs occur throughout the human genome - about one in every 300 nucleotide base pairs. This translates to about 10 million SNPs within the 3-billion-nucleotide human genome.

SNPs and disease-causing mutations: Not exactly the same!
If you know what a point mutation is, then the description of a SNP might sound similar. True, both are single-nucleotide differences in a DNA sequence, but SNPs should not be confused with disease-causing mutations. There are some differences:

First, to be classified as a SNP, the change must be present in at least one percent (1%) of the general population. No known disease-causing mutation is this common.

Second, most disease-causing mutations occur within a gene's coding or regulatory regions and affect the function of the protein encoded by the gene. SNPs are not necessarily located within genes, and they do not always affect the way a protein functions.

SNPs are divided into two main categories:

1. **Linked SNPs** (also called indicative SNPs) do not reside within genes and do not affect protein function. Nevertheless, they do correspond to a particular drug response or to the risk for getting a certain disease.

2. **Causative SNPs** (similar to disease-causing mutations) affect the way a protein functions, correlating with a disease or influencing a person's response to medication. Causative SNPs come in two forms:
   i. **Coding SNPs**, located within the coding region of a gene, change the amino acid sequence of the gene's protein product.
   ii. **Non-coding SNPs**, located within the gene's regulatory sequences, change the level of gene expression and, therefore, how much RNA and protein is produced.

CORE IDEA
(3) Energy and Equilibrium

HOMEOSTASIS & ENDOCRINE CONTROL

Content
- Homeostasis
- Hormonal control
- Cellular responses to signals

(p) Outline how insulin and glucagon regulate the concentration of blood glucose through the respective tyrosine kinase receptor and G-protein linked receptor.

References
- Reece *et al.*, Campbell Biology, 9th edition
- Sherwood, Fundamental of Human Physiology, 4th edition
- Alberts *et al.*, Molecular Biology of The Cell, 4th edition
- Green, Stout and Taylor, Biological Science Vol. 1 and 2, 3rd edition
- Lodish *et al.*, Molecular Cell Biology, 5th edition
- Raven and Johnson, Biology, 6th edition

*** This handout is the effort of several Biology teachers at RI(Yr 5-6). It has and will continue to be updated.**
**** Any information given in a double-lined box is for your information only.**

Part 1. Introduction

- In multi-cellular organisms where different parts of the body perform different functions, it is important to transmit information between these different body parts so that the body functions can be **well co-ordinated** and carried out **efficiently**.

- Communication is essential to ensure:
  (a) co-ordination between organs – one organ should facilitate the function of another so that the organism can function as a whole in response to changes
  (b) composition of the internal environment is maintained at a narrow, optimum range to support the functioning of the body cells
  (c) organs will not under-work or over-work to minimise energy wastage

- There are two major internal communication systems (Figure 1): **nervous system** and **endocrine** system. These two systems **detect** any changes known as **stimuli** in the external or internal environment, and **co-ordinate** appropriate response. Both systems can function independently or interact with each other to carry out certain physiological processes, including homeostasis.

- This section focuses on how the **endocrine** system mediates these interactions with the environment in maintaining homeostasis.

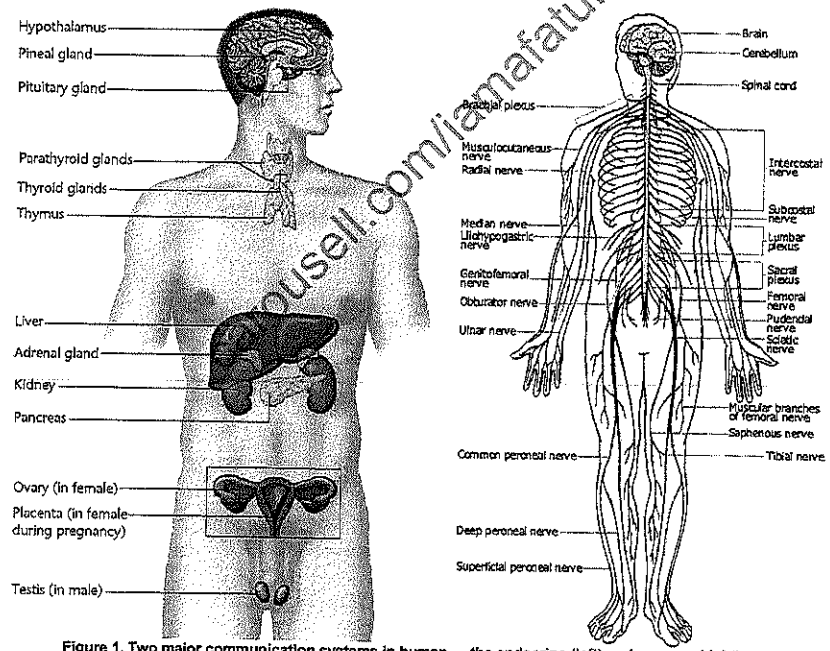


Figure 1. Two major communication systems in human — the endocrine (left) and nervous (right) systems

Part 2. Homeostasis

A. The Internal Environment

- In complex multicellular organisms, most living cells of the body are not directly exposed to the external environment. They are bathed in a liquid internal environment known as **interstitial fluid**.
- No matter how remote a cell is from the external environment, it can make life-sustaining exchanges with its surrounding interstitial fluid.
- Components of the internal environment which are homeostatically regulated and kept constant
  (a) chemical constituents e.g. ions, glucose
  (b) pH
  (c) temperature
  (d) volume and pressure
  (e) water potential (concentration of water, salt and other electrolytes)
  (f) concentration of respiratory gases e.g. O_2 and CO_2

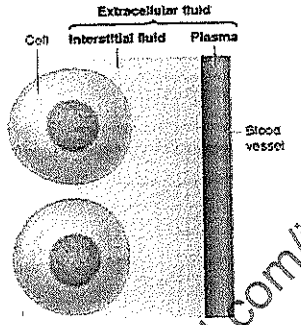


Figure 2. Extracellular fluid consists mostly of interstitial fluid, which fills the spaces between cells, and plasma, the fluid portion of the blood.

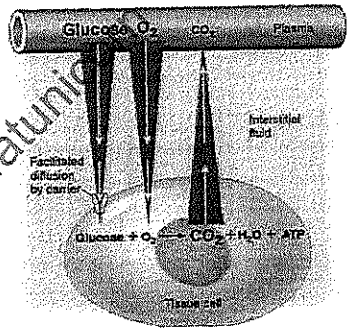


Figure 3. Independent exchange of small water-soluble substances between plasma in blood vessels and cells via interstitial fluid.

B. Need For Homeostasis

- Fluctuations in the external environment may affect the temperature, pH, concentration of the metabolites etc in the internal environment.
- **What is Homeostasis?**
  - Homeostasis is the maintenance of a **dynamically constant internal environment** by an organism **irrespective of changes in external environment**.
  - By maintaining stable optimal conditions to allow for proper functioning of the cells in a state of maximum efficiency.
  - It involves **self-regulating** and **negative feedback** mechanisms to control the level of substances that are to be maintained.

Question
What are the consequences of disruptions in homeostasis?
- altered conditions can lead to illness & death
- E.g. increased temperature / acidity more denaturation of enzymes or membrane transporters

C. Principles Of Homeostasis

Homeostatic mechanisms exhibits 2 principles:

1. **Self-regulation**
   - This involves the **control mechanism** that is triggered by the very parameter/entity that it serves to regulate.
     E.g. in the control of blood glucose level, the secretion of hormone (glucagon and insulin) involved in the control of blood glucose level is triggered by changes in the blood glucose level.
2. **Negative feedback**
   - Negative feedback is a corrective / regulatory mechanism in which a **stimulus** as a result of a **change in parameter / a deviation from the set point**, triggers a response.
   - The response is in the opposite direction to the stimulus, and it **opposes the initial deviation/ reduces the initial stimulus**.
   - In other words a **change sets off events that counteract the change.**
   - Homeostasis operates primarily via a **negative feedback control loop.**

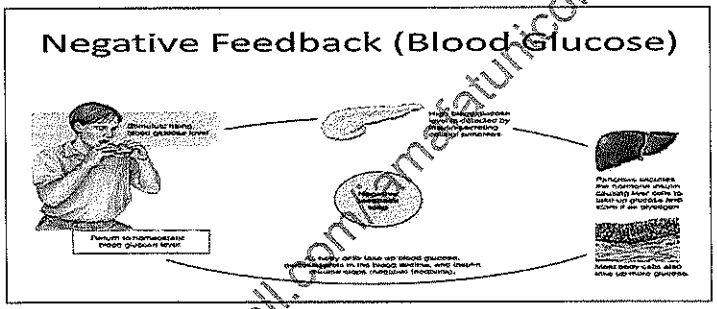


Figure 4 Components of a homeostatic control system/negative feedback loop

- The response/output (**changed condition**) serves as a negative feedback to the receptor for monitoring of any **displacement from the set/reference point** that is caused by the stimulus.
- Continuous monitoring of the parameter by the receptor produces continuous adjustments of the output which keep the parameter **fluctuating around the norm/set point**.
- Negative feedback is associated with increasing stability of the system because the disturbance triggers a sequence of events to **restore the system back to norm / set point**.
- Homeostasis is a dynamic process: it works by constantly making adjustments to compensate for fluctuations of output. Thus it is more accurate to describe such system as steady state or dynamic equilibrium.
  - E.g. when a parameter (e.g. glucose concentration in blood) increases above the set point, the corrective mechanism will act to cause that parameter to decrease and return to set point.

Part 3. Endocrine system

The endocrine system is a collection of **endocrine glands** (e.g. pituitary, hypothalamus, adrenal, pancreas) that maintains homeostasis via **slow** and **long-term control** using chemical signals known as **hormones**.

A. Hormones

- Hormones are responsible for many processes such as growth and development, metabolism, homeostasis and reproduction.

- Hormones have the following characteristics (**Figure 5**):
  - **secreted directly into blood** by **ductless** endocrine gland (a duct is like a tube).
  - transported by blood to exert its effect at a **specific** target site (or target cells). → complementary to receptor.
  - **effective in small quantity.**

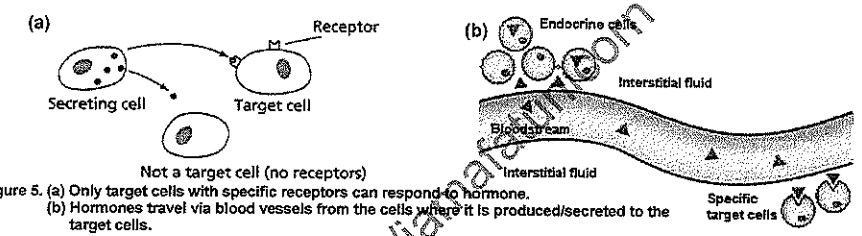


Figure 5. (a) Only target cells with specific receptors can respond to hormone.
(b) Hormones travel via blood vessels from the cells where it is produced/secreted to the target cells.

- The effects of hormones on their target cells can be complex:
  - hormones are **extracellular chemical messengers** that bring about **specific cell responses** through cell signaling (refer to Cell Signalling lecture). intracellular
  - hormone has a **complementary shape** that allows it to fit precisely into **receptor** molecules on the target cells. This explains why hormones are specific for a particular target cell. & charge ↓ ensures specificity of hormone.
  - a single hormone is capable of **eliciting different responses** from
    (1) different target cells / tissues,
    or even from
    (2) the same tissue but at different stages of development.

- Hormones are small, soluble organic molecules that can be classified into 3 main classes:
  (a) peptides/proteins, composed of amino acids
  (b) amines, derivatives of a single type of amino acid
  (c) steroids, derived from cholesterol

B. Endocrine Glands

- Endocrine gland has these features
  (a) **ductless**. Cohesive of tubes (openings aka ducts)
  (b) secrete hormones **directly** into the **blood** circulation to be brought to their **target site.**
  (c) endocrine glands are supported by a **rich supply of blood capillaries.** consists of a cluster of secretory cells.
  (or rich supply of blood required to transport hormones (secreted by secretory cells))

Question
Can you say endocrine glands excrete hormones?
No, excrete means to eliminate waste. Secrete means to produce a useful substance.

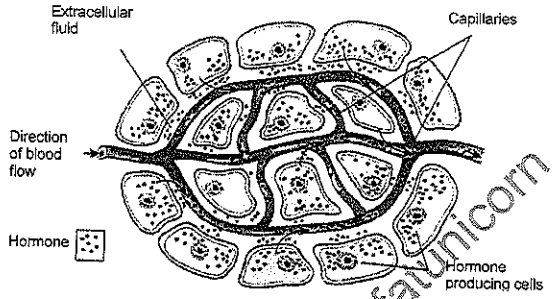


Figure 6. An endocrine gland consists of a cluster of hormone secretory cells embedded within a network of blood capillaries.

- An example of endocrine gland is **islets of Langerhans of pancreas.**
  - constitute only 1-1.5% of the mass of human pancreas. The rest of pancreas is exocrine tissue which secrete digestive enzymes. The pancreas is both an endocrine and exocrine gland.
  - each cluster of islet of Langerhans has a population of
    (a) **alpha cells** which secrete hormone **glucagon**
    (b) **beta cells** which secrete hormone **insulin**

alpha cells → darker.
beta cells → lighter coloured.

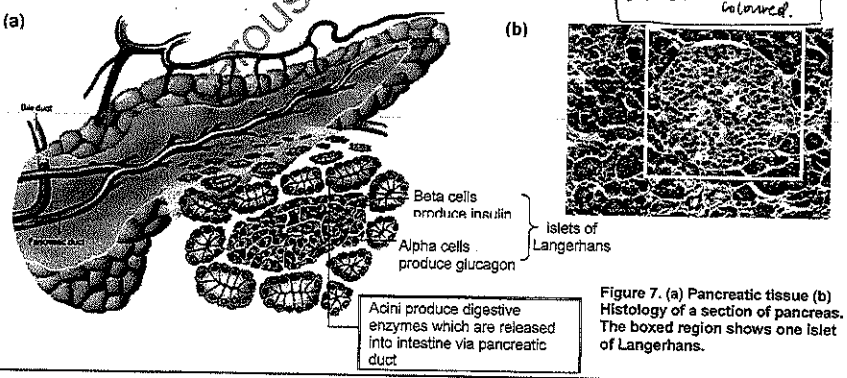


Figure 7. (a) Pancreatic tissue (b) Histology of a section of pancreas. The boxed region shows one islet of Langerhans.

Question
What happen to hormones after they have exerted their effects?
They are degraded by enzymes & excreted in the urine

Part 4. Control of blood glucose concentration

- Human blood glucose needs to be controlled:
  - energy must be constantly available for cell metabolism yet dietary fuel intake is intermittent, not continuous.
  - as a result, excess energy must be stored for use during fasting periods between meals. Stored energy fills in the gap between meals.
- Human blood glucose level is regulated by 2 hormones secreted from islets of Langerhans of pancreas
  (a) **insulin** from the **beta cells** and
  (b) **glucagon** from the **alpha cells.**
- Insulin and glucagon act in an **antagonistic** fashion. Their actions on metabolism oppose each other. It is the **ratio of their concentrations** that is actually more critical in the regulation of blood glucose concentration than their actual levels.

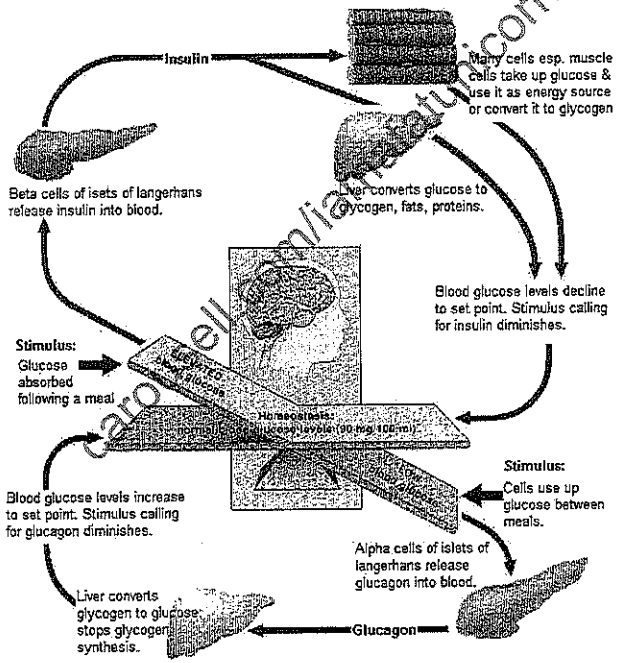


Figure 8. Regulation of blood glucose concentration by insulin and glucagon

A. Insulin – decreases blood glucose concentration

- The hormone insulin was isolated from animal sources in pure enough form or synthesised via recombinant DNA technology for administration and with dramatic life-saving effects.
- Deficiency of insulin will result in **Type 1 diabetes mellitus** in which there is hyperglycemia (abnormally elevated blood glucose concentration). Symptoms include glucose excretion in the urine as kidneys are unable to reabsorb all the glucose.

Role of insulin in regulating blood glucose concentration

- When blood glucose concentration rises above set point of around 90 mg/dL (or 90 mg per 100 ml) (stimulus), the displacement is detected by **beta cells of islets of Langerhans** in pancreas (receptor and control center).
- Beta cells in response will secrete more insulin (signal) by exocytosis into the bloodstream.
- Insulin will be transported via the circulatory system to its target tissues e.g. **liver cells and muscles cells** (effector) where it binds to its cell surface receptor. This triggers a cascade of intracellular signalling events to bring about a response
- for instance, one of the responses is the fusion of cytoplasmic vesicles (in muscle, adipose and liver cell) containing glucose transporters with the cell membrane. Thus with more glucose transporters on the cell membrane resulting in increased permeability of these cells to glucose.
- Excess glucose from bloodstream is transported into liver and muscle cells, thus **decreasing blood glucose concentration**.

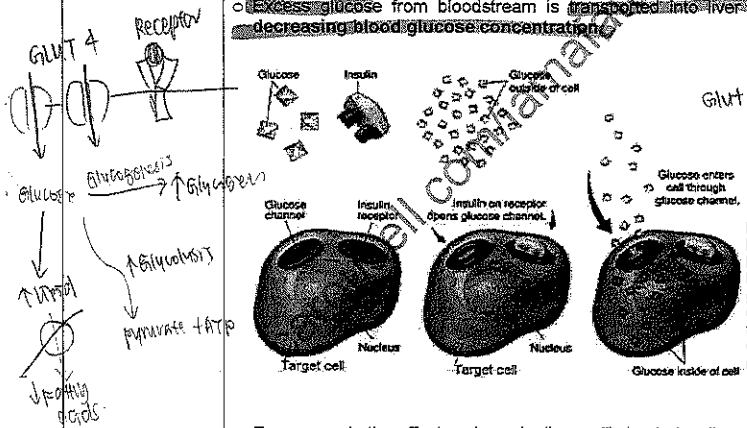


Figure 11. Insulin hormone-receptor interaction leads to increase in uptake of glucose via glucose channel/ transporter from the blood into its target cells (liver cells and muscle cells).

- Responses in the effectors (muscle, liver cell) due to insulin:
  (a) **increases glucose intake by tissues** (by increasing glucose transporters at the plasma membrane of target cells)
  (b) **increases glycolysis** (oxidation of glucose)
  (c) **increases glycogenesis** (synthesis of glycogen from glucose), mainly in liver and muscles
  (d) reduces free fatty acids release and increase lipid synthesis
  (e) increases transport of amino acids into cells and protein synthesis
  (f) decreases **gluconeogenesis** (synthesis of glucose from non-sugar carbon substrates such as pyruvate, lactate, amino acids etc).

- As the blood glucose concentration decreases to return to its set point around 90mg/dL via negative feedback mechanism, the output also serves as a negative feedback to the beta cells of islets of Langerhans in pancreas to decrease insulin secretion.
- Insulin secretion returns to normal when set point (normal blood glucose concentration) is restored.

Question
Is it correct to say insulin converts excess glucose to glycogen?

- No. Insulin is not an enzyme.
- It triggers the signal transduction in target cell resulting in the conversion of glucose to glycogen

B. Glucagon – increases blood glucose concentration

- Glucagon is a peptide hormone that is synthesized and secreted from alpha cells of the islets of Langerhans of the pancreas when the glucose level in the blood is low (hypoglycemia).

Role of glucagon in regulating blood glucose concentration

- When blood glucose concentration drops below the set point of around 90 mg/dL, the displacement is detected by alpha cells of islets of Langerhans in pancreas (receptor and control center).
- This usually happens between meals or after exercise (i.e. an increase in metabolic demand).
- Alpha cells in response will secrete more glucagon into bloodstream.
- Glucagon will be transported via the circulatory system to its target tissues e.g. liver cells (effector), where it binds to its cell surface receptor to trigger a cascade of intracellular signaling events to bring about the response — e.g. release glucose into bloodstream to increase blood glucose concentration.
- Glucagon will increase blood glucose concentration by **stimulating enzymes for** :
  (a) **glycogenolysis** (breakdown of glycogen to glucose)
  (b) **gluconeogenesis**
  (c) **breakdown of lipids** in adipose tissues
- As blood glucose concentration increases to return to its set point 90 mg/dL, this serves as a negative feedback to alpha cells of islets of Langerhans in pancreas to decrease glucagon secretion.

Question
What is the order of metabolic fuel usage in the body?
Protein/fat/glucose/glycogen

1. Glucose (first)
2. Glycogen
3. Fat (primary energy reservoir)
4. Proteins (last)

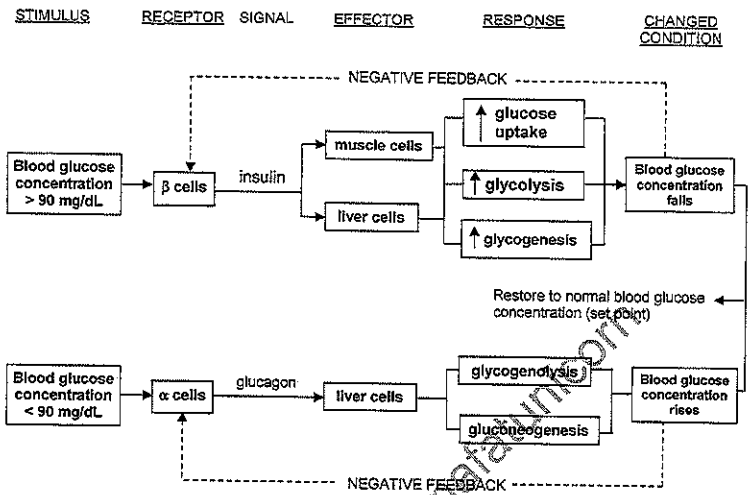


Figure 10. Overview on homeostatic control for blood glucose level using negative feedback mechanism

CORE IDEAS
(3) Energy and Equilibrium

CELL SIGNALLING

Content

An overview of cell signalling and communication
- Signal reception and the initiation of transduction
- Signal transduction pathways
- Cellular responses to signals

Learning Outcomes

Candidates should be able to:

(m) Outline the main stages of cell signalling:
  i. ligand-receptor interaction
  ii. signal transduction (phosphorylation cascade and signal amplification)
  iii. cellular response (change in gene expression)
  (knowledge of intracellular receptors is not required)

(n) Explain the roles and nature of second messengers (including cyclic AMP)

(o) Explain the role of kinases and phosphatases in signal amplification

(p) Outline how insulin and glucagon regulate the concentration of blood glucose through the respective tyrosine kinase receptor and G-protein linked receptor. (The outline should be limited to describing how the ligand induces a conformational change in a membrane-bound receptor to trigger downstream signalling pathways that elicit physiological changes in blood glucose concentration. Details of different second messengers and specific kinases activated in the pathway are not required.)

1 The Cell and Biomolecules of Life

(o) describe the molecular structure of the following proteins and explain how the structure of each protein relates to the function it plays:
  iii. G-protein linked receptor (signalling)

Use the knowledge gained in this section in new situations or to solve related problems.

References

- Reece *et al.*, Campbell Biology, 9th edition
- Sherwood, Fundamental of Human Physiology, 4th edition
- Alberts *et al.*, Molecular Biology of The Cell, 4th edition
- Brooker *et al.*, Biology, McGraw-Hill.
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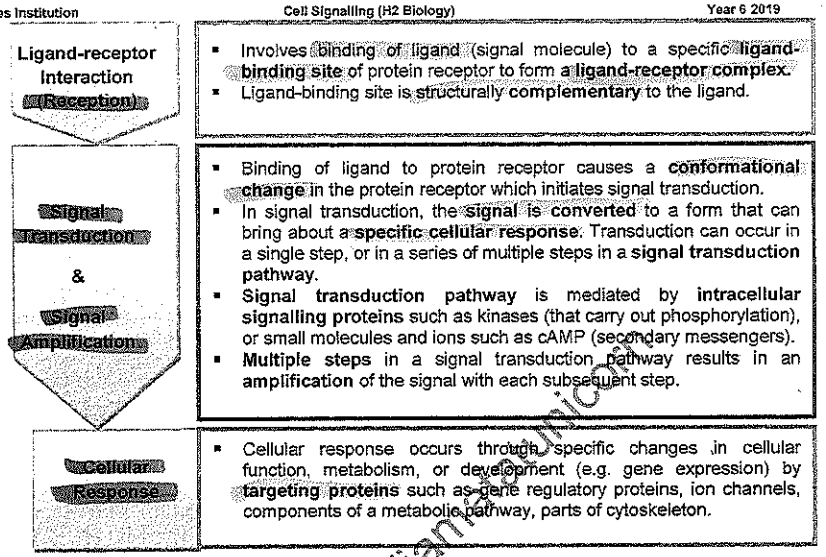


Fig. 1c. Overview of stages of cell signalling.

***Ligand** – a molecule that binds specifically to another molecule, usually a receptor
***Signal transduction** – a series of steps by which signals are conveyed into the target cell where they are transformed into a cellular response

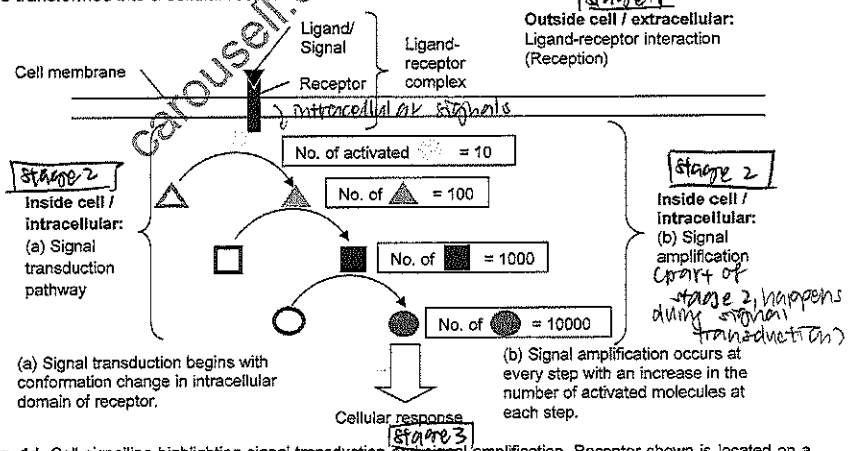


Fig. 1d. Cell signalling highlighting signal transduction and signal amplification. Receptor shown is located on a cell membrane.

Part B. Stages of cell signalling

LO(m) Outline the main stages of cell signalling:
- i. ligand-receptor interaction
- ii. signal transduction (phosphorylation cascade and signal amplification)
- iii. cellular response (change in gene expression)

1. Stage 1: Ligand-Receptor Interaction (Reception)

- **Ligands** include a variety of chemicals, such as hormones, neurotransmitters and small molecules (e.g. amino acids, amino acid or lipid derivatives) that can bind to the **ligand-binding site** of the **specific** receptor.
- Ligands are also known as extracellular chemicals/ **first messenger**/ signal.
- Reception involves binding of a specific ligand to a **specific, structurally complementary protein receptor** to form ligand-receptor complex.
- **Receptors** are proteins that bind and transduce the message of the signal molecule into a cellular response.
- Binding of the ligand to its specific receptor will result in the activation of the receptor protein.

Question
Why is ligand-receptor interaction specific?

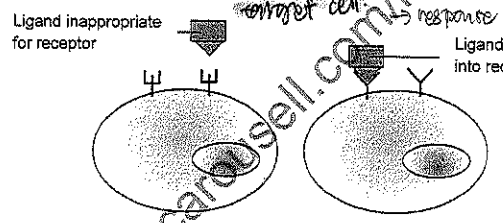


Fig. 2. Receptors bind only to ligands that can fit into them. Only one of the cells responds to the chemical messenger. The receptor shown is located on a cell membrane.

- Two types of receptors can be found in the body, depending on their location in a cell:

(i) intracellular receptors (not in syllabus)
- are located in cytoplasm or in nucleus
- bind to small and/or hydrophobic/ non-polar signal molecules (e.g. steroid hormones) that can diffuse through the hydrophobic core of phospholipid bilayer.

(ii) cell surface receptors (focus of syllabus)
- are located on plasma membrane.
- bind to large and/or hydrophilic/polar signal molecules which cannot diffuse readily across the phospholipid bilayer.
- are transmembrane proteins that span cell membrane.
- signal molecule binds to ligand-binding site in the extracellular domain (**Domains** are distinct functional and/or structural units in a protein). Binding induces a **conformational change** in the intracellular domain.

- there are three main types of cell surface receptors:
  (a) **G-protein-coupled receptors** (e.g. glucagon receptor)
  (b) **enzymatic receptors** (e.g. receptor tyrosine kinase) such as insulin receptor
  (c) **chemically-gated ion channels** (see Part C/ other receptors)

- Recipient target cells can be located near to the signalling cells or at some distances away in the body.

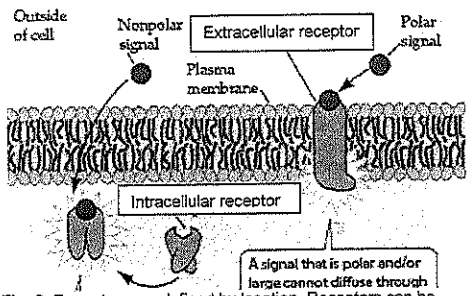


Fig. 3. Receptors are defined by location. Receptors can be located on the cell surface membrane or in the interior of the cell.

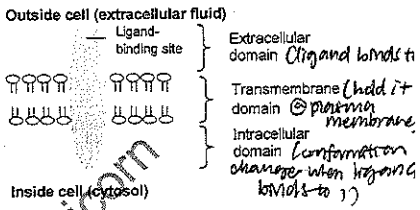


Fig. 4. Structure of a cell surface receptor.

Intracellular receptors

- Intracellular receptors may be located in the cytoplasm or in the nucleus.
- They are bound by small, hydrophobic molecules that can easily diffuse across the hydrophobic core of the phospholipid bilayer to enter the target cell.
- Upon activation, receptors may function as transcription factors or gene regulators (e.g. receptors for steroid hormones such as estrogen and progesterone).
- Signal transduction is usually a 1-step process: binding of signal molecule causes a conformational change that allows the receptor protein to function as a transcription factor.
  - No signal amplification is involved.

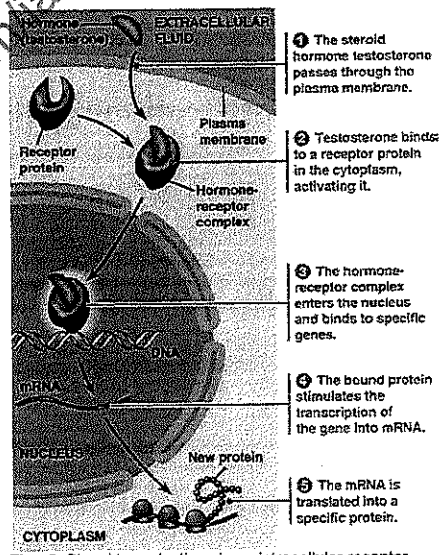


Fig . 5. Signal transduction via an intracellular receptor.

2. Stage 2: Signal Transduction

- Signal transduction refers to the series of changes in cellular proteins that converts **extracellular chemical signal** to a specific **intracellular response**. The function of a signal transduction pathway is to change the behaviour of a cell.

- Signal transduction pathway starts after reception that brings about
  (a) activating **receptor** proteins
    1. in the previous stage, a signal molecule binds to the ligand-binding site of receptor protein. This now causes a **conformational change** in the **intracellular domain of receptor protein**. It activates the receptor protein, enabling it to interact with other cellular molecules.

    2. the syllabus will focus on two main types of cell surface receptors:
       i. **G-protein-coupled receptors**
       ii. **enzymatic receptors**

  (b) activating other **relay proteins** which involves:
    1. **Multiple steps** process
       - activated receptor activates another relay protein, which activates another relay protein molecule and so on until the final protein that produces cellular response is activated.
       - involving specific molecular interactions.

    2. **Phosphorylation** cascade
       - A phosphorylation cascade is a sequence of events where one enzyme phosphorylates another, causing a chain reaction leading to the phosphorylation of thousands of proteins
       - **Phosphorylation** and **dephosphorylation** are ways of changing these proteins and they involve the addition or removal of phosphate groups respectively

    3. **Signal amplification**
       - each step increases the **proportion** of proteins that make up a cell or the **activities** of cellular proteins.

(o) Explain the role of kinases and phosphatases in signal amplification

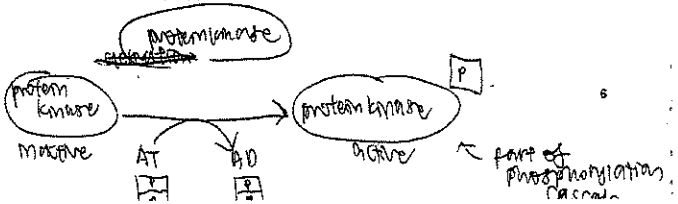
2.1 Phosphorylation cascade

- Many proteins are inactive when they are initially synthesised and require modification after synthesis for activation. In other cases, a protein may require modification for deactivation. Phosphorylation-dephosphorylation of proteins is a widespread **post-translational modification** for controlling protein function (See Organisation and Control of Prokaryotic and Eukaryotic Genome).

- Phosphorylation or dephosphorylation involves the addition or removal of phosphate groups, respectively.
  - Most proteins are **activated** by phosphorylation and **deactivated** by dephosphorylation. In other cases, the reverse occurs.

  - 2 enzymes are involved in the processes are **kinase** and **phosphatase**.
    i. **Kinase** is an enzyme which **adds phosphate** groups from ATP to the **protein (phosphorylation).**

    ii. **Phosphatase** is an enzyme that **removes phosphate** groups from **proteins** by hydrolysis **(dephosphorylation).** This action is directly opposite to that of kinase.



- Phosphorylation may directly alter activity of an enzyme, e.g., by promoting a **conformational change**. Protein kinase that has been phosphorylated becomes active. It then adds a phosphate group to the next protein kinase in line, activating it. The sequence of events is known as phosphorylation cascade.

- The phosphorylation cascade is an example of a multi-step signal transduction pathway. In every step of this pathway, **signal amplification** occurs (see section 2.2 below).

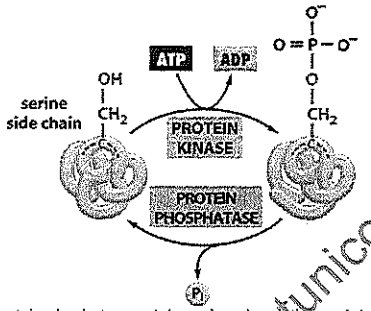


Fig. 6. Protein kinase and protein phoshatase catalyse phosphorylation and dephosphorylation respectively

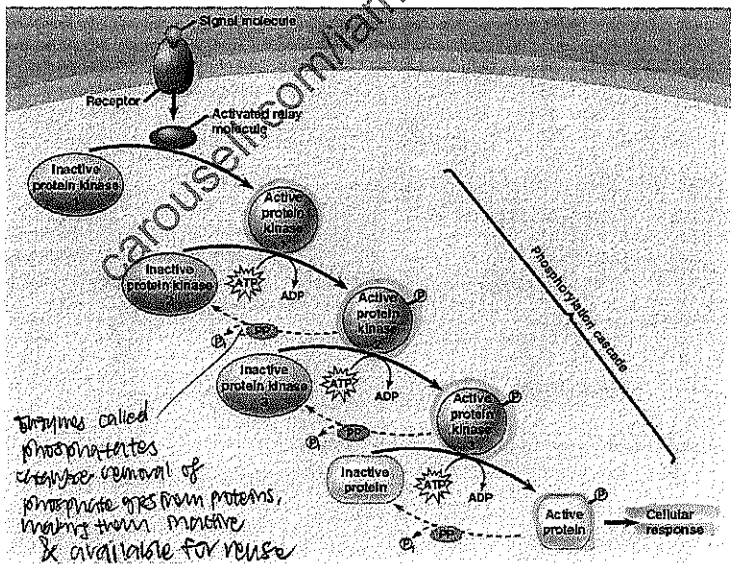


Fig. 7. A typical phosphorylation cascade containing 3 different protein kinases (numbered 1 to 3).

2.2 Signal amplification

more steps → more amplification.

- **Signal amplification** produces **a large number** of an intracellular mediator from a relatively small number of extracellular signals.

  - When a few of the molecules in a pathway transmit the signal to multiple molecules of the next component in the series, the result can be a **larger number of activated molecules** at the end of the pathway.

  - **Protein kinases** are usually involved as when these enzymes are activated, they catalysed the **phosphorylation** of several other specific proteins in the next step thus activating these proteins.

  - Each step increases the proportion of the molecule. In other words, a **small number of extracellular signal molecules** can produce a **large cellular response** in a signal transduction pathway. Starting with 1 signalling molecule, one cell surface receptor can trigger the production of 10^8 copies of the final product. activated enzyme can exist in activated form long enough before being deactivated, to activate other molecules.

  - Conversely, **protein phosphatases** serve to terminate the responses initiated by receptor activation of protein kinases.

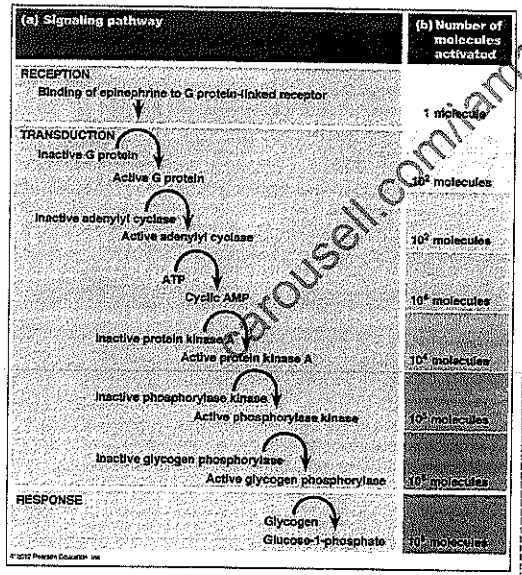


Fig. 5. Figure showing the number of activated molecules at each step of the signal transduction pathway.

Question

a. What is the basis of signal amplification?
At each step, one molecule acts on many other molecules → produces a large amount of the final product. Allows signal transduction to proceed even with v. little amt of signal molecules/receptors at the start

b. Why is amplification of signal required?
Allows ~~s~~ As there may not be enough receptors to yield the appropriate responses, signal amplification will help with converting the signals to appropriate cellular responses.

c. With reference to **Fig.5** which transduction step/s does not result in signal amplification? Explain.

3. Stage 3: Cellular Responses

- The transduced signal will eventually produce an appropriate **cellular response** in the cells, such as:
  - **Regulation of Gene expression** → up regulation of gene or down regulation of gene
  - **Metabolic pathway regulation** for activation/inhibition of enzymes
  - Changes in cytoskeleton such as assembly of microtubules for movement of vesicles to plasma membrane

Question
Even though the ligand and receptor may be the same, different responses may be elicited from different cells. Why?

- In different ~~cells~~ kinds of cells, different sets of genes are expressed thus resulting in
- presence of different relay molecules / signal pathways in different cells.

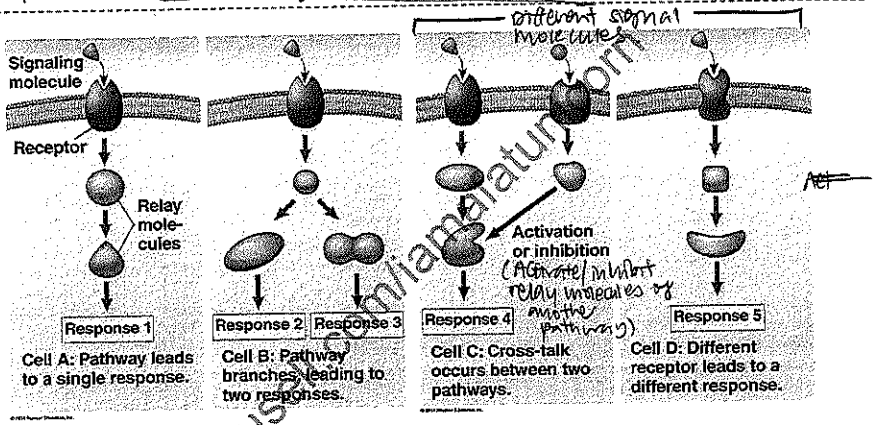


Fig. 6. Specificity of cell signalling.
The 4 cells respond to the same signal molecule in different ways.
Cells A, B and C all use the same receptor protein for the signal molecule (represented by a triangle); differences in other proteins account for their differing responses.
Cell B - a pathway triggered by a single kind of signal diverges to produce 2 responses.
Cell C - 2 pathways triggered by separate signals (represented by a triangle and a circle) converge to modulate a single response.
Cell D - same signal molecule recognises a different receptor molecule which transduces the signal via a different pathway, producing a different cellular response.

- When it comes to cellular responses :
  (a) different cell types can have different responses to the same signal
  - e.g. in liver cells, insulin will increase permeability to glucose, and stimulate conversion of glucose to glycogen. In adipose cells, insulin will inhibit breakdown of triglycerides.

  (b) same cells can have the same response to different signals
  - e.g. liver cells can be stimulated by both glucagon and epinephrine to mobilise glucose. These different signals act by the same transduction pathway to stimulate the breakdown and inhibit the synthesis of glycogen.

4. Termination of Cellular Response

- Cellular responses can be **terminated / regulated** at 2 points:
  (a) reception
  - extracellular first messenger can be **degraded by enzymes** in the extracellular space.
  - **endocytosis of ligand-receptor complex** prevents signal transduction from continuing.
  (b) during signal transduction pathway
  - Increase activity of the **phosphatases** that dephosphorylate proteins to inactivate the relay molecules so that propagation of the signal will be inhibited.

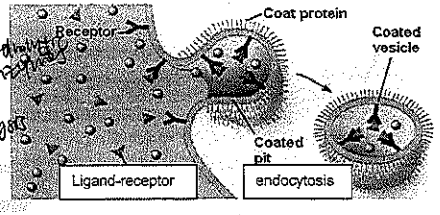


Fig. 7. Endocytosis of ligand-receptor complex.

  - Production of inhibitors that bind to the ligand-receptor complex and/or any of the intracellular signal proteins in the signal transduction pathway to prevent transduction of the signal.

Question
Why is it important to remove a bound ligand after a cellular response has been triggered?

- If the ligand remained bound to the receptor, it will continue to trigger signal transduction that leads to inappropriate / excessive cellular responses.
- only when the ligand is removed, the cell can then respond again to new ligands.

5. Advantages of the signal transduction stage are:

1. Facilitate signal amplification
   - only a small number of signal molecules needed to solicit a large response from cell
2. Multiple responses to 1 signal molecule (ligand) because 1 signal molecule (ligand) can trigger multiple signal transduction pathways to elicit different responses.
   - E.g. insulin binding to liver cell triggers increase GLUT 4 transporters at cell membrane to increase glucose uptake from blood, results in activation of enzymes for increased glucogenesis, glycolysis and decreased gluconeogenesis.
3. Provides multiple checkpoints for regulation
   - Several steps in the signalling pathway can be regulated and controlled E.g. activation/ inactivation of G protein, activation of adenyl cyclase and amount of cAMP produced. This will eventually regulate the cellular response of the pathway.
4. Ensures specificity because the specific signal molecule (ligand) binds to a specific receptor (ref. to specific conformation) to elicit specific reaction via specific pathway in each cell type;
5. Ability of a type of signal molecule (ligand) to co-ordinate activation of many different cells simultaneously
   - E.g. insulin molecules carried by the blood stream bind to receptors of several target cells such as liver, muscle and adipose cell triggering cellular responses from these cells.

6. Ability of a signal molecule (ligand) to activate genes in nucleus upon binding to cell surface receptor without the need to move into nucleus.

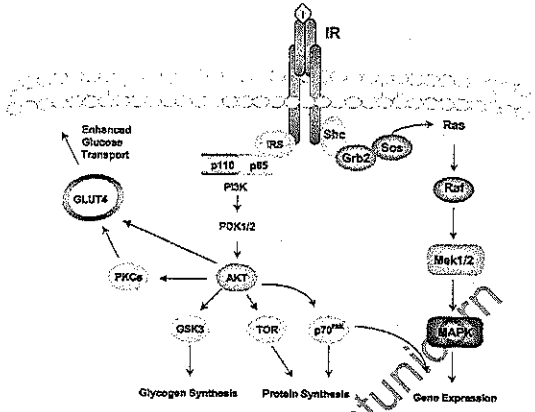


FIG. 8. Schematic representation of insulin signaling. Insulin (*I*) binding to the receptor leads to phosphorylation and activation of different relay molecules of different signal transduction pathways. Eventually leading to several cellular responses such as glycogen synthesis, increased GLUT4 at cell membrane, protein synthesis and gene expression.

(p) Outline how insulin and glucagon regulate the concentration of blood glucose through the respective tyrosine kinase receptor and G-protein linked receptor.

(o) Describe the molecular structure of the following proteins and explain how the structure of each protein relates to the function it plays:
iii. G-protein linked receptor

PART C Types of receptors:

1. G-Protein Coupled Receptor (GPCR)

- The G protein system is the largest category of receptor type in animal cells. Examples of ligands that bind to GPCRs: **glucagon**, adrenaline.
- G protein system comprises of 3 components – **G protein-coupled receptor**, **G protein** and another protein (usually an enzyme).
- **G Protein-Coupled Receptors (GPCRs):**
  - GPCRs consist of a single polypeptide that is folded into a globular tertiary protein.
  - is a **transmembrane protein** (which snakes through the membrane 7 times).
  - It consists of 7 **α-helices** connected by three intracellular and three extracellular peptide loops.
  - It has an extracellular N-terminus, and an intracellular C-terminus.
  - has an **extracellular ligand-binding site** that binds to **specific** hydrophilic ligands including hormones like glucagon, adrenaline/epinephrine.
  - The intracellular domain/cytoplasmic side of GPCR has a **G protein binding site** that allows binding of a G protein complex.

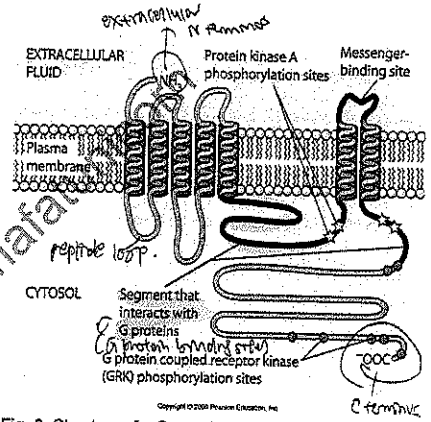


Fig. 9. Structure of a G protein-coupled receptor. This is one of 3 components in the G protein system.

- **G proteins:**
  - are found on the cytoplasmic side of the membrane.
  - are heterotrimeric complexes made up of alpha (α), beta (β) and gamma (γ) subunits
  - are GTP-binding proteins (guanine nucleotides).
  - alternate between 2 states:
    (i) **inactive** state – bound to GDP
    (ii) **active** state – bound to GTP
  - has intrinsic **GTPase** activity (capable of hydrolysing GTP to GDP).
- Signal transduction system:
  The following steps show how a chemical signal activates the entire G protein system.
  (a) **G protein system in inactive form**
  In the absence of extracellular signal molecule specific for the receptor, all 3 proteins are in inactive form.
  Inactive G protein has a GDP molecule bound to it.

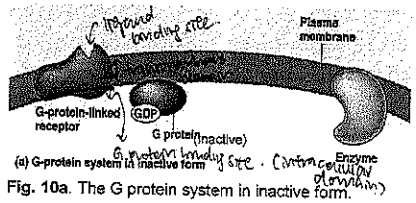


Fig. 10a. The G protein system in inactive form.

(b) **G protein system in action**

(1) When the signal molecule binds to receptor, the receptor changes conformation in such a way that it binds to an inactive G protein.
(2) A molecule of GTP displaces GDP on the inactive G protein. This activates the G protein.
(3) The active G protein dissociates from the receptor and moves along the cytoplasmic side of the cell membrane.
(4) It binds to and activates the enzyme which
(5) triggers the next step in the pathway leading to cellular responses.

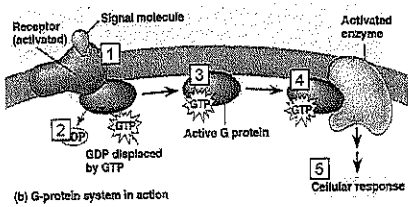


Fig. 10b. The G protein system when activated

(c) **G protein system returns to inactive form**

Due to intrinsic GTPase activity of the G protein, it catalyses hydrolysis of its GTP to GDP, returning the G protein to an inactive state.
Once inactivated, G protein dissociates from the enzyme and becomes available for reuse.
All 3 proteins remain attached to the plasma membrane.

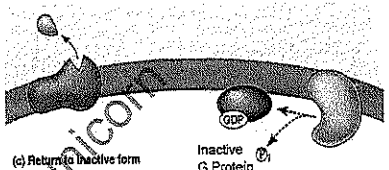


Fig. 10c. Return to inactive G protein

- Once G protein is activated, it can activate 2 enzymes in the signal transduction pathway:
  (a) **adenylyl cyclase**
  - catalyses conversion of ATP to cAMP (**secondary messenger**) which triggers a series of biochemical events within the cell to bring about the response dicated by the signal molecule (see Part C).

  (b) phospholipase C (not in syllabus).

Question
When a signal binds to the receptor of the G protein system, can inactive G protein becomes active by phosphorylating its GDP to GTP?
No. Inactive G protein becomes active by exchanging GDP with GTP; NOT by phosphorylating GDP.

(n) Explain the roles and nature of second messengers (including cyclic AMP)

1.1 Secondary Messengers

- Both G protein-coupled receptors (GPCR) and receptor tyrosine kinase (RTK)-initiated pathways involve second messengers.
- The extracellular signal molecule/ ligand that binds to the membrane receptor is a pathway's first messenger.
- Many signalling pathways also involve small, non-protein, water-soluble molecules or ions, called **second messengers.** They help to activate cellular proteins. Not all components of signal-transduction pathways are proteins.

Question
What are the characteristics of second messengers that enable to function effectively in the cytoplasm?
small & water-soluble → readily spread throughout the cell by diffusion.

- Second messengers in G protein-coupled receptors/ GPCR-initiated pathways:
  - **cAMP**
    - cAMP are produced by breakdown of ATP by activated adenylyl cyclase
    - Signal molecule is the 1st messenger, which activates a G-protein-coupled receptor, which activates a specific G-protein.
    - In turn, G-protein activates **adenylyl cyclase** (enzyme) which catalyses the conversion of ATP to cAMP.
    - cAMP acts as a second messenger. Its concentration is increased and activates another protein, usually protein kinase A.
    - **Protein kinase A** starts a **phosphorylation cascade** leading to cellular responses (see Part E).

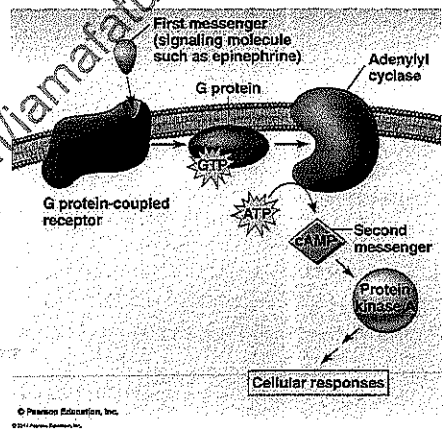


Fig. 11. cAMP is the second messenger in a G-protein signalling system.

GPCR → G protein → adenylyl cyclase → cAMP → protein kinase A.

- Ca^{2+}
  Acts as second messenger that initiates some cellular responses by binding to calmodulin (a cytoplasmic protein). Normal cytosolic Ca^{2+} concentrations are usually low. Many signalling molecules, e.g neurotransmitters and hormones induce responses in their target cells via signal transduction pathways that increase the cytosolic concentration of Ca^{2+}.
- IP_3 (Inositol trisphohsphate), DAG (diacylglycerol) are 2nd messenger molecule, which are formed by hydrolysis of PIP_2 (phosphatidylinositol 4,4-bisphosphate) by phopholipase C (**Fig. 12**)

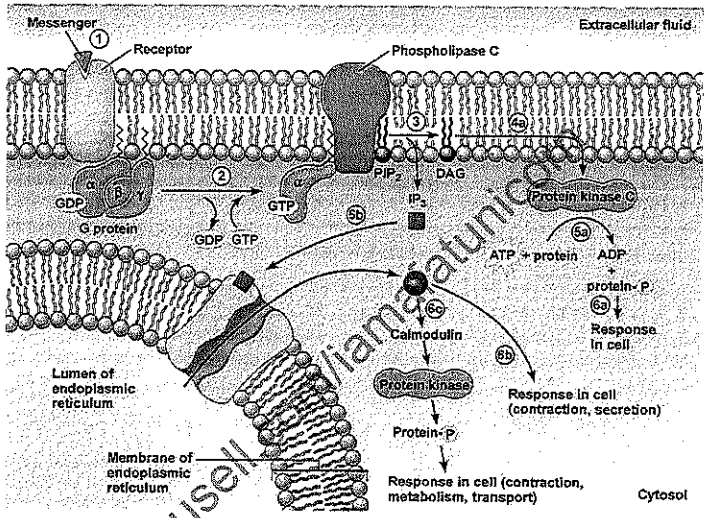


Fig. 12. Phospholipase C catalyzes conversion of phosphatidylinositol-4, 5-biphosphate (PIP_2) to diacylglycerol (DAG) and inositol triphosphate (IP_3) each of which serves as a second messenger. DAG remains in the membrane and activates protein kinase C. IP_3 moves into cytosol and triggers the release of Ca^{2+} from the endoplasmic reticulum. Ca^{2+} as second messenger by binding to calmodulin to activate a protein kinase.

Question
What happens if the second messenger e.g. cAMP remains in the cell?
Cellular molecules downstream of cAMP ~~activation~~ continues to be activated. cellular response may persist even without the binding of signal molecule to the receptor.

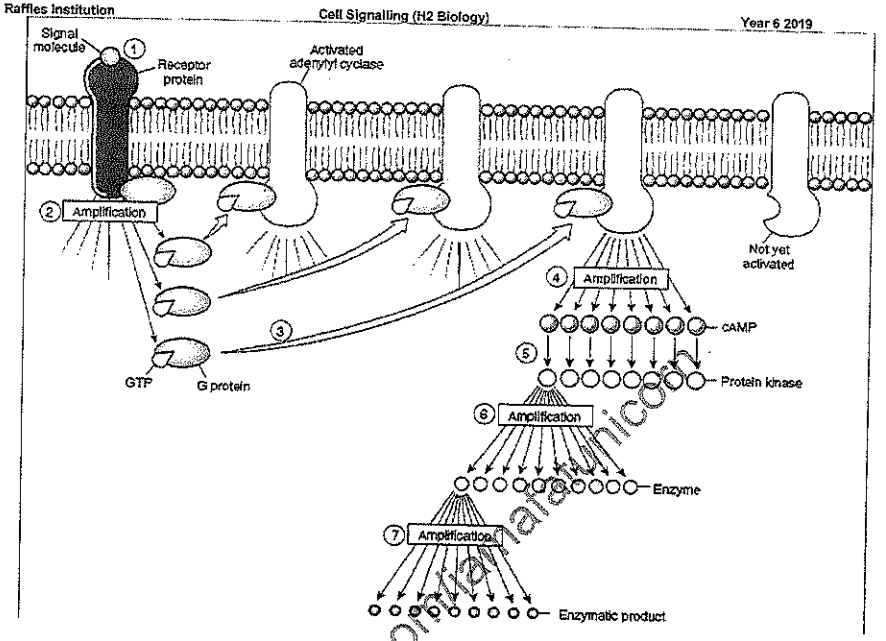


Fig. 13. Amplification at many steps ultimately produce a large response by the cell.
(1) Binding of one signal molecule to one cell surface receptor. (2) Activation of many G protein molecules, each of which activates a molecule of adenylyl cyclase (3), yielding an enormous production of cAMP (4). Each cAMP molecule in turn will activate a protein kinase (5), which can phoshorylate and thereby activate several copies of a specific enzyme (6). Each of those enzymes can then catalyse many chemical reactions (7).

Question
Suggest a benefit of having all 3 proteins in the G-protein system attached to plasma membrane.
Proximity & restriction of movements to increase chances of interaction

Question
Explain why the GTPase activity of the G protein is important in regulating the cellular response to an external stimulus.

2. Types of receptors: Receptor Tyrosine Kinase (RTK)

- Some cell surface receptors that bind to extracellular ligands either act as enzymes themselves or are linked to enzymes. These enzymes are necessary to activate a cellular response.
- Receptor Tyrosine Kinase (RTK) is a group of cell surface receptor that also functions as an enzyme. The cytoplasmic part of the receptor has a **tyrosine kinase** which phosphorylates the amino acid residues tyrosine. RTKs thus, have intrinsic kinase activity.
  [A protein kinase is a class of enzyme that adds phosphate groups from ATP to proteins.]
- Examples of ligands that bind to RTKs: **insulin**, epidermal growth factor.
- Abnormal RTKs that function even in the absence of signalling molecules are connected with many types of cancer.
- RTKs exist as 2 separate subunits or as a linked dimer:
  (i) RTKs with 2 individual subunits (**Fig. 14A**)
  (ii) RTK that exists as linked dimer (**Fig. 14B**)
  e.g. insulin receptor

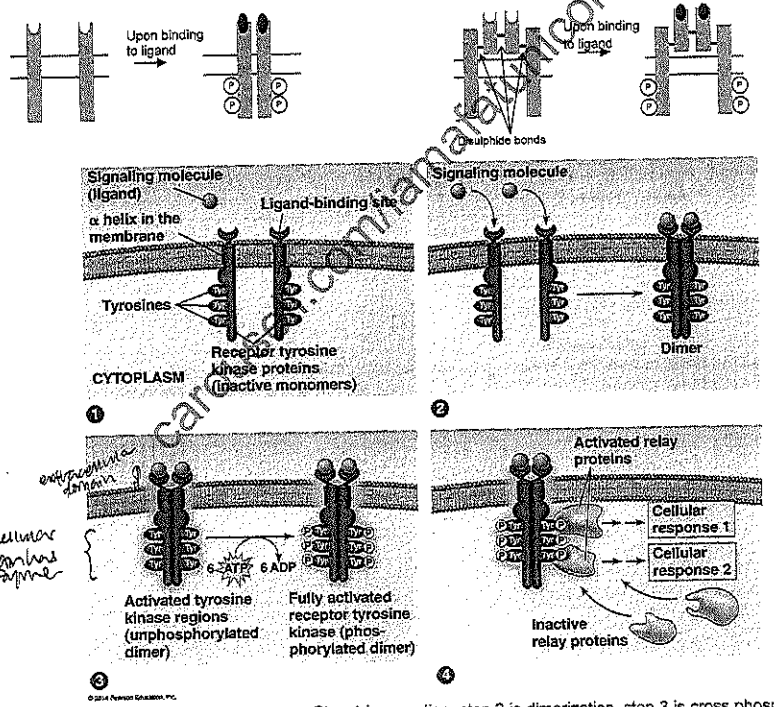


Fig. 15. Receptor tyrosine kinase system. Step 1 is reception, step 2 is dimerization, step 3 is cross phosphorylation of the dimer subunit tyrosine residues and step 4 is activation of relay proteins that bind to the phosphorylated tyrosine residues leading to signal transduction pathways and resulting with cellular responses.

Signal transduction mechanism:

As expected, before the signal molecule binds, the inactive receptor exists as individual subunits or as a linked dimer.

(1) When the specific signal/ligand molecule binds to **complementary extracellular ligand-binding site** of a specific receptor at the plasma membrane,

(2) it causes the **dimerization/formation of a dimer** where the two receptor subunits assemble into a pair (for receptors that existed as separate subunits before ligand binding).

(3) **Conformational change** in the **intracellular** domain of receptor results in **activation** of intrinsic **tyrosine kinase**
- Intrinsic kinase activity of each subunit in the intracellular domain, obtains phosphate groups from ATP, **cross-phosphorylates** the **tyrosine residues** on the other subunit **(autophosphorylation).** In other words, the receptor subunits phosphorylate each other.

Steps 1 & 3 → effect of signal molecule on receptor is **dimerisation** and **phosphorylation**.

(4) Now the receptor is fully activated, the RTK is recognised by specific relay proteins which bind to the **phosphorylated tyrosine residues** and in the process are themselves **activated.** The relay proteins are activated by binding or via phosphorylation by the RTK.
- One RTK dimer may activate 10 or more different intracellular relay proteins simultaneously triggering many different signal transduction pathways and cellular responses (see **part** B 2.1/ phosphorylation and 2.2/ amplification). RTK often activates different signal transduction pathways at once.

Part D. Other receptors

- There are three main types of cell surface receptors:
  (a) G-protein-coupled receptors (e.g. glucagon receptor)
  (b) enzymatic receptors (e.g. receptor tyrosine kinase)
  (c) chemically-gated ion channels
- In chemically-gated ion channels:
  - the receptor proteins usually also serve as an ion channel in the cell membrane.
  - when the receptor is activated by **binding of a chemical ligand** (e.g. neurotransmitters), the receptor changes conformation. This results in the opening or closing of ion channels to allow / prevent ions to pass through.
  - flow of ions across cell membrane results in a temporary change in membrane potential, which can affect the activity of other membrane proteins (such as voltage-gated channels). In this way, a cellular response is triggered.

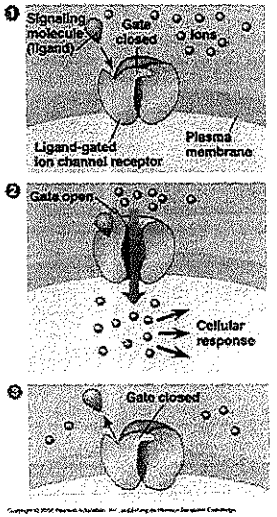


Fig. 16. Binding of ligand (1) triggers the opening of the ion channel (2). When the ligand dissociates, the channel closes (3).

Part E. Specific examples of Cell Signalling

1. Glucagon and G-protein signalling

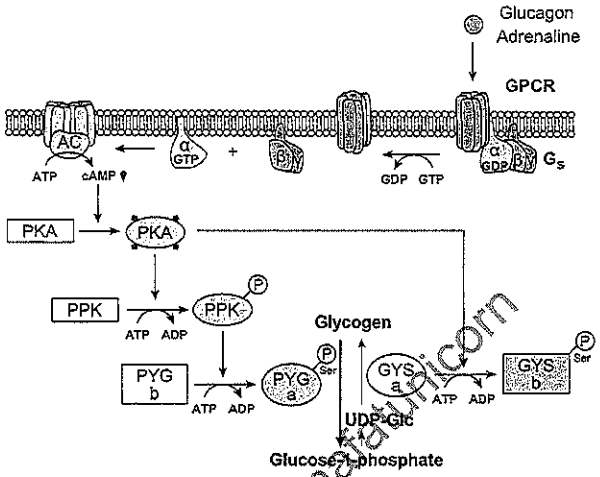


Fig. 17. The glucagon signalling pathway

Glucagon is a hormone which is released, from α cells of the islets of Langerhans in the pancreas, in response to low blood glucose levels. It acts mainly on liver cells to promote glycogen breakdown and to encourage glucose synthesis. The net effect of glucagon signalling is an increase in blood glucose levels.

Ligand – receptor interaction

- The glucagon receptor on the cell surface of liver cells is a G-protein coupled receptor (GPCR).
- This GPCR has two domains: an extracellular binding domain which is complementary to the shape of glucagon and an intracellular binding domain which is coupled to a G-protein.

Signal Transduction

- Upon binding of glucagon to the extracellular domain of the glucagon receptor, **conformational changes** occur in the **intracellular domain** of the transmembrane receptor leading to the replacement of a GDP molecule with a GTP molecule on the G protein, thus activating it.
- The activated G protein activates the next enzyme in the cascade, adenylyl cyclase. The activated adenylyl cyclase will then convert intracellular ATP into cAMP.
- This results in an increase in cAMP levels which also serves to amplify the glucagon signal.
- cAMP will then activate protein kinase A (PKA) which is a cAMP-dependent protein kinase. Activated PKA in turn, activates phosphorylase kinase, which, in turn, phosphorylates glycogen phosphorylase, converting it into the active form.
- The activated PKA will also inactivate glycogen synthase enzyme.

Cellular Response

- The activated glycogen phosphorylase will function to catalyze the breakdown of glycogen to produce glucose-1-phosphate. The glucose-1-phosphate will be converted into glucose within the cell before it is released into the bloodstream.
- Since the activated PKA will inactivate glycogen synthase, glycogen production will be inhibited as a result.

2. Insulin and RTK signalling

Insulin is the major hormone controlling critical energy functions such as glucose and lipid metabolism. Insulin functions to increase the permeability of muscle and liver cells to increase glucose uptake. It also stimulates glycogenesis, lipid and protein metabolism as well as many other cellular metabolic activities. The net effect of insulin signalling is to decrease the blood glucose levels.

Ligand - Receptor interaction

The insulin receptor is a RTK that exists as a **linked dimer**. Insulin binds to the extracellular domain causing a conformational change in the intracellular domain.

Signal Transduction

- This change in conformation of the intracellular domain leads to the cross-phosphorylation of the specific tyrosine residues on the receptor.
- The activated insulin receptor tyrosine kinases can phosphorylate and recruit different intracellular signal proteins which can activate different intracellular pathways.

Cellular response

Cellular responses of insulin include induction of glycogen synthesis via activated glycogen synthase and stimulation of glucose uptake in muscle and adipose tissues via translocation of vesicles containing GLUT4 transporters to the plasma membrane.

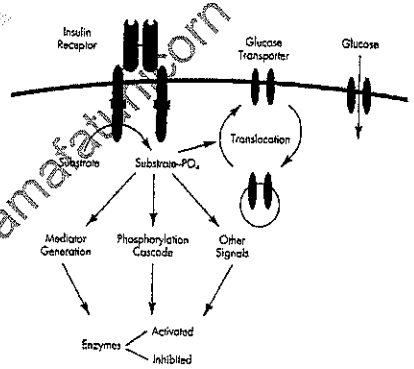


Fig. 18. Insulin signalling pathway

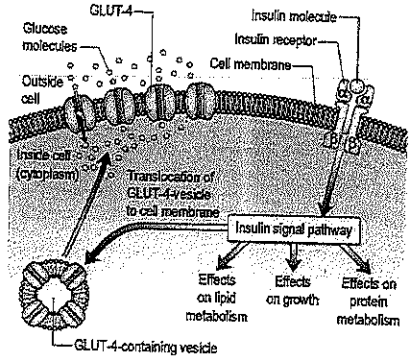


Fig. 19. Translocation of vesicles containing glucose transporters, GLUT-4 in response to the insulin signal.

Part F. Link

Cell Signalling and Cancer

Cancer may result from defects in critical signalling molecules that regulate many cell properties, including cell proliferation, differentiation and survival.

Oncogenes are proto-oncogenes that are mutated or abnormally expressed during cancer development. Oncoproteins, the product of the expression of the oncogenes, are in many cases, signal transduction proteins that normally function in the regulation of cellular proliferation.

Examples of signalling molecules that can become oncogenic span the entire signal transduction pathway. E.g. Ligands (growth factors), receptors, adapter and effector molecules and transcription factors.

Ras protein signalling pathway

One of the most common intracellular signaling pathways triggered by RTKs is known as the **mitogen-activated protein (MAP) kinase cascade.** The pathway starts with the activation of **Ras**, a small G protein anchored to the plasma membrane. In its inactive state, Ras is bound to GDP. However, the activated tyrosine residues on the RTK will in turn activate an adaptor protein. This activated adaptor protein will cause Ras to bind to GTP in place of GDP and become active.
Next, the GTP-bound Ras activates other intracellular signalling protein in the MAP kinase cascade. Then, the final enzyme in the signal transduction pathway phosphorylates transcription regulators, leading to a change in gene expression levels including the cyclin D gene. The cyclin D gene stimulates the progression of the cell cycle into the S phase and through the remainder of the cell cycle.

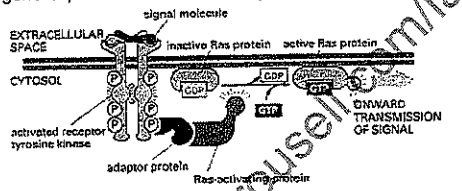


Fig. 19. Ras protein signalling pathway.

A mutation in the Ras gene can result in the Ras protein becoming oncogenic. This mutation causes the Ras protein to lose its ability to convert GTP to GDP, thereby inactivating the protein once the signal has been transmitted. Therefore, the Ras protein remains in the active state even with no extracellular signal molecule binding to the RTK receptor. The Ras protein then sends continuous signals to keep the cell cycle running with no checks and balances. The result is excessive cell proliferation and cancer.

1. INTRODUCTION TO STEM CELLS

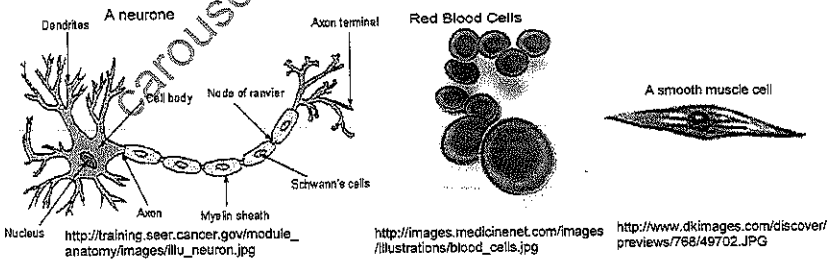
- In order for a multicellular organism to function properly, it must diversify cell types, control their production, and eliminate aged/damaged cells.
- Differentiation is a process by which a less specialised cell develops tissue-specific adaptation that enables it to become a more specialised cell type.
  - Many differentiated cell types have limited life spans. Disease can also lead to their loss. Since differentiated cells **do not usually divide**, their supply must be replenished in some ways.

- Stem cells
  - are a group of ***undifferentiated**** and ***unspecialised**** cells that are capable of ***self- renewal**** through mitosis.
  - can **differentiate into various specialised cell types** with specialised functions upon **stimulation** by the appropriate ***molecular signals****.
  - supply all the differentiated cells that construct tissues and organs from a fertilised egg. In adults, stem cells also replenish worn out or damaged cells.

- Therefore, stem cells are important **in development, cellular regeneration and repair.**

1.1 Unique Features of Stem Cells

- Stem cells are unique cells because they
  (a) are ***undifferentiated**** and ***unspecialised**** cells
  - Specialised cells in tissues are differentiated and have tissue-specific structures that allow them to be adapted to perform unique roles.
  - E.g. of specialised cells include red blood cells, muscle cells and nerve cells. (Fig. 1)
  - Stem cells are undifferentiated and unspecialised cells. This means that they do not have any **tissue-specific structures** (e.g. haemoglobin, myosin) for them to perform tissue-specific functions.



http://training.seer.cancer.gov/module_anatomy/images/illu_neuron.jpg
http://images.medicinenet.com/images/illustrations/blood_cells.jpg
http://www.dkimages.com/discover/previews/768/49702.JPG

Figure 1: Examples of specialised cells. Nerve cell with voltage-gated ion channels and synaptic knobs. Adult red blood cells with haemoglobin and no nucleus. Smooth muscle cell with extensive actin and myosin filaments. These structures help them perform their tissue-specific roles.

(b) can undergo ***extensive proliferation**** and ***self-renewal****

- A stem cell is capable of **dividing** and **renewing** itself many times by **mitosis**.
- There 2 possible types of cell division:
  - (i) **symmetrical division** (Fig. 2)
    - ➢ The stem cell divides to produce **2 daughter stem cells** that possess the **same developmental** and **differentiation potential** as the parent cell.
    - ➢ Both daughter stem cells have same characteristics as the parental cell (e.g. shape, behaviour, responses to molecular signals etc).
    - ➢ This enlarges the population of undifferentiated cells, **maintains a pool of stem cells for further division.**

  - (ii) **asymmetrical division** (Fig. 2)
    - ➢ The stem cell divides to produce 1 daughter stem cell that is identical to the parental stem cell and 1 progenitor[1] daughter cell.
    - ➢ Asymmetrical division occurs when the stem cell is stimulated by ***molecular signals**** for differentiation.
    - ➢ Hence the stem cell divides to produce:
      1. **1 daughter stem cell -** to ensure a **constant pool** of stem cells
      2. **1 progenitor daughter cell -** to **increase or renew** population of **specialised cells in a specific tissue.** The progenitor daughter cell is only capable of differentiating into related specialised cell type.
         E.g. progenitor daughter cells of hematopoietic (blood-forming) stem cells can only differentiate into various blood cells (e.g. white blood cells, red blood cells etc.) **(Fig. 3)**. This replenishes cells that have a finite life span, such as red blood cells.

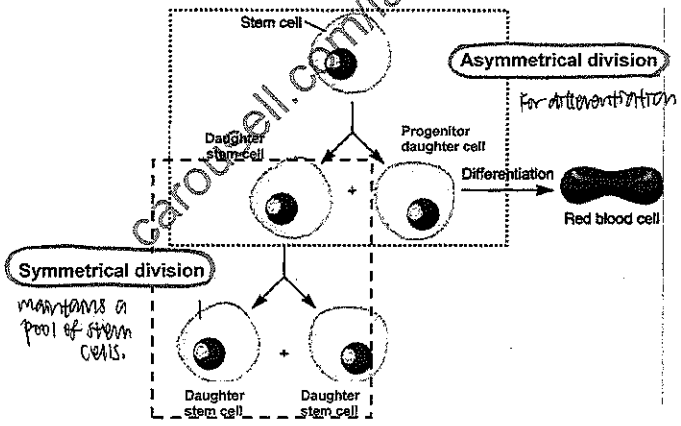


Figure 2: Asymmetrical and symmetrical divisions. In both scenarios, proliferation of cells (seen as an increase in number from 1 to 2 cells) occur. Developmental potential is maintained (where at least 1 of the daughter cells is a stem cell). Stimulus for each remains unclear.

[1] Progenitor daughter cell is a partially specialised cell whose potential fate is more restricted that the stem cell and can specialise into a smaller subset of cell types.

Q: What happens if a stem cell division resulted in two progenitor cells all the time?

There will no longer be self-renewal & all stem cells will eventually be depleted.

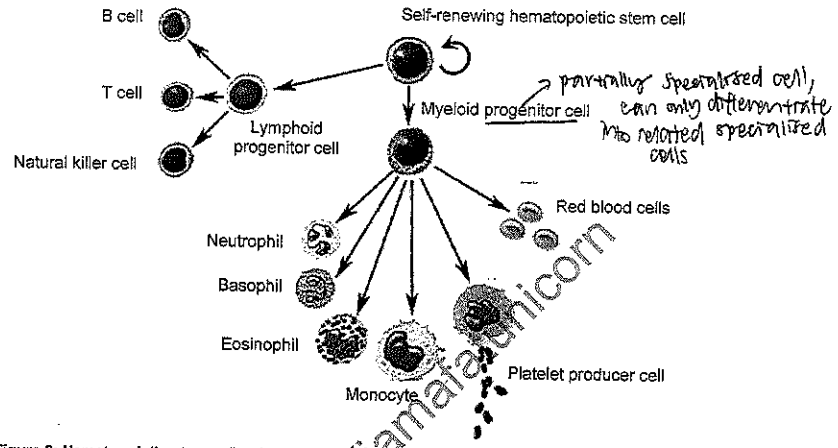


Figure 3: Hematopoietic stem cells gives rise to a variety of specialised cells (e.g.: B cell, red blood cell) but is restricted to blood cells only.

(c) are able to ***differentiate**** to produce specialised cells upon receiving appropriate molecular signals

- These molecular signals switch some genes on, and others off.
  E.g. molecular signals: transcription factors, growth factors, hormones, cell-cell signals, properties of neighbouring cells.

Q: What is the difference between the genome of a stem cell and a specialised cell? What accounts for the difference in characteristics?

There is no difference between the genome of a stem cell & a specialised cell. The difference in characteristics is as a result of differential gene expression

Q: Suggest how stem cells are able to differentiate into specialised cells upon receiving molecular signals such as hormones or growth factors.

Molecular signal may activate or shut down expression of specific genes that result in synthesis of tissue-specific structures that enable these specialised cells to perform certain functions.

1.2 Type of Stem Cells and their Differentiation Potential

In mammals, stem cells are commonly categorised according to their developmental stage and their ability to differentiate.

Type of Stem Cell / Differentiation Potential	Characteristics	Examples and Sources
Totipotent	Has the ability to ***differentiate**** into **all of the cell types** that make up an organism **including *extra-embryonic tissue**** such as ***placenta****, which nourishes the embryo. Via multiple cellular divisions, has the ability to give rise to an entire organism. ***Totipotent**** cells are said to be also ***pluripotent**** and ***multipotent****.	***Zygotic stem cell**** that is derived from a **fertilised egg, zygote.** Cells that are **produced within the first 3 division (*8 cell stage**)** after the egg is fertilised. (see **Fig. 4**)
Pluripotent	Has the ability to ***differentiate**** **into all of the cell types** that make up an organism **except *extraembryonic tissue**** such as the ***placenta****. ***Pluripotent**** stem cells alone **cannot form the entire organism** as extraembryonic tissues such as the placenta is required for foetal nourishment and development. ***Pluripotent**** cells are also said to be ***multipotent****.	***Embryonic stem cells**** (ES) are derived from **cells of *inner cell mass of blastocyst**** (200-300 cells) at about 4 to 5 days post fertilisation. (see **Fig. 4**)
Multipotent	Has the ability to ***differentiate**** **into several related cell types** but far **fewer types** than the pluripotent embryonic stem cell.	**Blood/ haematopoietic stem cells**, which are found primarily in **bone marrow**, can differentiate to all type of blood cells, including red blood cells, white blood cells, and platelets, but not other cell types such as kidney cells. **Neural stem cells** can differentiate into nerve cells and neural support cells called glial cells.

1.3 Source of Stem Cells for Research and Therapy

- Two types of stem cells are commonly studied and used in stem cell research - embryonic stem cells (ES cells) and adult stem cells.
  - (a) Embryonic stem cells (ES cells)
    - After fertilisation, the early embryo (no longer called a zygote after first division) moves down the fallopian tube. As the embryo moves to the uterus, it divides by mitosis.
    - After day 4/5 following fertilisation, a ball of cells (consist of 200-300 cells) known as **blastocyst** is formed (Fig. 4).

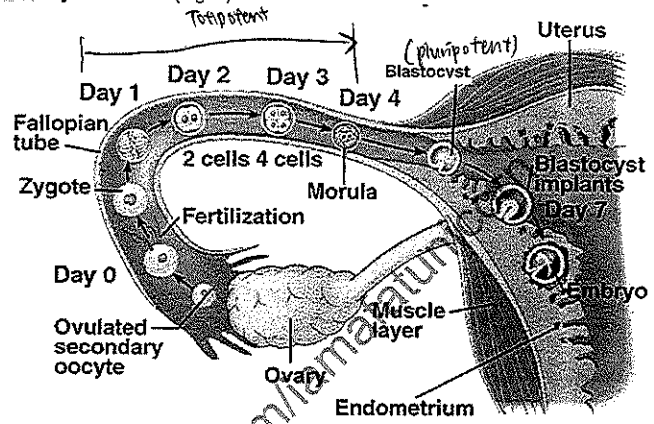


Figure 4: Stages of development of fertilised egg in human female. It eventually develops into a foetus.

- A blastocyst consists of inner cell mass, trophoblast and blastocyst cavity.
- The ***inner cell mass**** of the blastocyst will eventually develop into the foetus while ***trophoblast**** will develop into placenta and other supporting tissues. The inner cell mass alone **cannot** form an entire, intact organism. The trophoblast which gives rise to the placenta and supporting tissues (these are critical for development of the foetus) must also be present. (Fig. 5A)

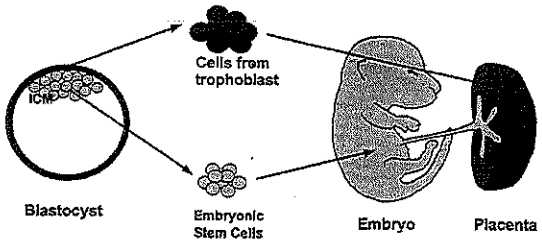


Figure 5A: The inner cell mass by itself cannot form the whole organism as the embryo cannot survive without the placenta.

- Therefore cells of the inner cell mass are not totipotent, but pluripotent.

- For stem cell studies, ***embryonic stem cells**** have to be isolated from inner cell mass of ***blastocyst****, which are subsequently cultured in the laboratory. (Fig. 5B, 6)

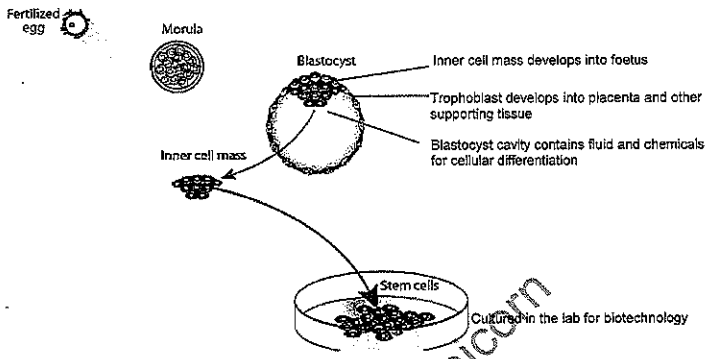


Figure 5B: A blastocyst is made up of 3 parts. It is the inner cell mass that is used to generate stem cells needed for science research.

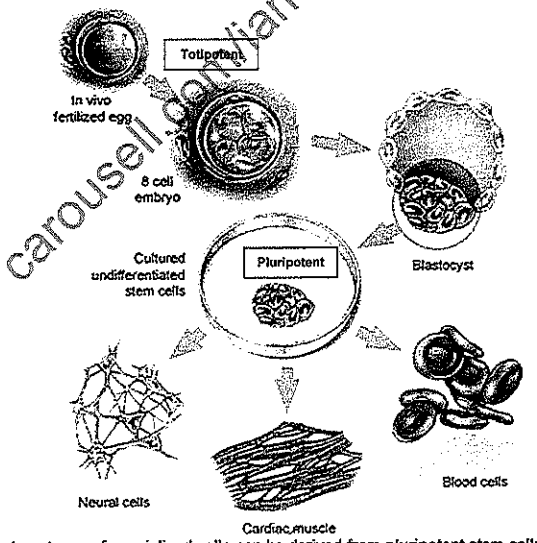


Figure 6: Various types of specialised cells can be derived from pluripotent stem cells; taken from inner cell mass of a blastocyst. http://www.stemcellresearchfoundation.org/WhatsNew/Pluripotent.htm

(b) Adult/somatic Stem Cells

- As the embryo continues to divide by mitosis, its cells become increasingly specialised.
- Embryonic stem cells ***proliferate**** and ***differentiate**** into ***adult stem cells**** (e.g blood/haematopoietic stem cells, neural stem cells).
- These adult stem cells are found in **different tissues** of the organism **after embryonic development**.
- They are found in small numbers (1 in 10,000 – 15,000) in diverse tissues such as bone marrow, blood, cornea and retina, teeth, intestine, liver, muscles, nervous system and brain, pancreas and skin. (These properties can make them difficult to harvest e.g. some organs are less accessible than others).
- Adult stem cells are multipotent and therefore can only **differentiate into several related specialised cell types.**
- Their main purpose is to produce **specialised cells for growth and development,** and for **regeneration/replacement of cells** that are lost due to **cell death and injury.**

Q: Adult stem cells are found only in adults – true or false?

False - Adult stem cells are not confined to adults who are 21 years of age! Infants have them as well.

Q: What is the differentiation potential of umbilical cord stem cells?

multipotent cells.

1.4 Embryonic Stem Cell Differentiation Process (general)

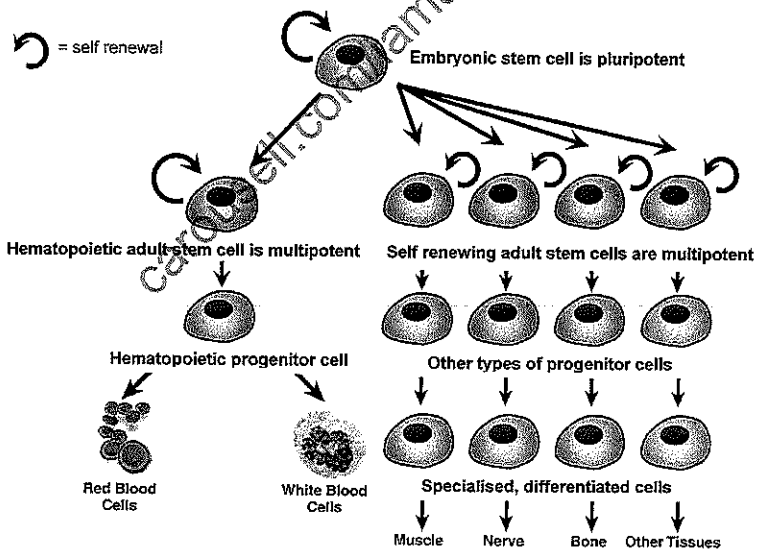


Figure 7: Adult stem cell differentiation process is similar to embryonic stem cells, except that the variety of specialised cells generated is smaller. E.g. A hematopoietic stem cell can generate a variety of blood cells, but not nerve cells or other types of cells. This is because adult stem cells are not pluripotent, but multipotent. Progenitor cells are more differentiated than stem cells and cannot renew themselves.

The diagram in Fig. 7 shows that progenitor cells are early descendants of stem cells. Unlike some sources which consider them to be the same as adult stem cells, the International Society for Stem Cell Research (ISSCR) defined them differently:

*"A progenitor cell, often confused with stem cell, is an early descendant of a stem cell that can **only differentiate, but it cannot renew itself** anymore. In contrast, a stem cell can renew itself (make more stem cells by cell division) or it can differentiate (divide and with each cell division evolve more and more into different types of cells). A progenitor cell is often more limited in the kinds of cells it can become than a stem cell. (**but some can be still be multipotent**) In scientific terms, it is said that progenitor cells are more differentiated than stem cells."*

Q: Do plants have stem cells?
Yes. They are located in the meristem

2. Normal functions of stem cells in a living organism.

- Normal functions of stem cells in a living organism are:
  (a) ***self-renewal**** to ensure a constant pool of stem cells,
  (b) ***growth and development****,
  (c) ***differentiate**** into ***specialised cells**** to ***regenerate****/replace cells that are lost due to cell death and injury.

- Stem cells found in a juvenile or adult animal is usually multipotent adult stem cells.
- The following tissues and organs in a human shows specific examples of the functions of adult stem cells:

✓spell correctly!! haematopoietic

2.1 Blood/haematopoietic stem cell in the bone marrow

- Blood is a tissue. It contains many different types of specialised cells, each of which has a different function: (**Fig. 3**)
  (a) red blood cells – transport oxygen
  (b) various types of white blood cells (e.g. lymphocytes, monocyte) – fight infection
  (c) platelets – blood clotting

- On average, the life span of red blood cells and white blood cells are about 112 days and 3-4 days, respectively. Haematopoietic stem cells constantly proliferate and differentiate into various specialised blood cell types to replace the blood cells lost through normal cell death. This maintains the immune and transport function of blood.

2.2 Neural stems cells in central nervous system (brain, spinal cord, nerves etc.)

- Neural stem cells from our central nervous system (CNS) can proliferate and differentiate into both neurons and supporting cells (called glial cells) to replace those that died (**Fig. 8**).
- The discovery of neural stem cells in mammals brings hope to sufferers of neurodegenerative diseases like Parkinson disease.
- Ageing appears to slow down the proliferation of neural stem cells.

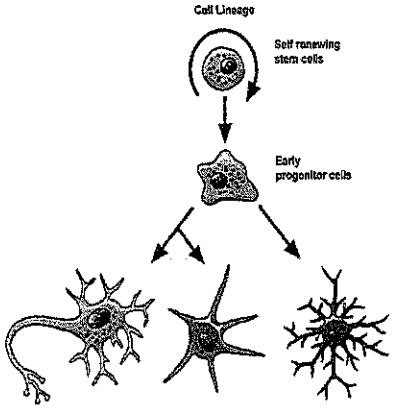


Figure 8: Progenitor cells are intermediates from stem cells - they are not capable of self-renewal but will undergo division to eventually yield specialised cells.

3. Treatments involving stem cells

- Multipotent and self-renewing nature of adult stem cells has been harnessed to provide therapy and treatment to a range of diseases. The use of embryonic stem cells remains controversial. Stem cell therapy has general advantages:
  (a) multipotent nature of adult stem cells ensure that the adult stem cells differentiate into the respective specialised cell type, thus restoring function of damaged or diseased tissue;
  (b) self-renewing nature of stem cells ensures that transplanted stem cells constantly replicate* in the patient to maintain a constant pool of stem cells (hence repeated stem cell transplants are not necessary to sustain the therapeutic effects).

- Two general approaches to stem cell therapies:

3.1 Stem cell transplant in regenerative medicine

(a) Bone marrow haematopoietic stem cells transplants (from normal healthy bone marrow donors) to leukaemia patients.
  - Leukaemia is a type of cancer of the blood or bone marrow characterised by abnormal increase in the number of immature white blood cells called blast cells. These abnormal immature blast cells are not functional, frequently crowd the bone marrow and prevent the production of functional white blood cells.
  - The treatment of choice is a **bone marrow haematopoietic stem cell transplant.** The patient is first irradiated to **remove** all existing haematopoietic cells and white blood cells from the body.
  - Bone marrow haematopoietic stem cells from **normal, healthy bone marrow donors** are multiplied, and **infused** into the patient. These transplanted haematopoietic stem cells will then populate the bone marrow and **differentiate** into normal blood cells. This treatment thus **restores function of blood.** As stem cells are capable of self-renewal, the stem cells will replicate and ensure a **constant pool** of stems cells which will differentiate into the various blood cells, **without the need for repeated** transplants.

Leukaemia

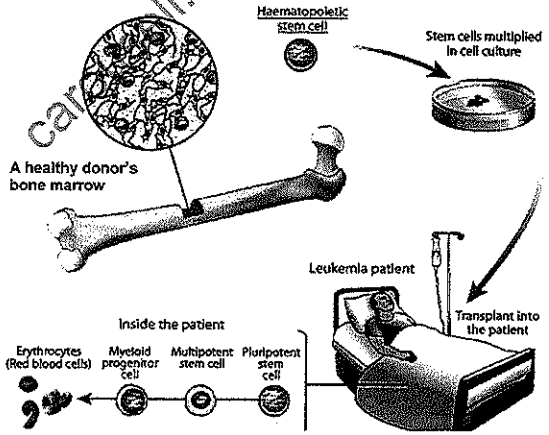


Figure 9: Stem cells from a healthy donor. The stem cells, are first cultured *in vitro* to increase their numbers. If successful, they produce a large number of red and white blood cells, to help the patient recover.

(b) Neural stem cell transplant for neurological disorders
  - Many neurological disorders such as Parkinson's disease and multiple sclerosis are caused by damage to neurones and nervous tissues.
  - Stem cell therapy involves introducing adult neural stem cells into damaged tissue in the hope that the introduced adult neural stem cells will **proliferate** and **differentiate** into neuron or nervous tissue type that is destroyed/ damaged.

3.2 Genetically modified stem cell transplant in gene therapy of genetic diseases.

- This strategy is used together with gene therapy to treat **genetic diseases.**
- It involves **removing stem cells from the patient,** genetically modifying the genome of the cells using gene therapy techniques to **insert** a ***normal, functional allele**** and then **reintroducing** modified stem cells back into the patient.
- The genetically modified stem cells are capable of **self-renewal,** and thus will **proliferate** to form more genetically modified stem cells that contain the therapeutic gene. These genetically modified stem cells will **differentiate** into specialised cell which **restores the functions** of the diseased tissues.
- This method is often used to treat blood genetic disorders.

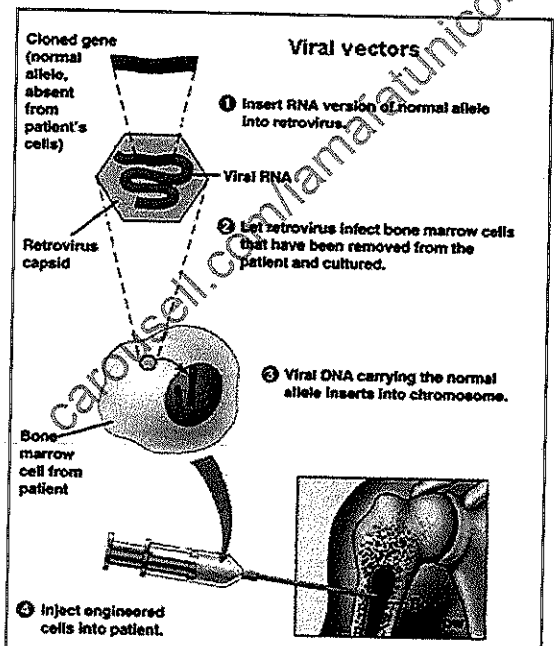


Figure 10: Stem cells (labelled as bone marrow cells) from a patient can be removed and genetically modified, before introducing it into the patient. This method takes advantage of the stem cell's ability to keep dividing and to differentiate into the specialised cells needed by the patient. A virus that cannot cause disease is used to deliver the therapeutic gene.

Other uses of stem cell research?
Ability of stem cells to self-renew extensively and to differentiate into specialised cells put them on the frontline of **developmental biology**[2] and regenerative medicine. Their unique properties are central to early development, which is when tissues are formed, and they are also required in the maintenance of the tissues in an adult.

Currently, search for **molecular signals** that cause stem cells to differentiate into specific cell types is an active area of research. This will allow us to understand the complex signaling events that occur during human development, and also if one can control and direct stem cell differentiation in a laboratory.

Why is the study of stem cells controversial?
Controversy does not lie with use of adult stem cells but embryonic stem cells. Harvest of embryonic stem cells involves usage and destruction of human embryo. Such techniques are considered by some to violate the sanctity of life and are tantamount to murder.

What are the latest updates in stem cell research?
It was once believed that an adult stem cell can only produce the specialised cell types of the tissue where the stem cell is found. However, recent evidence reported **plasticity**[3] of adult stem cells.

In an experiment, the population of hematopoietic cells in a mouse had been depleted by irradiation. When neural stem cells were injected into its bone marrow, the neural stem cells develop into hematopoietic cells. These results suggest
(a) importance of signaling cues from physiological environment in determining type of cell an adult stem cell will differentiate into. It is the same with embryonic stem cells.
(b) possibility to circumvent any ethical concerns in using embryos.
(c) that using stem cells from an adult patient will avoid any immune rejection because the transplanted cells are from the patient himself.

Researchers are also discovering that tumours can arise from cancer stem cells. These cancer stem cells (CSCs) divide and proliferate to sustain the growth of the tumour. It is believed that despite the clonal origin of many cancers, a common characteristic of primary tumors is cellular heterogeneity[4], which is probably the result of differentiation of these cancer stem cells.

Conventional chemotherapy destroys differentiated or differentiating cells, which form the bulk of the tumour. These cells are unable to generate new cancer cells because of their differentiated nature. However the surviving population of cancer stem cells, which exhibits self-renewal property of stem cells could cause a relapse of the disease.

Some believed that cancer stem cells arise from malfunctioned adult stem cells. Future cancer therapies will have to target these CSCs.

Recently scientists have been able to induce adult somatic cells to develop pluripotency through the "forced" expression of specific genes. These are called induced pluripotent stem cells or iPS cells or iPSCs. They are similar to natural pluripotent stem cells, such as embryonic stem (ES) cells, in many respects, such as the expression of certain stem cell genes and proteins but the full extent of their relationship to natural pluripotent stem cells is still being assessed.

[2] Developmental biology is the study of the process by which organisms grow and develop.
[3] A phenomenon used to describe a cell that is capable of becoming a specialised cell type of a *different* tissue.
[4] Existence of different type of cells, as opposed to just one kind i.e. clones

4. Ethical implications of application of stem cells

Introduction to Bioethics
Ethics is a field of study that looks at the moral basis of human behavior ("Why do we act as we do?") and attempts to determine the best course of action in the face of conflicting choices ("How do we decide what to do when people disagree about a complex issue?"). It is a key component of living within a society in a civilised way.

Bioethics is a subfield of ethics that explores ethical questions related to the life sciences. Bioethical analysis helps people make decisions about their behavior and about policy questions that governments, organisations, and communities must face when they consider how best to use new biomedical knowledge and innovations. Examples are:

"How should we decide who receives organ transplants?" or, "Should a terminally ill patient be allowed to end his/her life with physician-prescribed medication?"

Principles of Bioethics
This sets the foundation for the ***Principles of Bioethics: Respect for Persons, Maximise Benefits/ Minimise Harms, and Justice***

1. ***Respect for Persons*** (Respecting the inherent worth of an individual and their autonomy)
   Respect for Persons emphasizes the inherent worth and dignity of each individual, and acknowledges a person's right to make his or her own choices. It means not treating people as a means to an end.

2. ***Maximise Benefits/Minimise Harms*** (Beneficence/Nonmaleficence–non-harming / inflicting least harm)
   Maximising Benefits and Minimising Harms asks how we can do the most good and the least amount of harm. It considers how one would directly help others and act in their best interests, while "doing no harm."

3. **Justice** (Being Fair)
   Justice considers how we can treat people fairly and equitably. It involves the sharing of resources, risks, and costs according to what is "due" to each person.

   In addition to the Principles of Bioethics introduced here, ethicists use a number of different ethical perspectives and theories to defend their positions, including:
   - Moral Rules and Duties
   - Virtues
   - Outcomes
   - Care

Ethical Considerations Regarding Stem Cell Research

(v) Discuss the ethical implications of the application of stem cells in research and medical applications and how human induced pluripotent stem cells (iPSCs) overcome some of these issues (procedural details of how iPSCs are formed are not required).

Potential use of stem cells in therapy

I. Adult stem cell

Adult stem cells are obtained directly from the organ or tissue in which they are found. They can be used to investigate questions around cell differentiation that are specific to a certain tissue. Adult stem cells have provided many different therapies for illnesses such as Parkinson's disease, leukemia, multiple sclerosis, lupus, sickle-cell anemia, and heart damage. Currently there are some effective treatments that use adult stem cells from donors but these treatments carry a risk that donated cells will be rejected.
However, adult stem cells are also limited to differentiating into different cell types of their tissue of origin.

II. Embryonic stem cell

Embryonic stem cells (ES cell) are pluripotent and have the ability to differentiate into any type of cell except the extra embryonic tissues (e.g. placenta). They are used in the development of medical treatments for a wide range of conditions. Treatments that have been proposed include treatment for physical trauma, degenerative conditions (e.g. Parkinson's disease), and genetic diseases (in combination with gene therapy). Further treatments using ES cells could potentially be developed due to their ability to repair extensive tissue damage and develop organs to replace those lost in injury or disease.

Argument against using embryonic stem cells

(a) Some assert that the embryo has the status of a human being as it has the potential to become one. They believe that embryonic stem cell research violates the sanctity of life and is tantamount to murder.

(b) Some object to extracting stem cells from an embryo to make replacement body cells is treating the embryo as just a source of spare parts. Embryonic stem cell research takes a purely utilitarian view of the embryo.

(c) Justice and equity :
- Adult stem cell treatment is established and there are fewer ethical issues involved. Thus adult stem cell research may be able to make greater advances if more money and resources were channeled into it instead of embryonic stem cell research.
- As embryonic stem cell research is expensive, funds can be channeled to treat other more treatable diseases.

(d) Claims of the benefits of embryonic stem cell research are over-rated / few (if any) examples of success in medical applications.

(e) Current benign applications may lead to abuse in the future. Once human status is denied to embryos, this precedent may extend to other categories of human beings such as the profoundly disabled or the elderly infirm.

(f) Possibility of unforeseen consequences in treated patients such as possible risks of tumor formation, immunological reactions, unexpected behavior of the cells, and unknown long-term health effects.

(g) Unnecessary because alternative techniques such as adult stem cells, from sources such as umbilical cord blood, have already produced some results.

(h) Donation and consent - For those people involved in donating eggs, embryos or tissues, there are issues of informed consent, understanding of research aims and privacy.

Argument for using embryonic stem cells

(a) Embryonic stem cells can potentially treat a wide range of diseases as they have the potential to grow indefinitely in a laboratory environment and can differentiate into almost all types of bodily tissue. Treatments that have been proposed include treatment for physical trauma, degenerative conditions (e.g. Parkinson's disease), and genetic diseases (in combination with gene therapy).

(b) Embryos are not equivalent to human life:
- Embryos are not conscious, cannot feel and cannot survive outside the womb.
- Blastocysts are a cluster of human cells that have not differentiated into distinct organ tissue, making cells of the inner cell mass no more "human" than a skin cell.
- Some believe life only begins when the heartbeat develops (during the fifth week of pregnancy) or when the brain begins developing (at 54 days after conception).

(c) There is legislation on the period when ES cells can be extracted.
Eg: Current UK legislation does not allow use of embryos that are more than 14 days old. In fact, ES cells are obtained earlier from blastocyst (between 3-8 days after fertilisation).

(d) More than a third of zygotes do not implant after conception. Thus, far more embryos are lost due to chance than are proposed to be used for embryonic stem cell research.

(e) Surplus embryos created via *in vitro* fertility treatments are destroyed, or stored for long periods of time, long past their viable storage life. These can be used for creating new stem cell lines for research which would otherwise be destroyed.

(f) Instead of destroying new human embryos to establish new stem cell lines, there are existing stem cell lines that can be used.

Embryonic stem cell vs Adult stem cell

	Embryonic stem cells	Adult stem cells
Differentiation ability	Pluripotent: capable of becoming any cell in the human body except extraembryonic tissue e.g. placenta.	Multipotent: typically only give rise to the cells of the tissue in which they are found.
Role in body	To develop the embryo into an entire human.	To replace specific cells in the body which die throughout life due to wear and tear or injury and disease.
Sources	Unused IVF embryos which have been donated, or embryos created for the purpose from donated eggs and sperm.	Bone marrow, muscles and skin; and from the fetus, umbilical cord, placenta and amniotic fluid.
Advantages in research and therapy development	▪ Embryonic stem cells make up a significant proportion of a developing embryo and are easier to isolate and grow *ex vivo* than adult stem cells ▪ Have a strong ability to self-renew in the laboratory and divide more rapidly than adult stem cells, resulting in a constant supply of ES cells. ▪ Pluripotency means that ES cells have the potential to produce any cell type in the body, potentially allowing them to treat a wider range of diseases.	▪ If taken from the patient's own body for use in therapies, cells would be genetically identical to that of the patient, avoiding the problem of immune rejection. ▪ There are less ethical considerations compared with using embryonic stem cells.

	Embryonic stem cells	Adult stem cells
Disadvantages in research and therapy development	▪ Genetically different to cells of potential patients, so immune rejection could occur. ▪ Ethical issues over embryo destruction	▪ They usually only produce a limited number of different cell types. ▪ Conditions supporting self-renewal in the laboratory have only been identified for a few tissue stem cell types, for instance skin and cornea. ▪ Are found in small numbers and are difficult to isolate. ▪ Adult stem cells with genetic mutations from the patient's own body will not be effective in treatment of genetic disorders.

Alternatives to using human embryos

I. Somatic Cell Nuclear Transfer (SCNT)

Somatic cell nuclear replacement is a method that makes 'cloned' embryos from which stem cells could be produced. In SCNT, the nucleus of the fertilised oocyte is removed and the nucleus of a recipient's somatic cell (e.g. skin cell) is transferred into the enucleated oocyte. The somatic nucleus is reprogrammed by the competent cytoplasm and the oocyte (egg) with the donor nucleus starts cleaving and forms a morula that continues to develop into a blastocyst.

Some reasons for being ethical:

- Stem cell lines matched to persons with specific diseases can serve as *in vitro* models of diseases to help in understanding the development of diseases and screen potential new therapies.
- This 'therapeutic cloning' makes cell replacement possible as the body should not reject them since they are genetically matched for a patient.

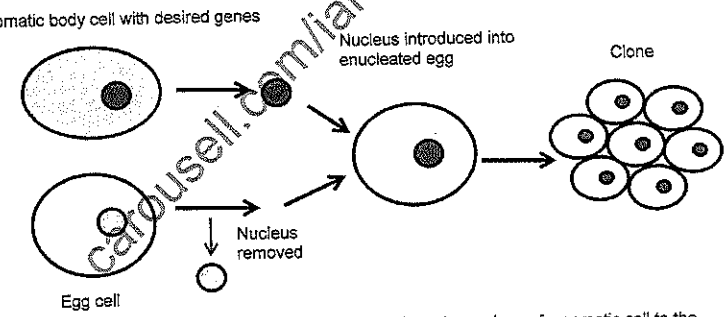


Fig. 1 The SCNT method of transferring the chromosomes from the nucleus of a somatic cell to the enucleated oocyte(egg)

However, creating human SCNT stem cell lines has not only been scientifically impossible to date but is also ethically controversial.

Argument that it is unethical:

(a) Unlike embryos from IVF that are created in surplus, the generation of embryos through SCNT involves the deliberate creation of early human life for the express purpose of destroying and using it.
(b) SCNT also raises ethical concerns regarding the exploitation and endangerment of egg donors.
(c) Furthermore, SCNT is a cloning technique: the embryos created will have a genome identical with some donor human being. As there are widespread ethical and safety objections to reproductive human cloning, SCNT thus opens the door not only to cloned embryos but to the birth of cloned human children.

II. Use of animal oocytes to create SCNT lines using human DNA

Because of the shortage of human oocytes for SCNT research, some scientists wish to use nonhuman oocytes (e.g. from cow) to derive lines using human nuclear DNA. These so-called "cytoplasmic hybrid embryos" raise a number of ethical concerns.

Argument that it is unethical:

(a) As animal DNA is present in the mitochondria of the oocyte, the presence of human and animal DNA means it is partly human and partly cow / a human animal.
(b) Others view such research as an inappropriate crossing of species barriers, which should be an immutable part of natural design.
(c) Some are concerned that there may be attempts to implant these embryos for reproductive purposes.

NB: pts (d) to (g) from Argument against using embryonic stem cells (not ethical) also apply here and SCNT plus (h) for SCNT.

Argument that it is ethical:

(a) The shortage of donated human eggs was holding up stem cell research and potential treatments as it would be unethical not to use cybrids to relieve, human suffering / e.g. diseases such as Parkinson's disease;
(b) the amount of non-human DNA is very small/negligible ;
(c) protocol limits keeping 'embryo' to 14 days (to guard against abuse) so cannot develop into a whole organism ;
(d) provides a more ethical alternative to the use of embryonic stem cells from humans ;
(e) eggs can be harvested repeatedly from the animal donor (e.g. cow) so the process can be repeated to eventually provide many more stem cells. In humans, supply of embryonic stem cells is more limited;
(f) ovum has bovine/other animal proteins which are antigens that would be rejected at implantation ;

Potential solution to overcome some of these issues

Induced pluripotent stem cells (iPSC)

Induced pluripotent stem cells are a type of pluripotent stem cell that can be generated directly from adult somatic cells (e.g. skin cells). The non-pluripotent cell is therefore induced to become pluripotent. The iPSC technology was pioneered by Shinya Yamanaka's lab in 2006 that the introduction of four specific genes encoding transcription factors could 'reprogramme' some specialised cells to become pluripotent so that they lose their specialist functions and behave in virtually the same way as embryonic stem cell.

Advantages of iPSCs/ Argument it is ethical

Induced pluripotent stem cells are perhaps the most prominent alternative source of stem cells proposed for therapy and research. It offers several advantages:

(a) Since iPSCs can be derived directly from adult tissues, it does not generate or destroy any human embryos.
(b) iPSCs can be easily procured from any type of adult/specialised somatic cell (e.g. skin cell) without risk to the donor.
(c) In contrast to ES cells extracted from human embryos, iPSCs derived from a patient's own cells would open the possibility of generating lots of patient-specific cells, which will not be rejected by the immune system upon transplantation.
(d) Further, it also allows the generation of pluripotent stem cell lines from patients with inherited diseases, in order to better understand why the diseases develop and use in personalised drug discovery efforts.
(e) An additional reproductive technology that may be enabled by iPSCs is the generation of sex cells (sperm and eggs) for treating infertility.

Possible problems

- Low efficiency: in general, the conversion to iPSCs has been incredibly low. For example, the rate at which somatic cells were reprogrammed into iPSCs in was 0.01–0.1%.
- Genetic modification of adult cells to obtain iPSCs may cause cancer by overexpression of proto oncogenes or switching off of tumour suppressor genes.
- There are ethical concerns (e.g. lack of consent, long term unexpected consequences) related to the creation of embryos and children from IPSC-derived sex cells.

Summary

Ethical complications are related to the means of procuring stem cells (e.g. techniques involving the destruction of human embryos), human cloning and the exploitation of embryo and egg donors. Alternative methods of obtaining stem cells need to be discussed along with scientific challenges to ensure that stem cell research is carried out in an ethically appropriate manner. The use of iPSCs still appears to overcome many ethical issues and provides viable solutions related to stem cell research.

A set of **four key questions** that can be used to clarify if there is an ethical problem:

1. What is the **ethical question?**
2. What are the **relevant facts?**
3. **Who** or **what** could be **affected by the decision?**
4. What are the **relevant ethical considerations?**
   - **-respect for persons**
   - **-minimizing harms while maximizing benefits,**
   - **-fairness**
   - **-authenticity, responsibility/stewardship, integrity**

RAFFLES INSTITUTION

H2 BIOLOGY LECTURE NOTES

Syllabus 9744

2018 – 2019

4

CORE IDEA 4: BIOLOGICAL EVOLUTION

Topics

1. Evolution I
2. Evolution II

Extension Topics

A. Infectious Diseases
B. Impact of Climate Change on Animals and Plants

Name: **Class:**

CORE IDEA
(4) Biological Evolution

EVOLUTION 1

Content
- The concept of the species
- The neo-Darwinian revolution
- Variation, natural selection and evolution

Learning Outcomes
Candidates should be able to:
(a) Explain why variation (as a result of mutation, meiosis and sexual reproduction) is important in natural selection
(b) Explain, with examples, how environmental factors act as forces of natural selection
(c) Explain the role of natural selection in evolution
(d) Explain why the population is the smallest unit that can evolve
(f) Define biological evolution as descent with modification and explain the link between micro-evolution and macro-evolution
(h) Explain the various concepts of the species (biological, ecological, morphological, genetic and phylogenetic concepts)
(j) Explain how new species are formed with respect to geographical isolation(allopatric speciation) and behavioural or physiological isolation within the same geographical location (sympatric speciation)

Use the knowledge gained in this section in new situations or to solve related problems.

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*** This handout is the effort of several Biology teachers at RI (Yr 5-6). It has and will continue to be updated.**
****Any information given in a double-lined box is for your information only.**

Table of Contents

"It's (evolutionary history) one of the great dramas in the history of Earth"
David F. Attenborough (Born: 1926)

English naturalist

A. Darwin's Theory of Natural Selection, Neo-Darwinism and Evolution

LO:
(f) Define biological evolution as descent with modification and explain the link between micro-evolution and macro-evolution

Darwin's Ideas

- Charles Darwin's ideas were published in "On the Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle for Life" in 1859.
- Darwin used concept of **natural selection** to explain **variation** he observed within and between species. Adaptations, which conferred survival advantages in a given environment, dominate a population, all else being equal.

Natural Selection

- Natural selection is a process in which **individuals that have certain inherited traits tend to survive and reproduce at higher rates than other individuals.** (favourable traits; differential reproductive success)
- Organisms with most favorable adaptations out-compete other organisms in a population and displace them, so only the fittest survive and reproduce i.e. differential reproduction will occur. So, when natural selection takes place over the course of **many generations**, it can change the basic attributes of original population of organisms.

Neo-Darwinism

- Darwin never knew or mentioned anything about genes, alleles of mutations.
- Neo-Darwinism is theory of evolution that represents a combination of Darwin's theory in terms of natural selection with **modern population genetics.** It supports natural selection as the major process that account for biological evolution.

Evolution

(Allele frequency = proportion of alleles of a gene in a population)

- Broadly, evolution is defined as **descent with modification from a common ancestor.**
  1. Modification: Evolution only occurs when there are **changes in allele frequency** within a population over time. (**Allele frequency** is a measure of chances of finding a particular allele of a gene within the population, Fig. 1a)
  2. Descent: Evolution involves passing on of these heritable genetic changes to the next generation.
  3. Common ancestor: Present day species are **related** through a common prehistoric ancestor that have changed and adapted over **time**. Therefore, all species come from preexisting species.

- Evolution can be divided into
  1. **microevolution**
     - is change in allele frequency within a population or species over **shorter periods of time**. It **does not give rise to a new species.**
     e.g.: antibiotic resistance in bacteria, pigmentation of peppered moth
  2. **macroevolution** (occurs beyond species)
     - is gradual changes in allele frequencies usually over **longer periods of time** and may eventually **give rise to a new species.** The defining feature of macroevolution is speciation.
     e.g.: Darwin's Galapagos finches where new species of finches arise from an ancestral finch from South America; mammals and birds diverged from reptiles

- Microevolution and macroevolution are fundamentally identical processes on a different time scale. Both are caused by 5 agents of evolutionary change (see next section).
- Microevolution may lead eventually to macroevolution, given enough time.

	Microevolution	Macroevolution
Scale of changes in allele frequencies	Small scale	Large scale
Time period	Few generations	Thousands/ millions of generations
Level of changes	Within a population/ a species	At/ above level of species
Formation of a new species	No new species is formed	New species is formed
Similarity between ancestors and descendants	Descendant is of the same type as ancestor	Descendants are different from ancestors as new species have emerged
Evidence	From experiments	From data such as fossil records

Table 1: Differences between microevolution and macroevolution

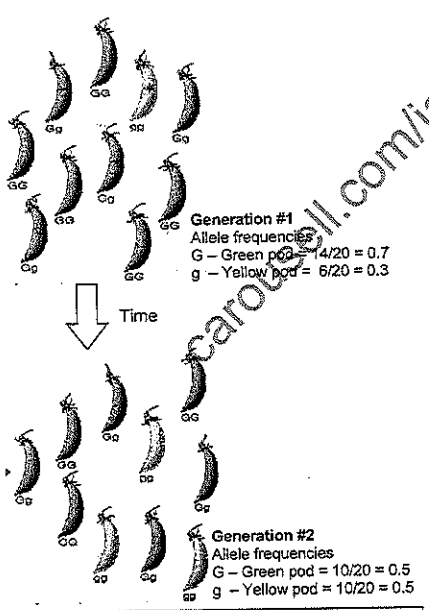


Fig. 1a. Changes in allele frequencies over time. This is an example of microevolution.

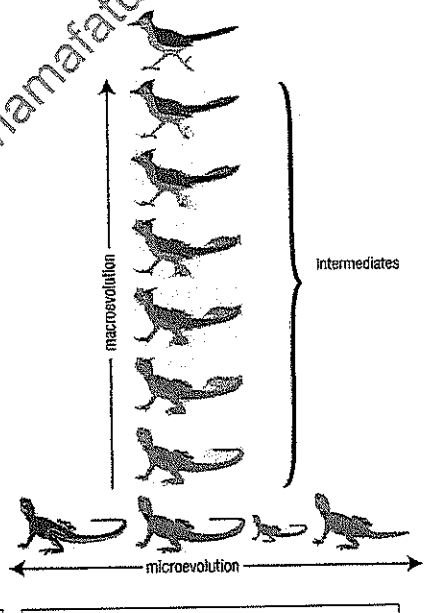


Fig. 1b. Macroevolution vs microevolution.

B. 5 Agents of Evolutionary Change

The 5 agents of evolutionary change are (1) natural selection, (2) disruption of gene flow, (3) genetic drift, (4) nonrandom mating and (5) mutation.

First: Natural selection

- Natural selection results in survival of individuals with most favourable adaptations / inherited characteristics, to a particular environment. This results in differential reproductive success in a population and, over many generations, can change the basic attributes of the population.

LO:
(a) Explain why variation (as a result of mutation, meiosis and sexual reproduction) is important in natural selection (Refer to pt. 4 of the 7 features)
(c) Explain the role of natural selection in evolution. (Refer to all 7 features)

How natural selection may bring about evolution

(1) Overproduction of offspring
- All organisms produce large numbers of offspring. This can lead to an exponential increase in size of any population, if all offspring survive.

(2) Constancy of numbers
- Despite tendency towards overpopulation, most populations remain relatively stable in size.
- This is because only a small fraction of the offspring population survives, matures and reproduces. Majority of offspring will die before reproductive age.

(3) Struggle for survival
- On the basis of points 1 and 2, individuals of a species constantly struggle to survive.
- Competition for finite resources such as food, soil nutrients, water, mates, habitats; and other factors such as disease and predators impose a limit on their number.

(4) Variation within a population (Fig. 2, 3)
- Variation is **differences between individuals of the same species** (due to presence of different alleles).
- It includes morphological, physiological, biochemical and genetic differences.
- It is the raw material for natural selection to act on. If not for variation, selection would not be possible as there would be no differences between individuals of a population.
- Variations arise spontaneously and are not dictated by the need of organisms to survive better in environment. Some variants are less adapted (due to presence of unfavourable alleles) to survive in an environment compared to others. Other variants are better adapted to survive.
- Variation helps to ensure perpetuation of species and hence safeguard species from extinction when environment changes.
- Meiosis and sexual reproduction, with the random fusion of male and female gametes, is a significant **source of variation.** A mating pair can give rise to offspring that are extremely varied. This is in contrast to asexual reproduction where the offspring are genetically identical to the parent.
- Meiosis and random fusion of gametes however **do not add new alleles** to the population gene pool. They merely reshuffle existing alleles among individuals, resulting in differences in phenotypes.

- **Mutations,** however, **introduce new alleles** and enrich the gene pool. Because new alleles are introduced, there will be new phenotypes and hence increased variation.

(5) Survival of the fittest by natural selection

- When **environmental changes** such as a change in climate or an introduction of new predators occur, the variation allows individuals with a **selective advantage (ie. those with favourable alleles that confer an advantageous characteristic to the individuals)** to survive and reproduce more successfully than others.
- These survivors have characteristics that are **selected for / favoured by the environment.** The traits help them adapt better to the new environment.
- Organisms which survive get a chance to produce **viable and fertile offspring.**
- Any cause that reduces reproductive success in a proportion of a population is called **selection pressure.**

(6) Like produces like

- Those which survive breed to produce offspring similar to themselves.
- Advantageous characteristics (determined by **favourable alleles**) are more likely to be passed on to offspring.
- For evolution to take place, the characteristics involved must be heritable.

(7) Formation of a new species over a long period of time

- Unequal ability of individuals to survive and reproduce will lead to a gradual change in a population.
- With each succeeding generation, the proportion of individuals having the advantageous characteristics (determined by favourable alleles) increases while those lacking the characteristics decreases. Favourable characteristics, and hence favourable genotypes accumulate over time, **changing allele frequency.**
- Over hundreds and thousands of **generations** spanning about 200,000 years, a new species may form. In organisms with a very short generation time such as bacteria, new species can be formed relatively faster.

smaller proportions in nxt generations

* neo-darwinism

reasons for variation
1) effect of polygenes (cumulative effects of diff genes)
2) additive effects of multiple alleles
3) ref. to environmental factors
4) mutations led to formation of new alleles result in variation in genotype
5) - crossing over in prophase I
- independent assortment during metaphase I/II
- random fusion of gametes

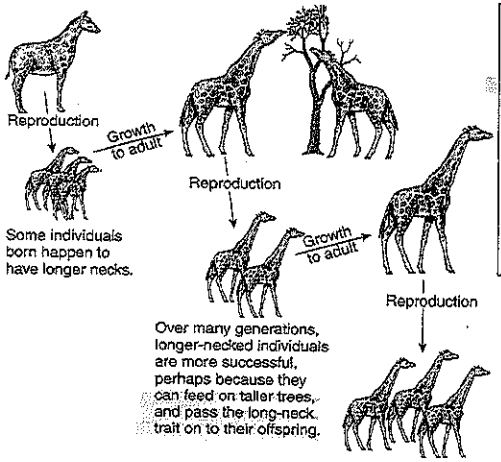


Fig. 2. Darwin's theory.
There is variation in neck length within population of ancestor giraffes. Food availability becomes selection pressure. Those with longer necks are more likely to survive and reproduce, passing on their genes for long necks to the next generation. Succeeding generations show an overall increase in number of individuals with longer necks. Over time, a new species arose.

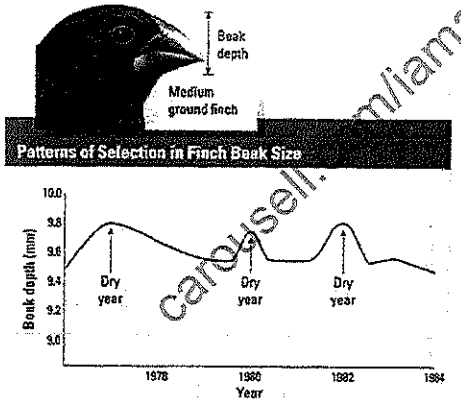


Fig. 3. Natural selection on Darwin's finches.
Graph record shows average beak size from *all* the finches captured over the years.
The medium ground finch, *Geospiza fortis*, feed preferentially on small, tender seeds produced in abundance in wet years. During droughts, plants produced few seeds and all available smaller, softer seeds were quickly eaten, leaving behind larger, harder seeds. The number of small-beaked finches declined correspondingly. Number of larger birds with more powerful beaks which can feed on the larger, harder seeds increases at the same time.

Types of Natural Selection

- Natural selection can affect frequency of a heritable trait in a population in 3 different ways, depending on which phenotypes are favoured (Fig. 4.):

Directional selection	Disruptive selection	Stabilising selection
▪ Phenotype at **one extreme** is repeatedly **selected for.**	▪ **Intermediate phenotypes** are **selected against.**	▪ **Extreme phenotypes** are selected against.
▪ Favours **what are initially relatively rare individuals.**	▪ Favours **individuals on both extremes of a phenotypic range.**	▪ Favours **more common intermediate variants** in a population.
▪ Once the new mean phenotype coincides with new optimum environmental conditions, stabilising selection takes over.	▪ Possible to result in polymorphism, where two or more forms are found in one species.	▪ Does not promote evolutionary change but maintain phenotypic stability with a population over time.
Fig. 4A. Directional selection Melanic form of the peppered moth has selective advantage in polluted areas. Dark colour is due to a melanic allele, whose frequency increases in the population.	**Fig. 4B. Disruptive selection** Snails with pale shells are selected for in dry grasslands whereas those with dark broad bands are favoured in areas with leaf litter.	**Fig. 4C. Stabilising selection** Babies who are heavier than and lighter than optimum birth weight are at a selective disadvantage. Slightly increased mortality works against extremes of birth weight in humans.

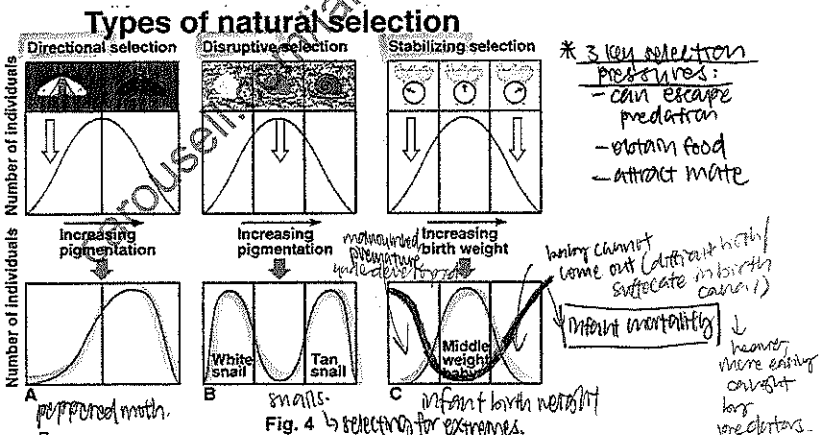


Fig. 4

⇩ Show selection pressure working against certain phenotypes

LO:
(b) Explain, with examples, how environmental factors act as forces of natural selection

Examples of how environmental factors act as forces of natural selection.

- **Selection pressure**: Environmental factors, biotic and abiotic, acting upon survival and reproductive success of members of a population,
  [Biotic factors: living elements of environment such as prey, predators;
  abiotic factors: non-living elements of environment such as soil, pH, climate etc.]

Example 1: Peppered moth

- Two varieties of peppered moths (*Biston betularia*) exists naturally (Fig. 5): lighter form (white with black spots) and melanic form (black with white spots). The black form arose from a spontaneous mutation.
  (Note: it is incorrect to imply that industrialisation caused melanic form of peppered moth to appear.)

- Before 1848, there was a greater proportion of lighter form. They were well-camouflaged from predators on light coloured, lichen-covered tree barks. The lighter form had a **selective advantage** and were better adapted to the environment. Hence, they were **selected for** and increased in frequency.

- With the industrial revolution, the environment changed. Lichens on bark of trees, being pollution -sensitive, were killed. Dark-coloured barks were exposed and soot from industries made the barks appear darker. Lighter form of moth became more visible and easy prey to birds. Lighter forms of moths were **selected against** and their numbers declined. Darker forms of moth however, were well camouflaged, selected for and proliferated.

- Hence individuals with favourable characteristic were more likely to **survive and reproduce** compared to individuals with unfavourable characteristics. Alleles that confer favourable characteristics are passed on the next generation.

- Human-induced change in which darker individuals predominate over lighter ones is known as "industrial melanism".

- Change in numbers of the two types of moths is due to **selection pressure** exerted by predatory birds which can now more easily spot lighter form of moths.

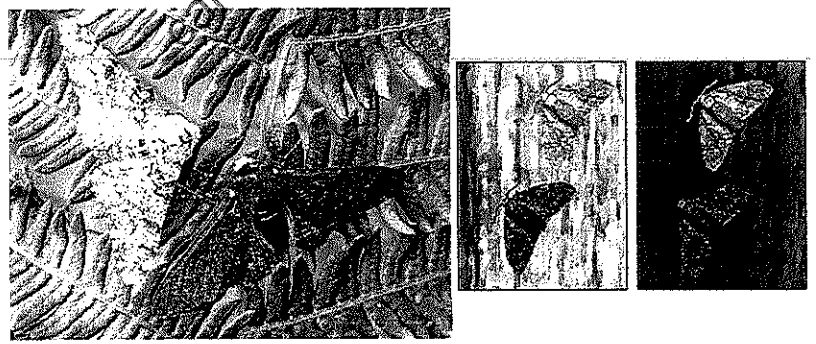


Fig. 5. Variation in the forms of peppered moth.
Biston betularia typica (lighter form) has selective advantage in non-polluted areas compared to *B. betularia carbonaria* (melanic form). This is a case of directional selection.

susceptible resistance

Example 2: Antibiotic resistant bacteria

- Antibiotics are widely used to treat diseases caused by bacterial pathogens. Antibiotic resistance refers to ability of a microorganism to withstand effects of an antibiotic.
- Antibiotic resistant and non-resistant bacterial strains i.e. **variation**, exists naturally. Resistant strains usually arise by spontaneous mutations.
  (Note: it is incorrect to imply that antibiotics caused bacteria to mutate into resistant strains.)
- Antibiotics kill most non-resistant bacteria. Thus non-resistant bacteria are **selected against** when antibiotics are added to the environment.
- Antibiotic resistant bacteria however, have a **selective advantage** in presence of antibiotics. They are best adapted to the environment as they contain antibiotic resistance allele. They are **selected for** and more likely to **survive and reproduce, passing on alleles** for antibiotic resistance to subsequent generations. Frequency of antibiotic resistance allele will increase in the population.
- Hence differential reproductive success occurs due to a **change in environment. Selection pressure** by antibiotics can lead to prevalence of antibiotic resistant bacteria. New antibiotics will now have to be developed to eliminate these resistant bacteria. In fact, new antibiotics will have to be continuously developed as newly-resistant bacteria keep emerging.

Q. Antibiotics are essential in combating bacterial infection. Their use is inevitable. However doctors insist we complete our course to prevent development of antibiotic resistance in the population. Explain how so?

Use of antibiotics → selective pressure.

→ Answer: completing course of antibiotics ensures dosage & sufficient to clear most of susceptible/ non-resistant bacteria → leaving just antibiotic-resistant bacteria for the immune system to deal with.

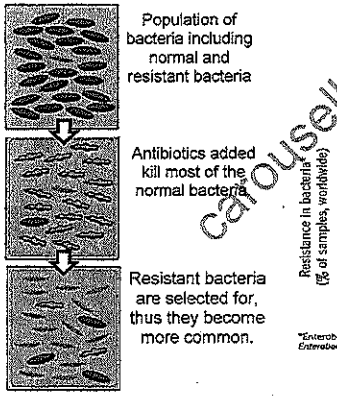


Fig. 6. Selection of antibiotic resistant bacteria.

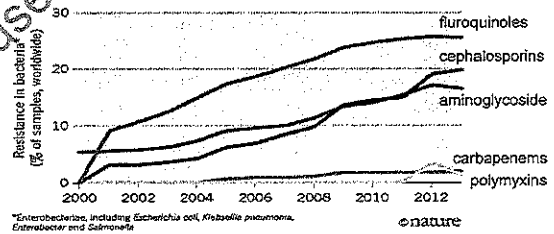


Fig. 7. Worldwide spread of resistance to common antibiotics.

"no interbreeding" [no interbreeding]

Second: Disruption of Gene flow

interbreeding occurs.

- Gene flow is **transfer of alleles from one population to another**. This is achieved through to movement of fertile individuals or their gametes.
  E.g.: An animal arriving from a different population may bring in new alleles, pollen grains blown across vast distances will fertilise plants of destination population.

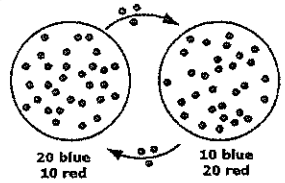


Fig. 8. Gene flow

- Gene flow depends on geographical proximity, mobility of individuals, number of individuals involved, extent of interbreeding and environmental harshness. In nature, individuals commonly migrate into or out of a locality.
- Gene flow tends to **reduce differences in allele frequencies between neighbouring populations.** Populations that mix their gene pools frequently tend to have similar allele frequencies.

– diff genetic changes in each sub population
– changes in allele frequencies in each sub population

- On the other hand, **disruption to gene flow can result in differences in allele frequencies** in two populations over time (see Part D: speciation).

pinky

small population → chance event.

Third: Genetic drift

- A change in allele frequency due to **chance events** - chance because which allele ends up lost from the original gene pool is an indiscriminate, random event. This is different from natural selection as alleles in this case are lost from **random individuals** instead of from individuals with disadvantageous phenotypes.
- Genetic drift tends to **reduce genetic variation** in populations through such losses of alleles. The **smaller** the original population is, the **greater the impact of genetic drift.**
- Founder effect and Bottleneck effect illustrate how genetic drift can affect allele frequency.

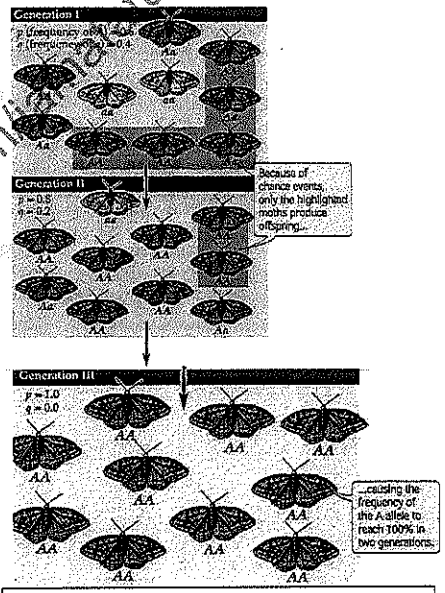


Fig. 9. Genetic drift
Frequency of alleles *A* and *a* are modified in the population of moths because only a sample of moths produces offspring by chance events.

typically on islands

(1) **Founder effect** (Fig. 10) → small no. of individuals leave ancestral populatn

- A **few, random** individuals from a larger population became **pioneers** of a newly isolated population and are **not likely to carry all the alleles present in the source/original population.**

- The new population is usually **small and reproductively isolated**. If there is continuous breeding within this pioneer population, **rare alleles** may become more **common.**
  e.g.: Amish community descended from a few founding individuals in 18th century; Darwin's finches on Galápagos descended from the distant mainland South American finches.

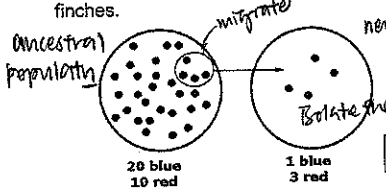


Fig. 10 Founder effect

(2) **Bottleneck effect** (Fig. 11) → small no. of individuals left behind after catastrophe.

- A **catastrophic** event leading to a **drastic reduction in population** results in the reduction of allele frequencies and even elimination of alleles in a random nature.
  e.g. disease epidemic, a natural disaster or any unfavourable environmental change/overhunting

- The **few, random surviving individuals** constitute a random genetic sample of the original pre-catastrophe population. Certain alleles may be **over-represented or under-represented** among the survivors.
  e.g.: European bison were hunted till 12 remained. Even though the population has rebounded to thousands, genetic variation has been lost.

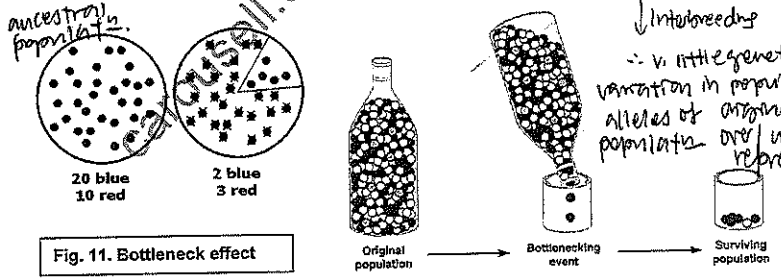


Fig. 11. Bottleneck effect

Q. Discuss if the following are examples of genetic drift.

1. *A global pandemic wipes out 90% of cheetah population.* Y
2. *Hunters hunting mink, for their fur, resulting in the decimation of their population.* Y
3. *Cheetahs hunting Thomson's gazelle.* N
4. *The warming of the ocean resulting in bleaching of coral reefs.* Y
5. *Seasonal flu epidemic resulting in 30,000 worldwide fatalities.* N
6. *Singaporeans setting up home in Kazakhstan.* N
7. *Singaporeans setting up home a deserted island.* Y

Fourth: Nonrandom mating

- In many natural populations, individuals preferentially choose mates. Random mixing of gametes in natural populations therefore **does not occur.**
  e.g.: presence of one or more inherited phenotype improves chances of successful mating. This is also known as sexual selection. This allele may then be more prominent in the next generation.

- Artificial selection, where humans select the breeding pair, is a type of nonrandom mating.

Fifth: Mutation

- A mutation is a random change in an organism's DNA and are a **source of new alleles.**

- For mutations to have an impact on evolution, it must occur in gametes.

Q. What happens if mutations occur in somatic (non-gametic) cells?
The mutations are not inherited & passed on in the subsequent generations.

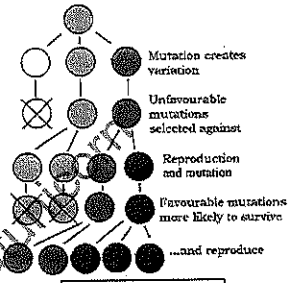


Fig. 12. Mutation

- Mutation rates are generally very low, odds of a gene mutating are in the order of 1 in 100,000 genes per generation. Although mutations at a particular gene locus are **rare, cumulative impact** of mutations at all loci over long periods of time can be **significant.**

- Mutation is unique amongst all the 5 agents of evolutionary change in that it is the only one that results in **formation of new alleles**. It doesn't act directly on allele frequencies.

- Once a **mutation generates a new allele**, evolutionary forces like natural selection can take over and change the allele frequencies.

- Mutations that are deleterious are likely to confer a selective disadvantage. If mutations confer a selective advantage, such individuals survive and produce a disproportionate number of offspring. The mutated allele thus becomes more common.

In Summary:

- There are **5 agents** of evolutionary change. The first 4 factors (natural selection, disruption of gene flow, genetic drift and non-random mating) result in **changes in allele frequencies**.
- **Natural selection** is one of the most powerful drivers of evolutionary change. It requires variation within a population and is influenced by the organism's interaction with environment.
- Disruption of **gene flow** involves isolating populations through various isolating mechanisms (to be discussed later). This allows isolated populations to evolve independently and over time, allele frequencies of the isolated populations will change. This concept is key to explaining speciation.
- **Genetic drift** takes into account unpredictable, random events that changes allele frequencies.
- **Non-random mating** takes into account mate selection which can influence evolution.
- **Mutations** are an important component for speciation to take place. It is the only one of the 5 agents that leads to formation of new alleles.
- Changes in allele frequency alone cannot result in speciation. Two populations need to have sufficient genetic differences before they can be considered a separate species.

Populations evolve, not individuals

LO:
(d) Explain why the population is the smallest unit that can evolve.

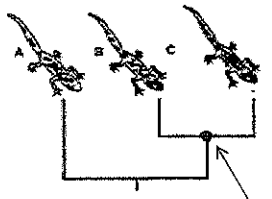
- A population is a group of **interbreeding individuals belonging to a species** and sharing a common geographic area.
- Evolution is a measure of changes in **allele frequencies** in a population over **successive generations**.
- Long-term effects of natural selection are at the level of gene and population rather than the individual. The survivors interbreed, thereby passing on favourable alleles to the next generation. Over time, these individuals contribute to allele frequencies changes that can be measured in a population. **Time** (beyond the lifespan on an individual) is needed to measure these allele frequency changes. **Allele frequency** cannot measured in one individual, but it can be measured in a population.
- Whilst natural selection occurs through interactions between individual organisms and their environment, it is the **collective genetic response** of the population that determines survival of the species and formation of new species.
- Hence a population is the smallest unit that can evolve.

C. Concept of the Species

LO:
(h) Explain the various concepts of the species (biological, ecological, morphological, genetic and phylogenetic concepts)

Species concept	NB: For most purposes, including your exams, biological species concept is used.
Biological	A species is a group of organisms capable of **interbreeding** and producing **fertile, viable offspring** and are **reproductively isolated** from other such groups. **Advantage:** Organisms can be studied to see if they can interbreed and produce fertile, viable offspring. **Limitations:** Definition cannot be applied to (i) asexually reproducing organisms and extinct species as their breeding behavior cannot be observed in nature. (ii) some plants - two different plant species can mate and give rise to viable hybrid offspring that are potentially fertile as they are capable of polyploidy (see autopolyploidy in pg 24). Polyploidy is rare in animals.
Genetic	A species is a group of **genetically compatible interbreeding** organisms in a natural population that is **genetically isolated** from other such groups. Organisms in a species have sufficient similarity in their DNA sequences and share same number of chromosomes. Focus on genetic isolation rather than reproductive isolation distinguishes Genetic Species Concept from Biological Species Concept. **Genetic isolation** results as 2 genomes diverge to the point that they are genetically distinct and evolve independently of each other. e.g. genetic changes that lead to behavioural changes or changes in such as pheromones and odours associated with species recognition. Genetic isolation maintains integrity of gene pools in natural population. However, if the 2 sister species are mated on rare occasions, fertile viable offspring may still be possible. There are instances where a species has achieved genetic isolation but not reproductive isolation. e.g. Two related species, grizzly bear and polar bear are genetically isolated. They have distinctive morphology, mating behavior and habitat preference. As the environment warms, grizzly bears move north, bringing them into contact with polar bears. In 2006, hybrids (known as pizzly / grolar bears) with characteristics intermediate between grizzly and polar bears came into the spotlight. Genetic isolation is achieved as these 2 species have genetically determined mechanisms in place to minimise breeding but reproductive isolation has not been achieved because if fertilisation occurs, fertile offspring is still possible. In this case, grizzly bear and polar bear are genetically isolated but it turns out they are not reproductively isolated. Two reproductively isolated species, however, will always be genetically isolated.

	Advantage: Genetic data from mitochondrial and nuclear DNA to identify species can be unambiguous in deducing evolutionary relationships. **Limitation:** Technology required to study DNA sequences is relatively expensive.
Ecological	A species is a group of organisms **sharing same ecological niche.** [Niche - both the place where an organism lives and its interactions with the environment [i.e. roles that an organism plays in its habitat (predator, prey, primary producer or consumer, decomposer etc).] **Advantage:** Every organism has a niche hence identity of a species is unambiguous. **Limitations:** (i) Cannot be applied to unrelated species that occupy similar niches e.g. striped possum, a marsupial mammal from Australia and aye-aye a placental mammal from Madagascar. (ii) Many animals are elusive and determining niches can be time-consuming and difficult.  **Striped possum** **Aye-aye**
Morphological	A species is a group of organisms sharing similar **body shape** and other **structural features.** **Advantage:** Every organism has morphological features that can be easily studied. **Limitations:** (i) difficult to determine degree of difference that is required to indicate separate species as well as what structural features should be used to distinguish the differences. (ii) some organisms may be superficially similar but have different evolutionary origins e.g. marsupial mole from Australia and placental mole from Africa.  **Marsupial "mole"** **Placental mole**

Phylogenetic * A phylogenetic tree, also known as a phylogeny, is a diagram that depicts lines of evolutionary descent of different species from a common ancestor.	A species is **smallest group of organisms that share a most recent common ancestor** and can be distinguished from other such groups. Phylogenetic history of a species can be obtained by comparing **homologous morphological structures and/or homologous molecular sequences**, with those of other organisms.  This node represents most recent common ancestor of species B and C. **Advantages:** (i) avoids mistakenly classifying organisms based on superficial morphological similarities as the characteristics that are compared are based on common ancestry/ homology. (ii) to determine phylogeny, genetic, DNA sequences, amino acid sequences, morphological, fossil evidence, hybridisation (mating) studies are conducted, so phylogenetic concept of species potentially presents most accurate representation of a species. (iii) provides accurate historical information about speciation history that biological concept of a species cannot. **Limitations:** Accuracy of phylogenetic tree is dependent on availability, diversity and accuracy of source data. If for instance, DNA evidence was not used, the phylogenetic tree may not be that reliable.

D. Speciation

LO:(j) Explain how new species are formed with respect to geographical isolation (allopatric speciation) and behavioural or physiological isolation within the same geographical location (sympatric speciation)

- **Speciation** is a process by which **one or more new species arise** from a previously **existing species.**
- A term often used in the discussion of speciation is gene flow.
  Gene flow is the transfer of alleles from one population to another, due to the movement of fertile individuals or their gametes. If members of a population migrate and interbreed with members of another population 2, gene flow has occurred.
- **Disruption of gene flow is required for speciation to occur**, and occurs via **isolating mechanisms** (e.g. geographical isolation, behavioural isolation and physiological isolation).
- Speciation can occur in two main ways – **allopatric speciation** and **sympatric speciation** (Fig.14.). Both depend on disruption of gene flow between populations of the existing species via an isolating mechanism.

Allopatric speciation	Sympatric speciation
A population in one geographic area ↓ Members are **geographically-isolated** due to a **physical barrier**. For example, geologic changes, like the formation of a deep canyon over time, will result in geographically-isolated sub-populations. ↓ **Gene flow is disrupted.** ↓ **Evolutionary changes occurring independently** within each sub- population. i.e. **different genetic changes** from **accumulation of mutations**, as well as changes in allele frequencies through **genetic drift** and natural selection occurred within each sub-population. ↓ Over time, **new species** form.	A population in one geographic area ↓ Members are **not geographically-isolated**. However, a **reproductive barrier emerges** that isolates a subset of a population from the remainder of the population in the same area. For example, some birds in a population may develop a new bird call and only mate amongst themselves. ↓ **Gene flow is disrupted.** ↓ **Evolutionary changes occurring independently** within each sub- population. i.e. **different genetic changes** from **accumulation of mutations**, as well as changes in allele frequencies through **genetic drift** and natural selection occurred within each sub-population. ↓ Over time, **new species** form.

Table 2. Comparison between allopatric speciation and sympatric speciation.

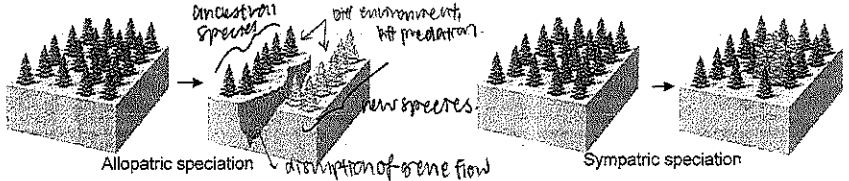


Fig. 14. Allopatric speciation and sympatric speciation.

Allopatric Speciation

- How geographical isolation can lead to speciation:

Example 1: Porkfish

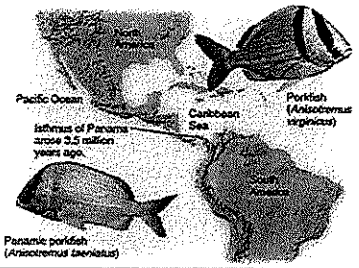


Fig. 15. Geographical isolation occurred due to formation of a barrier, the Isthmus of Panama.

* An **ancestral fish population** was split **into two sub-populations** by formation of Isthmus of Panama about 3.5 million years ago.
* This **physical barrier disrupted gene flow** between the two sub-populations.
* The divided sub-populations were exposed to **different environments** and thus **different selection pressures.**
* Since there was **variation within the populations, favourable** characteristics were **selected for** and **unfavourable** characteristics were **selected against** resulting in **changes in allele frequencies**
* Thus **evolutionary changes occurred independently** within each sub-population i.e. **different genetic changes** from **accumulation of mutations**, as well as changes in allele frequencies through **genetic drift** and **natural selection** occurred within each sub-population.
* **Over hundreds and thousands of successive generations**, each sub-population became genetically distinct species. These species are **reproductively isolated** and **unable to interbreed to form fertile, viable offspring.**
* The Caribbean porkfish (*Anisotremus virginicus*) is found in Caribbean sea, whereas Panamic porkfish (*Anisotremus taeniatus*) is found in Pacific ocean (Fig.15.).

Example 2: Finches

* An **ancestral population** of Darwin's finches strayed from the South American mainland of Ecuador to one of the Galapagos Islands. They then **colonised other islands/parts of the islands**.
* The sub-populations were **geographically isolated** in various islands/parts of the islands and did not interbreed. Hence **gene flow was disrupted.**
* The divided populations were exposed to **different environments** in the different islands and were under **different selection pressures.**
* Since there was **variation within the populations, favourable** characteristics were **selected for** and **unfavourable** characteristics were **selected against** resulting in **changes in allele frequencies.**
* Thus **evolutionary changes** occurred **independently** within each sub-population i.e. **different genetic changes** from **accumulation of mutations**, as well as changes in allele frequencies through **genetic drift** and **natural selection** occurred within each sub-population.
* **Over hundreds and thousands of successive generations**, each sub-population became genetically distinct species. These species are **reproductively isolated** and **unable to interbreed to form fertile, viable offspring.**

* There are now at least 13 species of finches on the Galápagos Islands, each filling a different niche on different islands/parts of the islands. Islands generally favour speciation as they are geographical isolated from the mainland (disrupting gene flow), and have different environmental conditions.

* This kind of evolutionary pattern in which there is a rapid increase in the number of species produced from a common ancestor upon introduction into new environments is known as **adaptive radiation**.

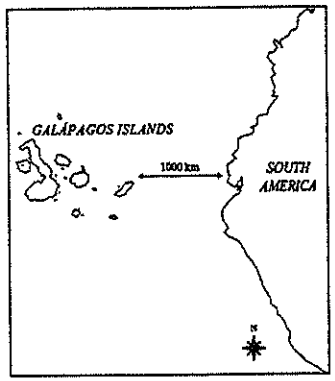


Fig. 16. Ancestral population of finches migrated from the South American mainland to Galápagos Islands.

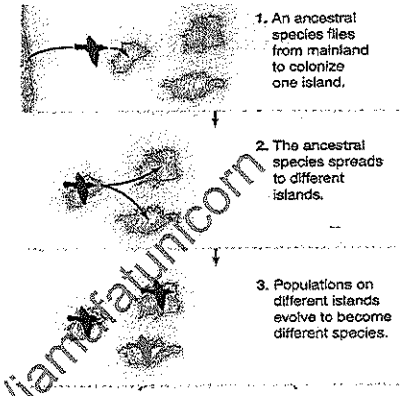


Fig. 17. Adaptive radiation on island archipelagoes.

endemic species

Why islands favour speciation?

① geographically isolated esp from mainland (disrupting gene flow)

② hv diff environmental conditions (no predators from mainland, unlikely to hv diseases common in mainland, diff habitats)

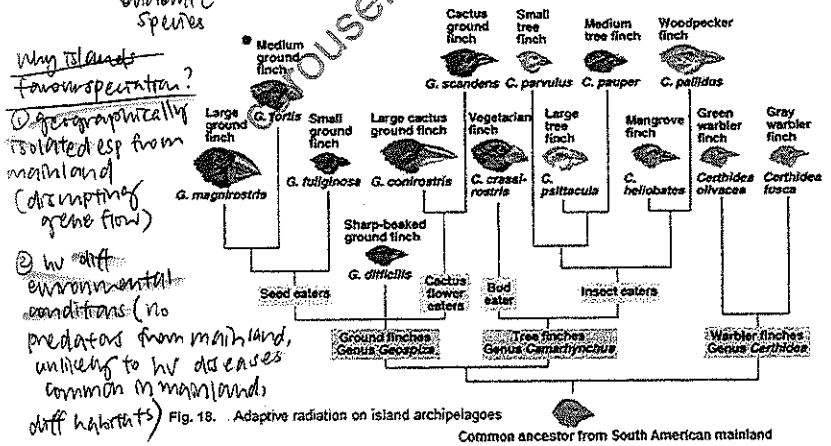


Fig. 18. Adaptive radiation on island archipelagoes

Sympatric Speciation (& Physiological Isolation)

- **Physiological isolation** is where mating between individuals of different sub-populations existing in the same geographical area is not possible, due to unique physiology (internal functions).

- The physiological difference serves as a **reproductive barrier**, thereby **blocking gene flow** between sub-population existing in the same area.

- How physiological isolation could have led to speciation

  Example: Palms

  * An **ancestral** palm grew on Lord Howe Island off the coast of Australia.

  * The island had **two soil types** in close proximity to each other - older volcanic soil and younger calcareous soils.
    When the palms that normally grew on volcanic soil started to grow on calcareous soil, flowering time difference may have arisen as a **physiological response** to growing on a different soil. This **prevented interbreeding** between the two sub-populations of palms growing in the two types of soil although they were in close proximity (Fig. 19, 20.).

  * This **disruption of gene flow** resulted in **evolutionary changes occurring independently** within each sub- population. i.e. different genetic changes from **accumulation of mutations**, as well as changes in allele frequencies through **genetic drift** and **natural selection** occurred within each sub-population.

  * Since there was **variation within the population, favourable** characteristics were **selected for** and **unfavourable** characteristics were **selected against** in the 2 different environments.

  * **Over hundreds and thousands of successive generations** each sub-population became **genetically distinct species**, *Howea forsteriana* and *Howea belmoreana* (Fig. 21). These two species are **reproductively isolated** and **unable to interbreed to form fertile, viable offspring**.

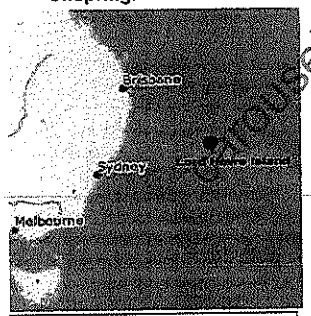


Fig. 19. Lord Howe Island is north east of Sydney, Australia

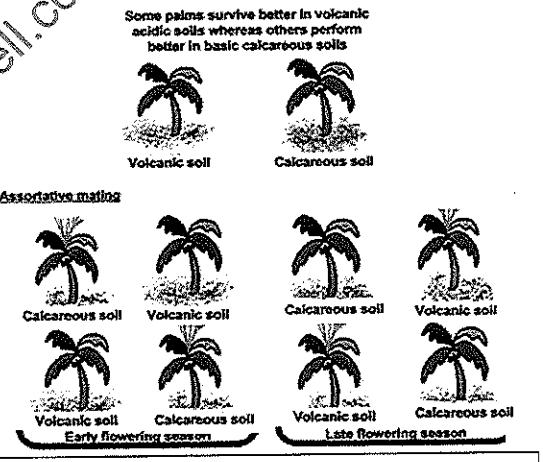


Fig. 20. Palms growing in calcareous soil have an early flowering season while palms growing in volcanic soil have a late flowering season (left).

Fig. 21. The two sister species of palms that formed on Lord Howe: *Howea forsteriana* (left) and *H. belmoreana* (right). The former, also known as Kentia Palm, is grown throughout the world as an ornamental plant. *H. belmoreana* (right) has more an upright, recurved leaves.

Sympatric Speciation (& Behavioural Isolation)

- **Behavioural isolation** is where mating between certain individuals of a population existing in the same geographical area does not occur, due to due to **unique mating rituals and preferences**.
- The behavioural difference serves as a **reproductive barrier**, thereby **blocking gene flow** between sub-population existing in the same area.
- How behavioral isolation could have led to speciation
  Example: Eastern & Western Meadowlark
  - In an **ancestral population** of meadowlark, **some members** of the population **developed a new call**.
  - Over many generations, the new bird call became more distinct. Birds began to distinguish between the two calls and tended to **mate preferentially with members with the same call**.
  - This **disruption of gene flow** resulted in **evolutionary changes occurring independently** within each sub-population. i.e. different genetic changes from **accumulation of mutations**, as well as changes in allele frequencies through **genetic drift** and **natural selection** occurred within each sub-population.
  - **Over hundreds and thousands of successive generations** each sub-population became **genetically distinct species**, eastern meadowlark (*Sturnella magna*) and western meadowlark (*Sturnella neglecta*) (Fig. 22.). These two species are **reproductively isolated** and **unable to interbreed to form fertile, viable offspring**.
  - Their differences in songs enable meadowlarks to recognise potential mates as members of their own species even though both species are nearly identical in shape, colouration and habitat and their ranges overlap in the central United States.

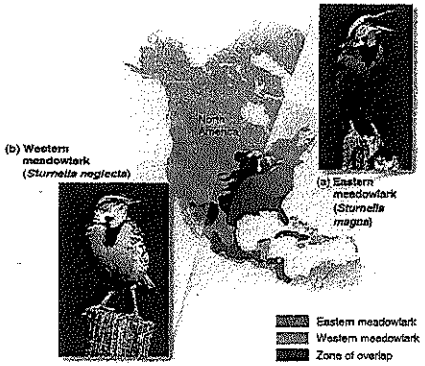
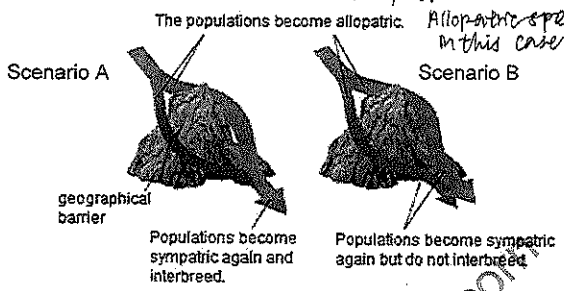


Fig. 22. Similarity between different species. Eastern meadowlark (*Sturnella magna*, left) and western meadowlark (*Sturnella neglecta*, right) have similar body shapes and colorations.
However interspecies mating does not occur due to differences in their songs. For many years they were thought to be the same species. When biologists discovered that Western meadowlark was a different species, it was given the species name *neglecta* to reflect the long delay in its recognition.

Q. Has speciation occurred in scenario A or scenario B? Explain.

A: speciation has occurred in scenario B.
If 2 geographically isolated populations meet up again and cannot interbreed to form fertile, viable offspring, speciation has occurred.



Reproductive barriers (can lead to sympatric speciation)

- **Emergence of a reproductive barrier can lead to sympatric speciation** (Table.1). This is because the reproductive barrier (e.g. a new bird call) can isolate a subset of the population from the rest of the population. The resulting two sub-populations can then undergo evolutionary changes independently of each other and so after many generations form two different species in the same geographical area.
- **Reproductive barriers** also **prevent different species from interbreeding,** that is, they keep different species **reproductively isolated.**
- Reproductive barriers can be classified into (Fig. 23):
  (1) prezygotic mechanism (prevents mating or fertilisation)
  (2) postzygotic mechanism (causes zygote fatality or prevents hybrids from developing into fertile adults).

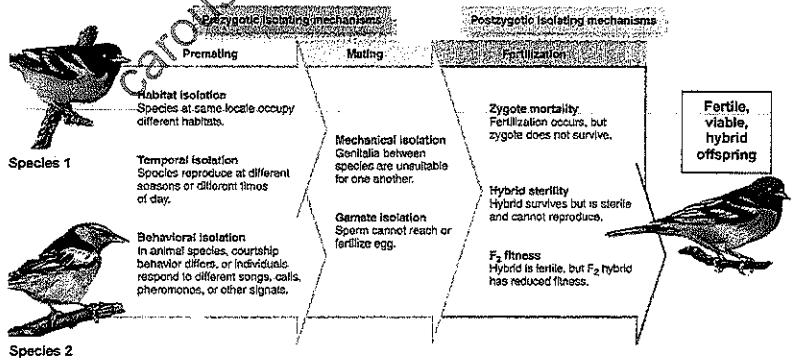
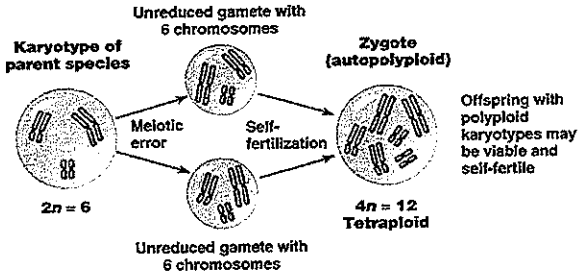
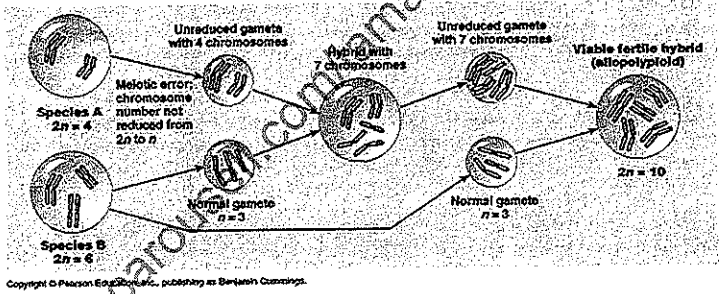


Fig. 23. Prezygotic and postzygotic reproductive barriers. The barriers prevent interbreeding between species 1 and 2.

Appendix
[Not required in syllabus]
Nondisjunction resulting in reproductive barriers and novel fertile offspring



Autopolyploidy in plants.
An organism that has more than two sets of chromosomes, but all sets are derived from the same species. For example, if a nondisjunction in production of gametes by either mitosis or meiosis, results in diploid gametes. If these plants self-fertilize, offspring would have four sets of chromosomes, they would be tetraploids. These tetraploids would be able to mate with other teraploids, but if they mated with either of the diploid parent species the offspring would be triploid, and would be sterile due to impaired meiosis of unpaired chromosomes. Such as instantaneous special genetic event would thus produce a postzygotic barrier, which would isolate gene pools of mutant tetraploids from diploid parent population.



Allopolyploidy in plants. A hybrid of two different species is usually sterile because its chromosomes are not homologous and cannot pair during meiosis. However, such a hybrid may be able to reproduce asexually. The diagram traces one mechanism that can produce fertile hybrids (allopolyploids) as new species. This example is an exception to the rule where macroevolution can only occur over a long period of time. In this case a new species can arise after 2 generations through chance events.

KEYWORDS
Some of the key words you should know include:

alleles gene flow geographically isolated interbreeding natural selection	reproductive isolation selection pressures variation viable, fertile offspring

Allopatric speciation

Physical barrier
(e.g. river, mountain)

⇩

interbreeding prevented, gene flow disrupted

⇩

independent evolutionary changes in each sub pop

⇩

Genetically distinct species; reproductively isolated due to reproductive barriers

Sympatric speciation

Reproductive barriers
(e.g. physiological / behavioural diff)

⇩

interbreeding prevented, gene flow disrupted

⇩

independent evolutionary changes in each sub pop

⇩

Genetically distinct species; reproductively isolated due to reproductive barriers

physical barrier ⇒ allopatric
reproductive barrier ⇒ sympatric

CORE IDEA
(4) Biological Evolution

EVOLUTION 2

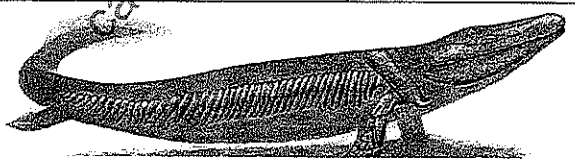
Content
- Evidence of evolution
- Classification

Learning Outcomes
Candidates should be able to:
(e) Explain how genetic variation (including recessive alleles) may be preserved in a natural population
(g) Explain how evidence based on homologies identified in biochemical data (molecular homologies) and the fossil record (anatomical homologies), together with biogeography, supports Darwin's theory of evolution
(i) Define biological classification as the organisation of species according to shared characteristics and describe how evolutionary relationship is established
(k) Define phylogeny as the organisation of species to show their evolutionary relationships
(l) Explain the importance of the use of genome sequences in reconstructing phylogenetic relationships and state the advantages of molecular methods, including multiple sequence alignment (nucleotide and amino acid), in classifying organisms.

Use the knowledge gained in this section in new situations or to solve related problems.

References:

- Reece *et al.*, (2011). *Biology*, 9th Edition.
- Kenneth V Kardong, *An Introduction to Biological Evolution*, 2nd Edition.
- Mark Ridley, *Evolution*, 2nd Edition.
- Taylor, Green et al, *Biological Science 2*, 3rd Edition
- *Understanding Evolution*. http://evolution.berkeley.edu/



The *Tiktaalik* is a transitional fossil that shows an evolutionary link between fish and four-legged land animals (amphibians, reptiles, birds and mammals). It is evidence of evolution.
http://evolution.berkeley.edu/evolibrary/news/060501_tiktaalik

*** This handout is the effort of several Biology teachers at RI(Yr 5-6). It has and will continue to be updated.**
****Any information given in a double-lined box is for your information only.**

Table of Contents

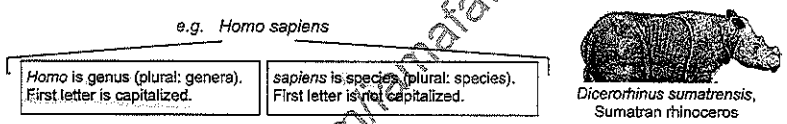
A. Classification

LO:
(i) Define biological classification as the organisation of species according to shared characteristics and describe how evolutionary relationship is established
(k) Define phylogeny as the organisation of species to show their evolutionary relationships

- Biological classification or **taxonomy** is the **organisation of species** according to **shared characteristics.** It involves **naming, identification and classifying organisms.**

Nomenclature (naming)
- Common names like cat, bear etc. can be ambiguous because there are many species of each of these organisms. Scientific names however, avoid ambiguity.

1) **Each species has a 2-part** Latin name, or a **binomial name.**



Dicerorhinus sumatrensis, Sumatran rhinoceros

2) When asked to name a species, you should write binomial name with both the genus and the species.
3) When handwritten, the binomial name for humans is underlined with a space in between the genus and the species, i.e. Homo sapiens

When printed in books or journals using computers, the binomial name is *italicized* and not underlined, i.e. *Homo sapiens*

- The person who came up with the Binomial system was Carolus Linnaeus, a Swedish Biologist, and physician. He first published this system in his book *Systema Naturae* in the Netherlands in 1735.

Identification
- The traditional Linnaean classification of living things looked mostly at **morphologies/physical structures.** E.g. the leaf arrangement of a plant, shape of limbs and appendages, teeth and bone arrangements etc.
- With the advent of **molecular methods**, DNA and amino acid sequence analysis, and DNA barcoding is now being used to tag and identify individual species.

Classification into taxonomic groups
- Once you can identify an organism based on certain traits mentioned above, you can **organise** them **into groups** according to **shared characteristics.**
- Closely related organisms are grouped together in the same taxon.

- A taxon consists of a group of organisms at a particular level. The assumption is that the more homologies two organisms share, the closer they must be in evolutionary distance.
- Linnaeus therefore grouped organisms into a hierarchy of **increasingly inclusive categories**. Of course during his time, he used mostly **morphological characteristics** to determine their grouping.

e.g : human beings

Taxon		
Domain:	Eukarya	Most recently-added taxon.
Kingdom:	Animalia	In Linnaean classification, this is the largest and most inclusive grouping; consists of grouping of phyla.
Phylum:	Chordata	Consists of grouping of classes.
Class:	Mammalia	Consists of grouping of orders
Order:	Primates	Consists of grouping of families
Family:	Hominidae	Consists of grouping of related genera
Genus:	*Homo*	Consists of grouping of related species
Species:	*sapiens*	To specify an organism, the binomial name should be used.

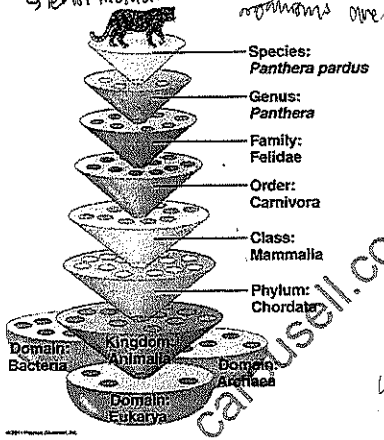


Fig. 1. Features of Linnaean classification

It is hierarchical
Species that are related are grouped into the same genus *Panthera*, which in turn is grouped into a **more inclusive, larger category**, the family *Felidae* and so on.

Binomial nomenclature
Another key feature of Linnaean classification is the use of the **binomial name** to identify a species. In this case the Leopard (common name) is more precisely named *Panthera pardus* (scientific name) where the **genus name is followed by the species name.**

Advantages of Linnaean classification

1. Hierarchical classification is a systematic way of grouping organisms. A newly discovered species can be **easily categorised and named**.
2. The binomial nomenclature provides an accurate identity to each species. It is far more **precise** to identify a species using its binomial nomenclature than its common name. For example a single species *Carica papaya* may have several common names like papaya, paw paw, depending on language and ethnicity of the person referring to it.

Disadvantages of Linnaean classification

1. Based on Linnaean classification, we **cannot infer the evolutionary relationships** between members of each category. We also **cannot tell how distantly related one species is to another** and the name **does not tell us the evolutionary history** of that species.

- In order to see where each species stands in relation to other species, we need to present the organisms in a branching evolutionary tree or phylogram.

Phylogeny

- A phylogeny is **organisation of species** to show their **evolutionary relationship**. This is usually presented in the form of a **phylogenetic tree /evolutionary tree /cladogram /phylogeny /phylogram.** (Fig. 2)
- The underlying principle of a phylogenetic tree is that species are related by descent from common ancestry. A **branch point/node** of a phylogenetic tree represents the **divergence** of two species from a **common ancestor.**

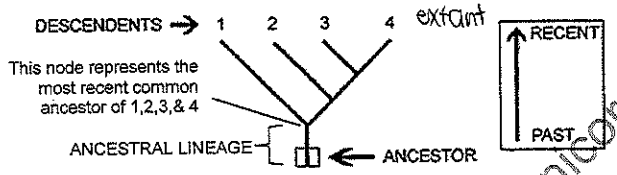


Fig. 2 shows a phylogenetic tree.
The root of the tree represents the ancestral lineage from which the descendants 1-4 arise from.

- Understanding a phylogeny is like reading a family tree. The **root** of the tree represents the ancestral lineage, and the tips of the branches represent the descendants of that ancestor. As you move from the **root to the tips**, you are moving **forward in time**.
- When a **lineage splits (i.e. a speciation event occurs)** (Fig. 3), it is represented as branching on a phylogeny. When a speciation event occurs, a single ancestral lineage or common ancestor gives rise to two (sometimes more) daughter lineages.

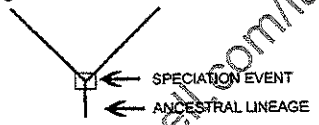


Fig. 3 shows a speciation event.
The ancestral lineage splits to give rise to two daughter lineages.

Understanding phylogenetic trees.

- Phylogenetic trees make use of **homologous characters** to group species into clades (or branches). Homologous characters are **phenotypic and genetic similarities** due to **shared ancestry**.
- **Molecular and anatomical data** including fossil records are used to infer evolutionary relationships. (We will explore these in the next section.)
- We need to be careful with the use of morphological or physical resemblances to determine if two organisms are related. It does not mean that when two organisms resemble each other, they are necessarily closely related. E.g. the Marsupial mole and the Placental mole resemble each other physically and functionally but are from different ancestral lineages.
- In determining phylogeny we look out for **homologous structures** with **shared ancestry**.
- An example of a homologous character is the **pentadactyl limb**. (Fig. 4) It has a basic structure of 5 digits, and limb bones that have evolved to become different structures such as the flipper of a dolphin, the wing of a bat and the human hand. These structures are homologous as they are **derived from a common ancestor. Modification** of the basic structure allowed for different functions and appearances.

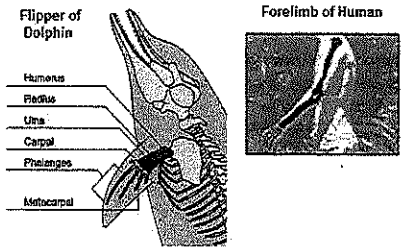


Fig. 4 Pentadactyl limb
The flipper of a dolphin is homologous to the forelimb of a person even though they may serve **different functions**, swimming and grasping.

- A common mistake in establishing evolutionary relationships is to use **analogous characters**.
- Two different species that **do not share a recent common ancestor** can **independently evolve similar traits** as a result of having to adapt to similar environments or ecological niches. This process is called **convergent evolution**.
- The pectoral fins of the shark, ichthyosaur and dolphin, superficially resemble each other. (Fig.5) The selection pressure posed by a need to swim fast in a watery media, resulted in the **structures that come from different ancestors resembling each other**. This is an example of an **analogous structure**. Closer examination of the internal structure of the fin reveals their **different origin** (Fig.6). The flipper of the dolphin is in fact derived from a mammalian forelimb, the pentadactyl limb with five bony finger-like projections, while the fins of the shark are made up of rays of cartilage.
- The danger of using analogous structures is that the distinct species are wrongly placed as being closely related.

Fig. 5 Show convergent evolution. The shark is closely related to the fish, the Ichthyosaur to a reptile and a dolphin to a mammal, yet they appear outwardly similar.

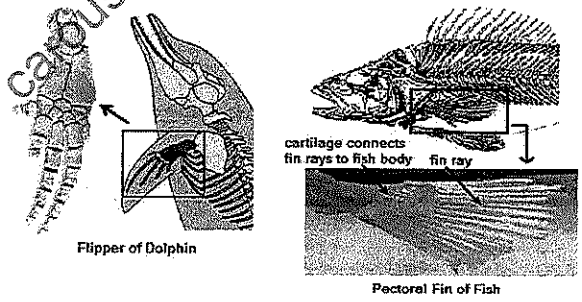


Fig.6 Analogous structures
The pectoral fin of a fish is analogous to the flipper of a dolphin (analogous structures usually have evolved to serve **similar functions** through *convergent evolution*)

- There are two kinds of **homologous characters** that are used in cladistics and these are
  1) shared **ancestral characters** and
  2) shared **derived characters**.

- A **shared ancestral character** is a character that **originated in an ancestor** and is **shared by all its descendants**.
- A **shared derived character** is a **unique** character of the group but **not found in the ancestors**. or their ancestors' other descendants
- All vertebrates share the **ancestral character** of having a **backbone** but members of the vertebrates **distinguish** themselves **from the common ancestor** with their **derived characters** which their descendants will possess. **Hair** is a shared derived character **unique to mammals** but is **not found in their ancestors** or their descendants like turtle, salamander, tuna, and lamprey. (Fig. 7)

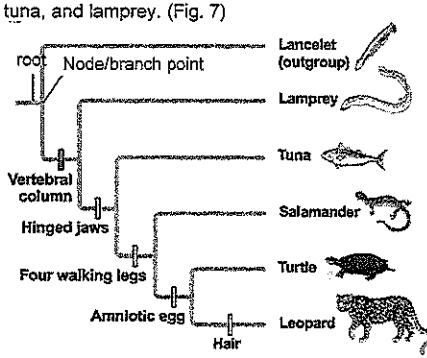


Fig. 7 Shared derived characters that are used to construct a phylogeny of vertebrates (i.e. animals with backbones. The lancelet is used as an outgroup as a reference point for the tree as it is considered an early lineage close to the vertebrates but not within it. It does not have a backbone but has a notochord which is considered a precursor to the backbone.

Phylogenetic trees reveal relatedness of species.

- Two living species are **closely related** if their **most recent common ancestor lived close to the present**, and more distantly related if their most recent common ancestor lived in the more distant past. Technically every living thing is thus related to every other, it is just a matter of degree.

Q. With reference to Fig. 7, is tuna more closely related to the lamprey or salamander? Explain your answer.

salamander, as it shares a more recent common ancestors with hinged jaws as compared to more distant common ancestor with lamprey with vertebral column.

Phylogenetic trees may reveal how long ago a species diverged from a common ancestor.

- A phylogenetic tree may show a timeline and the point **from which species have diverged** from their common ancestor. Each branch length is drawn proportional to the amount of change on that branch. Fig. 8 shows one such phylogenetic tree.

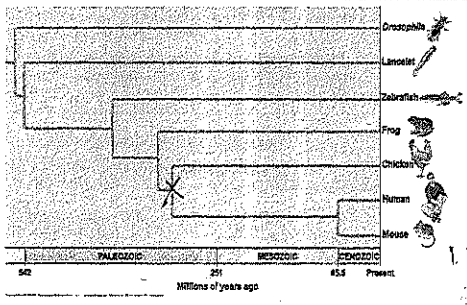


Fig. 8 The right way to read a tree.

Q: When did the common ancestor of the mouse and man started to diverge?

65.5 million yrs ago.

Q: Mark with an "X" the position of the most recent common ancestor of the chicken, human and mouse.

Advantages of Phylogeny

1. Determines evolutionary history of an organism based on the study of homologous characteristics.
2. Degree of relatedness can be inferred.
3. Can tell how long ago 2 species diverged from their common ancestor.

LO:
(I) **Explain the importance of the use of genome sequences in reconstructing phylogenetic relationships and state the advantages of molecular methods, including multiple sequence alignment (nucleotide and amino acid), in classifying organisms.**

http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=genomes&part=A8863

1) Basis of molecular methods

- All **known life** is based on **nucleic acids** thus studies involving any types of taxa can use DNA sequence data. Although amino acid sequences are still used today, DNA has now become by far the predominant molecule since it yields more phylogenetic information than protein. RNA molecules such as ribosomal RNA can be used, but RNA being easily degraded, are harder to handle than DNA or protein.

Q. Why does DNA yield more info than amino acid sequences?

- Species diverge as changes occur in nucleotide bases and subsequently amino acid sequences, with each species acquiring its own set of genetic changes. **Evolutionary changes are captured in the genetic sequences.** Thus, we would predict that **species that are closely related** have **more similar nucleotide sequences** in their nucleic acids and a greater number of **similar amino acid sequences** in their proteins than do distantly related species.
- Nucleotide and amino acid sequence **databases are accumulating at an increasing rate** because of recent advances in molecular biology. Molecular phylogenetics have led to extensive revision of the classification of all life-forms.

2) Advantages and importance of molecular (nucleotide and amino acid sequences) methods in classifying organisms and determining their phylogenetic relationships:

* *more important points.*

1. *They can be used to compare species so **morphologically indistinguishable** due to **convergent evolution** or are **very closely related.**
2. *On the other hand, **remotely related organisms** such as bacteria, humans and sunflower can also be compared because they **share some proteins** such as cytochrome c.
3. *They are **objective.** Molecular **character states are unambiguous** as A, C, G and T are easily recognisable and cannot be confused with another whereas some morphological characters, such as those based on the shape of a structure, can be less easy to distinguish because of overlaps between different character states.
4. *They are **quantitative.** Molecular data are easily **converted to numerical form** and hence are amenable to mathematical and **statistical analysis** and hence computation. The **degree of relatedness** can be inferred and **quantified** by **calculating the nucleotide differences between species.**
5. *Changes in the nucleotide sequences accumulate over time with clockwork regularity. We can thus **estimate the time of speciation** of modern to ancient species.
6. **Some molecular differences** may **not be reflected as a difference in the morphological character** and hence may not be picked up by morphological analysis. e.g. a nucleotide difference in the third base of a codon, or intron.
7. While **small genetic differences**, may result in **major phenotypic differences.** In such instances, vast differences in morphology can exaggerate the evolutionary distance between two species but not in the case of molecular methods. e.g. in snakes, the loss of forelimbs and elongation of the body, both radical changes in body form, are due to mutations in several *Hox* genes that affect the expression of body patterns and limb formation.
8. Offers **a large set of characters** to be studied relatively **quickly.** Each nucleotide position can be considered a character with 4 character states, A, C, G and T, and organisms have

millions - billions of nucleotide positions. There are limited morphological characters that can be studied, e.g number of segments, number of legs, shape of thorax etc.

9. Amino acid sequences for many proteins and nucleotide sequences for a rapidly increasing number of genomes can be accessed from **electronic databases** and used for comparative study and classification. No physical specimen is required unlike in morphological analysis. Issue of accessibility to a physical specimen is no longer an issue.
10. Both **living and dead tissue may be used** so long as the DNA or protein remains intact and you do not even need an entire specimen. Depending on what morphology you are studying, you sometimes need the entire specimen or a specific part of the specimen. e.g. skull, dentition. (dental organisation) Sometimes living specimens are needed as some morphological structures that you are studying, such as the eye, are difficult to preserve. The animal may be very elusive or are endangered, further compounding the problem of obtaining specimens. However if they left traces such as hair or shed skin behind, you can still study their DNA sequences.

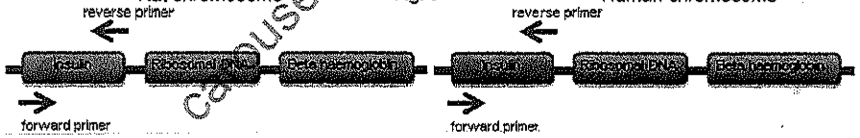
3) Drawing an evolutionary tree using molecular homology

- One can draw an evolutionary tree of a group of organisms using molecular data. The most common way in this day and age is to use **homologous DNA sequences or amino acid sequences.**

a) **Choosing a gene** that is **common to the groups of organisms being compared** and is **derived from a common ancestor – a homologous gene.** Examples of such genes are cytochrome C gene and ribosomal DNA.

b) **Amplifying and sequencing the gene.** This involves amplifying the gene from the different organisms by PCR and then sequencing the DNA. The forward and reverse primers are designed such that they amplify homologous genes from different species. e.g. (Fig. 9) the primers below are complementary to the flanking regions of the insulin gene in the rat and human genome so they only amplify the insulin gene. In this case insulin is the homologous gene being compared.

Fig. 9



c) **Sequence alignment.** The DNA sequences are aligned and a computer programme then calculates how many differences there are between them and converts the alignment data into a distance matrix. The alignment isn't always that straightforward as shown below, with gaps representing deleted nucleotides.

Example of a multiple alignment

Species 1	A	G	G	C	C	A	A	G	C	C	A	T	A	G	C	T	G	T	C	C
Species 2	A	G	G	C	C	A	A	G	A	C	A	T	A	C	C	T	G	A	C	C
Species 3	A	G	G	C	C	A	A	G	A	C	A	T	A	G	C	T	G	T	C	C
Species 4	A	G	G	C	A	A	A	G	A	C	A	T	A	C	C	T	G	A	C	C

Things to note in a multiple alignment:

1. The above alignment shows a segment of homologous genes sequenced from 4 species.

2. Each column is referred to as a nucleotide position (or amino acid position if it's an amino acid sequence alignment)
3. Conserved positions have the same nucleotides and variable positions (marked with an asterisk*) indicate a difference in the nucleotides at that position for the different species.
4. From the multiple alignments, the differences or similarities among the species can be generated and be sometimes used to draw the phylogenetic tree. Below are some of the forms of the matrix generated from an alignment.

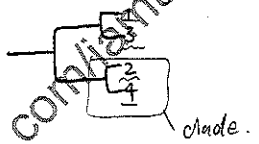
Distance matrix

	1	2	3	4
1	-	0.15	0.05	0.20
2		-	0.10	0.05
3			-	0.15
4				-

closely related.

- The evolutionary distance in the distance matrix is expressed as the number of nucleotide differences per nucleotide site for each sequence pair. For example, sequences 1 and 2 are 20 nucleotides in length and have three differences, corresponding to an evolutionary difference of 3/20 = 0.15 smaller number → more closely related.

Q. In the space below, draw a phylogenetic tree of the 4 species based on the above matrices



cladogram
Cclade → for a common ancestor & its descendants)

d) **Generating the evolutionary tree.** The programme than computes the differences between each species from the alignment and generates the tree.

- Fig. 10 shows an alignment of **haemoglobin beta chain amino acid sequences** from a variety of species. The alignment is used to calculate the molecular distances each species is from the others based on differences in the amino acid sequences. The relationships among the species are visualized in the form of a tree (phylogram) (Fig. 11). [distance matrix not shown].

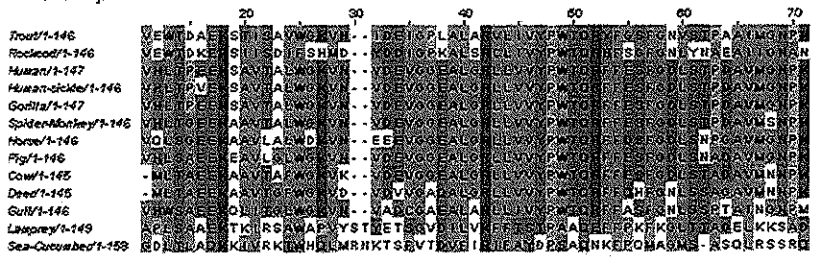


Fig. 10

Hypotheses as to why mutation rate higher than genomic DNA

1) mitochondria is a site of oxidative activities that result in lots of reactive oxygen species that damages DNA & causes mutation.
2) The DNA repair mechanisms although present are not as robust as that found in the genome.

Phylogram

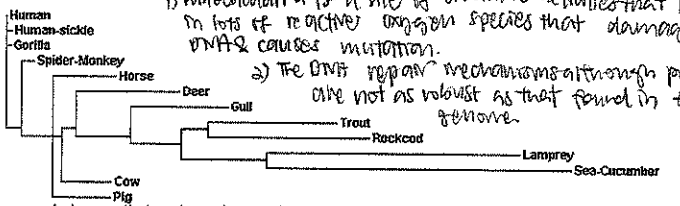


Fig. 11 shows a phylogenetic tree based on molecular homology. This tree explicitly represents number of nucleotide differences through its branch. ~~the~~ assumes estimation of time of speciation. ① no genetic recombination ⇒ any Δ → mutation

4) An application of molecular phylogeny (a study of mitochondrial DNA in humans)

Why use mitochondrial DNA? (you need to appreciate the advantage of using mtDNA)

- Unlike nuclear DNA, in which alleles are rearranged in the process of recombination between maternal and paternal chromosomes, there is usually **no such rearrangement of alleles in mtDNA from parent to offspring**. Thus **changes in DNA sequence** is **solely** due to **accumulation of mutations** over time. ② 10 times higher than nuclear genome
- mtDNA is not highly conserved and has a **rapid mutation rate**, making it useful for studying the evolutionary relationships of organisms that are closely related. It accumulates mutations at the rate of approximately one every 3,500 years. This is important when **comparing populations within a species** and for comparisons of **species that are closely related**. After all, there must be discernible differences for you to work out the degree of relatedness. This feature in fact works against studies involving distantly related species. ~~therefore~~ cannot compare

Mitochondrial Eve

- Phylogeny using the mitochondrial DNA has been used to trace the evolution of the earliest maternal line.
- Mitochondrial Eve is defined as the woman who is the **most recent common ancestor of the maternal lineage (matrilineal)** of all living humans.
- As the mother's egg contains the cytoplasm including the mitochondria, while the father's sperm contributes only the nucleus, all mitochondrial DNA (**mtDNA**) **is passed on from mother to children of both sexes.** The line can be traced back all the way to Mitochondrial Eve.
- Using **SNP markers** that are highly polymorphic, scientists could use mtDNA sequence analysis data to sort populations into more or less related groups, forming the **phylogenetic tree**.
- By looking at the **number of mutations which have accumulated in different branches** of this tree, and looking at which **geographical regions** have the **widest range of least related branches**, the region where Mitochondrial Eve lived can be proposed. (Fig. 12)
- This means that the **origin of our common ancestors** which was Africa,had;
  - The **greatest variety of lineages**, L0a, L0k, L0d, L1, L2, L3, M & N
  - And the **greatest degree of nucleotide differences**. So if you compare L0a and N, you will see many differences in their mtDNA.
- This is because the common ancestor had a **longer time to accumulate** those **differences** as compared to human lineages in Australia.

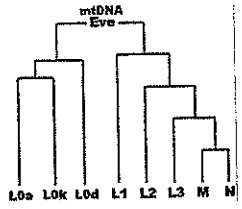
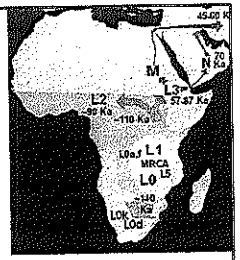


Fig. 12 "Out of Africa"

The maternal-lineage may trace back to a single female, such as Mitochondrial Eve. This is done by tracing the evolution of the mitochondrial genome sequence in a phylogenetic tree. By studying the lineages throughout the world, the place that showed the widest range of least related lineages was in Africa. This must have been the origin of modern humans.



- The male counterpart is the **Y-chromosomal Adam**, the most recent common ancestor of the **paternal lineage (patrilineal)** of humans. Only males inherit the Y chromosome.
- Of course, this Eve and Adam were not equivalent to the biblical characters and were by no means the only people alive at that time: they were simply the individuals who carried the ancestral mitochondrial DNA and Y chromosomes that gave rise to all the mitochondrial DNAs and Y chromosomes in existence today. Their contemporaries failed to produce a direct unbroken line to people living today. (Fig. 13)
- The mitochondrial DNA and Y chromosome studies appear to provide strong evidence in support of the **Out of Africa theory**. Rather than evolving in parallel throughout the world, as suggested by the **multiregional hypothesis**, Out of Africa states that *Homo sapiens* is likely to have originated in East Africa, with members of this species then moving into the rest of the Old World around 100,000 years ago, displacing the descendants of *Homo erectus* that they encountered.

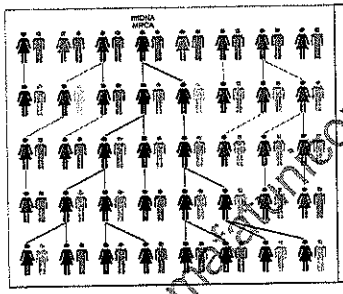


Fig. 13 Through random drift or selection the maternal-lineage will trace back to a single female, such as Mitochondrial Eve. In this example all humans descended from one female while other maternal lines became extinct.

B. Evidence of Evolution

LO:
(g) Explain how evidence based on homologies identified in biochemical data (molecular homologies) and the fossil record (anatomical homologies), together with biogeography, supports Darwin's theory of evolution

Recall: **Homology** refers to similarity inherited from a **common ancestor** unlike **analogy** which refers to similarity due to **convergent evolution**.

1) Anatomical Homology

- Organisms with **anatomical homology** have **morphological structures** such as bone, organs and gross structural features that are derived from a common ancestor.

- Homologous structures represent **variations on a structural theme** that was present in their **common ancestor**. Characteristics present in an ancestral organism are **altered (by natural selection)** in its descendants over time as they face different environmental conditions.
- Hence although homologous structures are derived from a common ancestor, they **may serve different functions & may superficially look very different** but **retains a common underlying similarity**. Examples of anatomical homology are;

a) The pentadactyl (five-digit) limb of tetrapods

- Tetrapods are the group of vertebrates with four legs. Amphibians, reptiles, birds, and mammals are tetrapods. In the case of birds and mammals like bats, the forelegs have been modified for a new function – wings for flight.
- They occupy a wide variety of environments, and use their limbs for many differing functions.

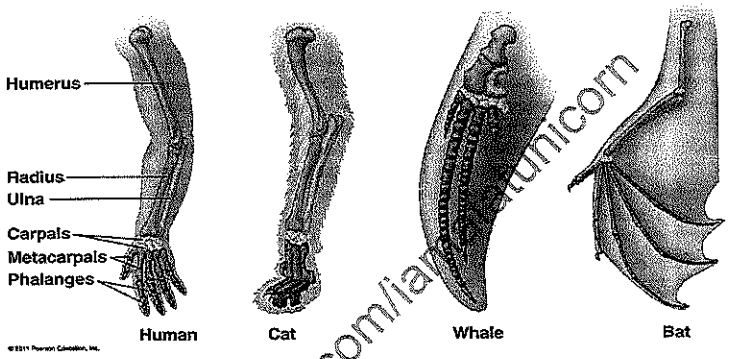


Fig. 14. Different functions but similar underlying forelimb anatomy.

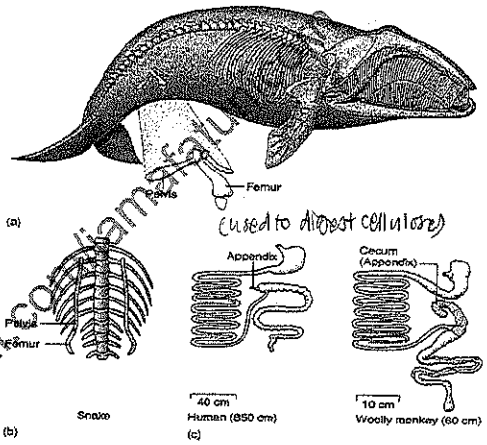
- The forelimbs of humans, cats, whales and bats show the **same arrangement of bones** from the shoulder to the tips of the digits, even though these **appendages have very different functions**: lifting, walking, swimming and flying and don't resemble each other.
- Such striking anatomical homology would be highly unlikely if these structures had arisen from scratch in each species.
- There is **no clear functional or environmental reason** to explain **why all these animals should need a five-digit**, rather than three-, seven- or more digit limb. Yet all modern tetrapods have such limbs, while fossil tetrapods that possess six-, seven-, and eight-digit limbs were found in the Devonian period but their lineage did not continue as they became extinct.
- There is however **clear functional reason** to explain the **different morphology.** An articulate hand to grasp, a hydrodynamic fin to swim and a broad flexible wing to fly.
- In this example we can clearly see evidence that **natural selection had resulted in the adaptive variations of the basic structural theme (the pentadactyl limb)** to suit their **specialised functions** and that it was a **modification of the five-digit forelimb of the common ancestor** which they all descended from.

b) Vestigial structures.

- Vestigial structures are anatomical features that **resemble structures** of their presumed **ancestors.**
- They are **typically degenerate, atrophied or rudimentary**. They may have **lost some or all of their functional roles** that they played in ancestral organisms.

- Organisms having vestigial structures **share a common ancestry** with organisms in which the structure is still functional as they are **homologous structures.**
- The existence of vestigial structures can be attributed to changes in the environment and **behaviour patterns of the organism** in question.
- In a changed environment, the **structure may no longer be beneficial** for the organism's survival, (e.g. legs for walking will not benefit a whale in water. Fig. 15) and hence the likelihood that the future offspring will **inherit the "normal" form decreases.**
- This is because all structures has a **cost** in terms of **development, maintenance, burden of weight,** and are also a **risk of disease,** (e.g. infection, cancer) thus contributing some **selective pressure** for the **removal of parts** that do not contribute to the organism's fitness.
- In other instances the structure actually becomes **detrimental** to the organism (e.g. the eyes of a mole can become infected in a subterranean lifestyle).
- Therefore vestigial structures are evidence that Darwin's theory of **natural selection is at work to select for reduced or degenerate structures with limited/no function.** These structures are **modified** (typically degenerated) from functional structures in the **common ancestor.**

Fig. 15 Vestigial Features
(a) Whales evolved from terrestrial ancestors with four legs. But in whales, the hips and hindlimbs are reduced to small bones with no function. (b) Snakes evolved from lizards with four legs. But in primitive snakes, the remnants of hindlimbs persist (forelimbs are absent). (c) The human appendix is a vestigial structure, reduced from the cecum of primate ancestors, which was involved in digestion of significant plant material.
Source: An Introduction to Biological Evolution. Kenneth V. Kardong 2nd Edition



c) **The Fossil Record** is the collective record of changes to the anatomy or biological development as reflected in the fossilized remains of organisms over geological time. The anatomy and morphology of living species are compared with the fossil record to trace their origin.

How fossils form

- When aquatic and terrestrial organisms swept into the seas and swamps die, they settle to the bottom together with sediments (sand and mud) from land. Deposits pile up and compress the older sediments below into rock. Sedimentary rocks are therefore, a rich source of fossils.
- New layers of sediment cover older ones and compress them into superimposed layers of rock called **strata**. Erosion will reveal buried strata.
- Organic substances of dead organisms decay rapidly but parts rich in minerals such as shells, bones and teeth remain as fossils. Minerals in water may also seep into tissues of dead organisms and replace their organic material. These minerals subsequently crystallize forming a cast in the shape of the organism.

ancient ↑ recent complex ↑ simple

- There are other ways of fossilisation such as encasement in tar or amber or preservation in acidic bogs.

Fossils are relics or impressions of extinct organisms preserved in rock.

- While most fossils only preserve the resilient structures of an organism such as its bone anatomy, some well-preserved specimens actually preserve other anatomical structures such as feathers, hair, skin texture and even skin pigmentation.
- Each stratum contains its own unique group of fossil species.
- Radioactive dating techniques allow us to place these strata and fossils they bear in a chronological sequence.
- Succession of organisms is shown as you progress up the layers. Deeper strata contain older organisms that are usually simpler in morphology.
- We can see in fossils how **homologous structures have been modified through time** (descent with modification).
- As we look at many examples of fossil evidence, we will realise that as we progress forward in time, there is an **ordered sequence of progression.** Each species is preceded by a logical and related ancestor through **descent with modification.** This is compelling evidence in support for evolutionary deductions based on homologies.
- A study of fossil evidence showed that fish arose first as they appeared in the deeper strata, followed by amphibians, then reptiles and finally mammals (Fig.16) which appeared in the upper strata.
- Fossil evidence reflects the anatomical milestones we see in evolutionary development.
- If species indeed arose spontaneously and not through descent with modification from pre-existing organisms, then that ordered sequence of progression in fossil record would not hold. Yet we find no evidence of this.

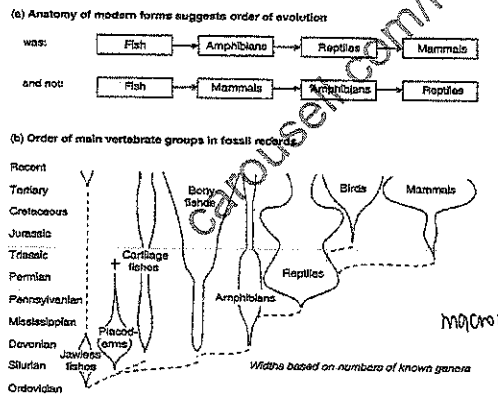


Fig. 16 (a) Anatomical analysis of modern forms indicates that amphibians and reptiles are evolutionary intermediate between fish and mammals. This order fits with (b) the geological succession of the major vertebrate groups. The width of each group indicates the diversity of the group at that time. Fossils found in the deepest strata were fishes and as we move up the layers, we begin to see amphibians, followed by reptiles and then mammals.

macroevolution

Example 1 The Evolution of Horses as seen through the fossil record

- One on the best studied examples of evolutionary change is based on an **almost complete fossil record** of the horse and its ancestors.
- The earliest form of the horse were small (the size of dogs), with short legs and broad feet. These species occurred in wooded habitats and probably browsed on leaves and herbs and escaped predators by dodging through the forest vegetation.

- The evolution to workhorses of today involved **increase in size, lengthening of limbs, toe reduction and increase in tooth size.** (Fig. 17)
- Much of the horse evolution occurred where large areas of land **changed from dense forest to open grasslands.** The increase in size and **changes in foot structure** allowed horses to **escape predators** and **travel great distances** in search of food. **Changes in horse teeth** are consistent with a **shift in diet from tender leaves to grasses** that need more chewing.
- The *Equus* descended through a series of speciation episodes that included several adaptive radiations, not all of which led to large, one-toed, grazing horses of today. Some branches like the *Anchitherium* were dead-end branches that became extinct.
- It was only when grasslands became widespread and there was a need to escape predators that there was a **strong selection for grazers that could run faster.** This led to the only surviving genus of today, the *Equus.*

In summary:

1. The extensive fossil record for horses supports the theory of **progressive change** of **homologous structures** such as **forelimbs and teeth. (descent with modification)**
2. The **driving force** of this change is **natural selection** where there was a need to escape **predation** and a change in diet due to **changing environment.**

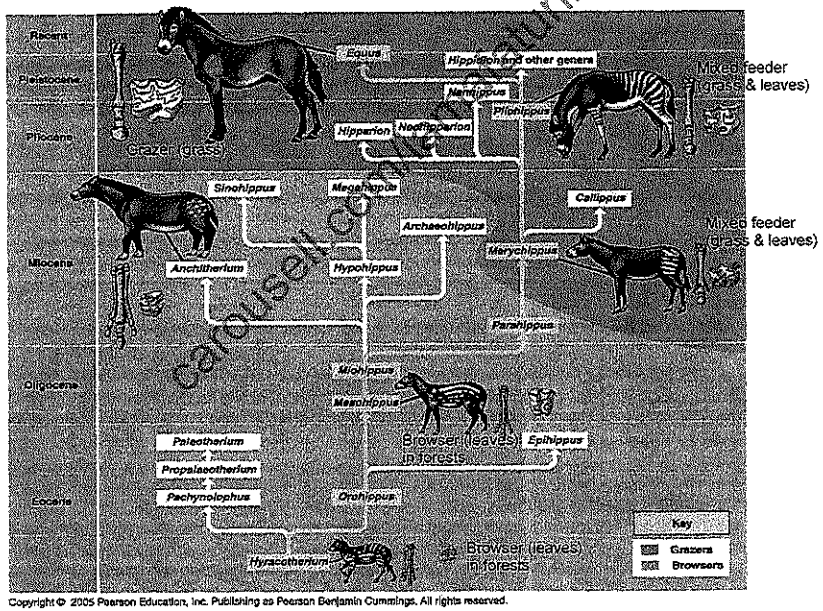


Fig. 17 Showing the forelimb bone arrangement as well as the teeth of the various horse genus

Transitional Fossils

- **Transitional fossils** are any fossilised remains of a life form that exhibits traits common to both an ancestral group and its derived descendant group.
- Unlike the largely complete fossil record of the horses, many other fossil records are incomplete for some of the following reasons.
  a. You need specialised conditions for fossilisation to occur
  b. Dead organisms decompose rapidly and even bones get consumed for their calcium
  c. Soft-bodied organisms do not fossilise easily
  d. Only a fraction of fossils have been discovered
- This lack of continuous fossils in the record, especially of transitional fossils, is a major limitation in tracing the descent of biological groups. This is especially important when the descendant group is **sharply differentiated by gross anatomy and mode of living from the ancestral group**. Such "missing" fossils are often called the "missing link."
- It is a matter of time before such "missing links" are uncovered. They support evolutionary deductions best as they **illustrate an evolutionary transition** between the two forms. They provide evidence for **descent with modification**.

Example 2 *Archaeopteryx*

- The most famous of the missing links is the oldest known bird, *Archaeopteryx* that lived 165 million years ago.
- This specimen is an **intermediate between birds and dinosaurs.**
- Features like a reptile – possession of teeth, a bony tail, link it to carnivorous dinosaurs. Thus, the *Archaeopteryx* linked birds and dinosaurs to a common ancestor.
- Features like a bird - it has feathers like a bird, bone structure that suggest that it walked on its hind legs like most birds do.
- *Archaeopteryx* reveals a pattern commonly seen in transitional fossils – it exhibits **some traits like their ancestors and others like their descendants.**

Fig. 18 shows one of the Archaeopteryx fossils. Since the 1990s many more genera of non-avian feathered dinosaurs were unearthed. In 2011, some excellent samples of preserved feathers were discovered encased in amber. Initial analysis suggests that some of these feathers were used for insulation and not flight but they could be the precursors to flight feathers.

Example 3 *Tiktaalik roseae* the "Fishapod"

- In 2005 fossils of *Tiktaalik roseae* was discovered by Daeschler, Shubin and Jenkins.
- *Tiktaalik* illuminates the steps that led to the evolution of tetrapods. *Tiktaalik* is an example of a transitional fossil animal between fishes & tetrapods. It provides strong evidence that fish are the ancestors to modern tetrapods.

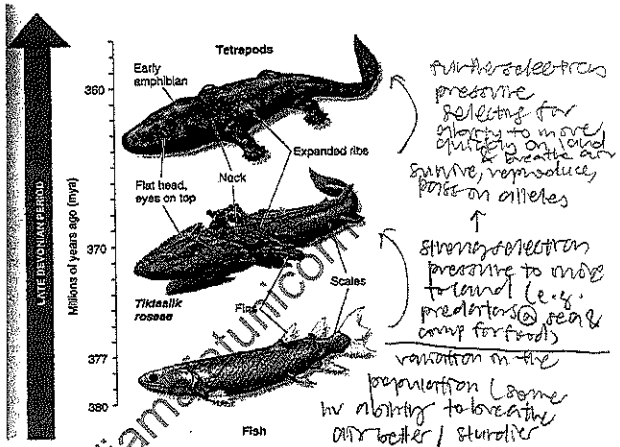


Fig. 19 Evolutionary change in the tetrapod lineage, showing a transitional form. This figure shows two early tetrapod ancestors, a Devonian fish and the transitional form *Tiktaalik roseae*, as well as one of their descendants, an early amphibian. An analysis of the fossils show that *T. roseae*, also known as a fishapod, had both fish and amphibian characteristics, so it probably was able to survive brief periods out of the water.

- It was **similar to its fish ancestors** as it had fish gills & scales.
- It was **similar to its tetrapod descendants** as it had
  1. a broad skull with eye mounted on top of its head, like a crocodile
  2. a mobile neck
  3. an interlocking rib cage suggesting it had lungs.
  4. pectoral fins which revealed the beginnings of a primitive wrist and five finger-like bones. i.e., one of the earliest records of the pentadactyl limb.

- These appendages would have allowed *T. roseae* peek its head above shallow waters to look for prey. This would be an advantage in the marshy floodplains of the Devonian period.
- Thus, the study of fossil record allows us to see the **evolution of species through the modification of homologous structures from a common ancestor to the present descendant through a series of transitional forms.**

2) Molecular Homology

- All forms of life use the same genetic language of DNA and RNA, and the **genetic code is essentially universal.** "AAA" in DNA codes for "UUU" in mRNA, which codes for phenylalanine in organisms as diverse as shrimp, humans, bacteria and tulips.
- Thus, it is likely that all species descended from **common ancestors that used this code.** It has been maintained and transmitted through all branches of the evolutionary tree since its origin in some extremely early (and successful) organism. If organisms had indeed not descended from a common ancestor, there is no reason why they should all share the same genetic code. To date, no other code has been found in any organism.

- When scientists examined **DNA and amino acid sequences**, similarities known as **molecular homologies** can be seen even in organisms as dissimilar as humans and bacteria indicating that they shared a very distant common ancestor.
- When looking for molecular homologies, homologous genes (or their proteins) of different organisms are being compared. Examples of **homologous genes** used are cytochrome c genes, ribosomal genes etc, typically genes important enough that every organism possesses them.
- Just like anatomical homologies, an **ancestral gene** would be **modified** in terms of **nucleotide sequence** over many generations. The more closely related the species, the fewer differences they have. Similarly, a higher degree of differences in nucleotide sequences would imply a more distant relationship.
- Earlier in page 8, we compared the molecular homology of DNA of various organisms and determined their relatedness. Here we will use an example using amino acid sequences.

	Short amino acid sequence within the p53 protein	Percentages of amino acids in the whole p53 protein that are identical to human p53
Human (*Homo sapiens*)	Val Pro Ser Gln Lys Thr Tyr Gln Gly Ser Tyr Gly Phe Arg Leu Gly Phe Leu His Ser Gly Thr	100
Rhesus monkey (*Macaca mulatta*)	Val Pro Ser Gln Lys Thr Tyr His Gly Ser Tyr Gly Phe Arg Leu Gly Phe Leu His Ser Gly Thr	95
Green monkey (*Cercopithecus aethiops*)	Val Pro Ser Gln Lys Thr Tyr His Gly Ser Tyr Gly Phe Arg Leu Gly Phe Leu His Ser Gly Thr	95
Rabbit (*Oryctolagus cuniculus*)	Val Pro Ser Gln Lys Thr Tyr His Gly Asn Tyr Gly Phe Arg Leu Gly Phe Leu His Ser Gly Thr	86
Dog (*Canis familiaris*)	Val Pro Ser Pro Lys Thr Tyr Pro Gly Thr Tyr Gly Phe Arg Leu Gly Phe Leu His Ser Gly Thr	80
Chicken (*Gallus gallus*)	Val Pro Ser Thr Glu Asp Tyr Gly Gly Asp Phe Asp Phe Arg Val Gly Asn Val Glu Ala Gly Thr	53
Channel catfish (*Ictalurus punctatus*)	Val Pro Val Thr Ser Asp Tyr Pro Gly Leu Leu Asn Phe Tyr Leu His Phe Gln Glu Ser Ser Gly	48
European flounder (*Platichthys flesus*)	Val Pro Val Val Thr Asp Tyr Pro Gly Glu Tyr Gly Phe Gln Leu Arg Phe Gln Lys Ser Gly Thr	46
Congo puffer fish (*Tetraodon miurus*)	Val Pro Val Thr Thr Asp Tyr Pro Gly Glu Tyr Gly Phe Lys Leu Arg Phe Gln Lys Ser Gly Thr	41

Fig. 20 shows a comparison of short amino acid sequence within the p53 protein from nine different animals. This figure compares a short region of the p53 tumour suppressor protein which plays a role in preventing cancer.
Amino acids are represented by three-letter abbreviations. Certain amino acids in the sequences are identical to those in the human sequence (e.g. valine and proline in the first two positions).
The numbers in the right column indicate the percentage of amino acids within the whole p53 protein that is identical with the human p53 protein, which is 393 amino acids in length. For example, 95% of the amino acids, or 373 out of 393, are identical between the p53 sequence found in humans and that in Rhesus monkeys.
Amino acids in other species that are identical to humans are in lighter print.

- The sequences from the two monkeys are closest to humans, followed by the two other mammal species (rabbit and dog). The three fish sequences are least similar to the human sequence but tend to be similar to each other.

- The figure illustrates that:
  1. **Certain homologous genes are found in a diverse array of species** such as mammals, birds and fish. This suggests **common ancestry.**
  2. The **sequences of closely related species tend to be more similar to each other** than they are to distantly related species.

- Molecular homology reinforces earlier studies on evolutionary relationships based on morphological structures and fossil data.

In summary:

- **Homologies** (anatomical and molecular) provide evidence of **common ancestry** and **descent with modification** which is what Darwin's theory of natural selection suggests.
- The different types of **homology** show that they were linked to a common ancestor but **developed into different forms** as the **result of natural selection.**

3) Biogeography

- The study of the **past and present geographic distribution of organisms** is called biogeography. It supports the evolutionary deductions based on homologies.
- Plant and animal species are **discontinuously distributed** throughout the world. Elephants occur in Africa and Asia but not North or South America. Ecological factors alone cannot account for this discontinuous distribution. This was demonstrated when rabbits, that are not naturally occurring in Australia, were brought over by colonists. The rabbits thrived in their new habitat and their numbers subsequently exploded.

Evidence 1 Biogeographic realms

- The discontinuous distribution of organisms is based on the concept of **species originating in a given area** and then **spreading (dispersing) outwards from that point. (centres of origin)** The extent of the dispersal will depend on the success of the organism, the efficiency of the dispersal mechanism and the existence of natural barriers such as oceans, mountain ranges and deserts. → barriers to migration.
- Alfred Russel Wallace and his contemporaries studied the distribution of flora and fauna in various parts of the world and based on the **distinctive assortments of fauna and flora, biogeographic realms** were recognised, wherein each continent today sports its defining complement of species. (Fig. 21)
- **Biogeographic realms support the evolutionary deductions** of descent from a common ancestor as the related species that evolved from a **common ancestor** are more or less **confined to their geographical area**. You don't see different elephant species in America for instance.

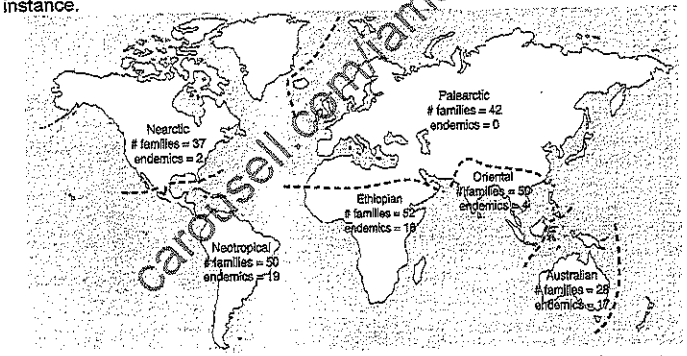


Fig. 21 Biogeographic Realms Continental land areas support characteristic assortments of plants and animals, which in turn define six biogeographic realms. The number of mammalian families is indicated, along with the number of endemic families.

Evidence 2 Darwin's finches

- The **Galapagos Islands** are situated in the Pacific Ocean, almost 1200km west of Ecuador and form an archipelago. These islands were formed by volcanic activity which thrust them up above sea level, so that they have **never had any direct geographical links with any land mass.** Plant species must have arrived on the island by wind dispersal as seeds while birds, bats and flying insects would have fewer problems of dispersal to these islands. Animals without the ability to fly probably drifted in as passengers on floating debris.
- When Darwin arrived at the Galapagos Islands, he was drawn to the variation among the finches (a bird).

- There were **13 distinct species of finches**, with diverse adaptive features. What he found intriguing was that the **finches on Galapagos bore a similarity with the finches from the South American coast** despite the vast differences in habitats. The Galapagos Islands are dry and rocky and the nearest part of South America is humid and has a lush tropical rain forest.
- Darwin concluded that a group of **ancestral finches from the mainland colonised the islands**. Here they flourished, and the inevitable competition produced by increase in numbers, and the availability of **vacant ecological niches**, favoured occupation of niches by those organisms showing the appropriate **adaptive variations**.
- This phenomenon of **rapid diversification** into a multitude of new forms, **filling the different environmental niches**, is called **adaptive radiation.**
- Incidentally, Darwin also visited yet another group of volcanic islands, the **Cape Verde Islands, off West Africa**, that resembled the Galapagos in climate, soil and size. Yet the inhabitants are nothing like the inhabitants of Galapagos but resembled African species.
- Biogeography explains why two islands with similar environments in different parts of the world are populated not by closely related species but rather by totally different species that resemble those of the nearest mainland, the centre of origin.
- The **distribution of finches support the evolutionary deduction** of descent with modification from a common ancestor because the finch species didn't emerge from the Galapagos islands but **came from a common ancestor from the mainland** which then **evolved into the different species** on the archipelago.

- **However** there are instances where **related forms are found in widely separated regions**. These **exceptional occurrences** can be **explained** by the hypothesis of **continental drift**, based on the concept of plate tectonics which we will discuss next.

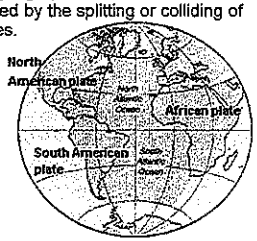
Continental drift

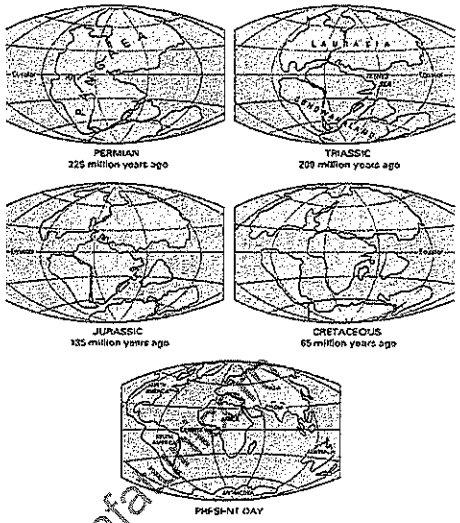
- Continental drift is the slow movement of the Earth's continents relative to each other over time as a result of movement of plates on which the continents float.
- About 250 million years ago, Earth had a single supercontinent called Pangaea. (Fig. 22) Over time, the landmass started to split to give rise to smaller continents.
- As continents drift apart, they will affect the migratory patterns and hence distribution of organisms.
- Our understanding of evolution and continental drift can be used to predict where fossils of different groups of organisms might be found. (Fig. 23)

Fig. 22 The continents have moved over the surface of the globe through geological time. The position of the main continents since the Permean period is shown in the figure on the right.

Geologists, who discovered that the movement of tectonic plates in the earth's crusts causes the migration of continents, later confirmed the continental drift hypothesis.

Many biogeographical observations can be explained by the splitting or colliding of landmasses.





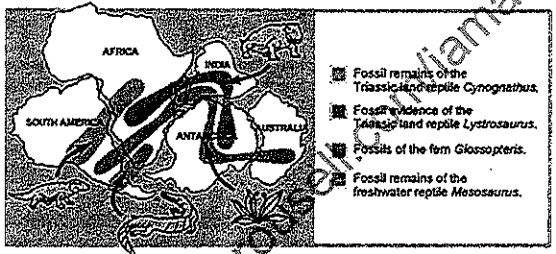
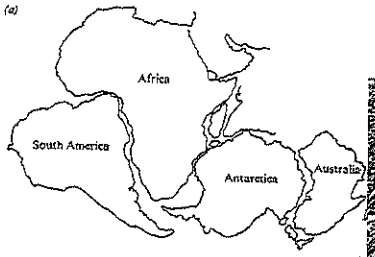


Fig. 23 Wegener, the person who in 1915 proposed the idea of continental drift found that the distributions of fossils of several organisms supported his theory that the continents were once joined together. Conversely, continental drift can be used to explain how species that spread out from one location ended up in different continents.

Evidence 3 Lungfish distribution as explained by continental drift.



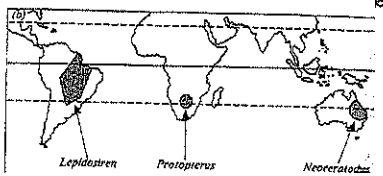


Fig. 24
a) Relative positions of South America, Africa and Australia during early stages of continental drift, indicating proximity of areas where lungfish may have originated.
b) Present distribution of species of lungfish.

- Related species are normally distributed within a region but in the case of the lungfish, they are found on different continents. How did this occur?
- *Neoceratodus* today is found only in Australia. However, fossils of this genus and other lungfish relatives have been found almost worldwide in Mesozoic strata, indicating that this group once had a much wider distribution. This being possible because the landmasses were connected in the past.
- Hence from the study of biogeography (and fossil record), it can be seen that the lungfish had a **common ancestor** that **dispersed from a centre of origin** when the continents were **still merged**. However due to **continental drift**, these ancestors of the lungfish become **geographically separated** and started to diverge and evolve through allopatric speciation.

In summary:

- To appreciate how biogeography supports the theory of evolution, you need to be able to see a **pattern in the geographical distribution of species, extinct or extant** (still existing), and **link** this pattern to an **evolutionary concept** such as descent from a **common ancestor**.

C. Preservation of Genetic Variation

LO:
(e) Explain how genetic variation (including recessive alleles) may be preserved in a natural population

1) How genetic variation *arises* in a natural population. (A recap.)

A. Mutations (Gene mutations, chromosomal mutations)

(i) Gene mutations

- These include **substitution**, **deletion** or **addition** of a nucleotide that **changes** the **triplet code** & hence the **amino acid**. Mutations in non-coding regions such as the promoter & enhancer can result in phenotypic variation as well.

(ii) Chromosomal mutations (worse than gene mutations)

a) **Polyploidy** - when **more than 2 homologous sets** of chromosomes are present e.g. triploids: 3n, tetraploid: 4n.
b) **Aneuploidy** - when a **particular chromosome** is **over-represented** or **under-represented** e.g. Trisomy 21(resulting in Down syndrome in humans)
c) **Deletion** - when a **segment of a chromosome** is **missing** e.g. a deletion in chromosome number 5 resulting in Cri-du-chat syndrome
d) **Duplication** - when an **extra segment** of a **chromosome** is **present**.
e) **Inversion** - when a chromosome segment is **detached, flipped around 180 degrees and reattached** to the rest of the chromosome
f) **Translocation** - when a segment from one chromosome is **detached & reattached to a different chromosome**. Therefore linkage relationships are altered.

B. Meiosis

(i) Independent assortment

- **Independent assortment & separation of homologous chromosomes** during metaphase I & anaphase I respectively.
- **Independent assortment & separation of sister chromatids** during metaphase II and anaphase II respectively.

→ results in gametes with numerous combinations of maternal & paternal chromosomes.

(ii) Crossing over between non-sister chromatids of homologous chromosomes results in more allelic combinations.

C. Sexual Reproduction

- **Random fusion of gametes** adds to the variety of genotypes. Different genotypes will result in different phenotypes and these will act as raw materials for natural selection to act on.

2) How genetic variation (including recessive alleles) may be *preserved* in a natural population

- Natural selection tends to reduce genetic variation by culling unfavourable phenotypes.
- What prevents natural selection from removing unfavourable alleles (including some recessive alleles)? The tendency for directional and stabilizing selection to reduce variation is countered by mechanisms that preserve or restore it.
  A. Diploidy/ Heterozygote Protection
  B. Balancing selection
     i. Heterozygote Advantage
     ii. Frequency-dependent selection

· dominant ⇒ D will be eliminated
· recessive ⇒ d will be preserved because

A. Diploidy / Heterozygote Protection

- Most eukaryotes are diploid, and a considerable amount of genetic variation is hidden from selection in the form of recessive alleles. **Recessive alleles** that are **less favourable than their dominant counterparts or even harmful** in the current environment **can persist** because they are propagated in heterozygous individuals where the **dominant allele masks the effect of the recessive allele**.
- Natural selection only acts on phenotypes. Recessive alleles are exposed to natural selection only when an individual carries 2 copies of this allele (i.e. homozygous recessive).
- Heterozygote protection maintains a huge pool of alleles that might not be favoured under the present conditions but some of which **could bring new benefits when the environment changes**.

B. Balancing selection

- Balancing selection occurs when natural selection **maintains two or more alleles at a locus**. Balancing selection can arise by the heterozygotes having a selective advantage, as in the case of sickle cell anemia – heterozygote advantage. It can also arise through frequency-dependent selection, where fitness depends on how common an allele is.

(i) Heterozygote Advantage (e.g. sickle-cell anaemia)

http://www.ornl.gov/sci/techresources/Human_Genome/posters/chromosome/hbb.shtml
http://sickle.bwh.harvard.edu/malaria_sickle.html

- When individuals who are **heterozygous** at a particular locus have **greater fitness than both kinds of homozygotes** (i.e., homozygous dominant and homozygous recessive), they exhibit heterozygote advantage. Heterozygote advantage is seen in sickle cell anaemia.

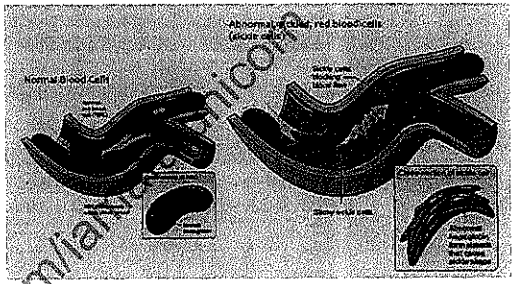
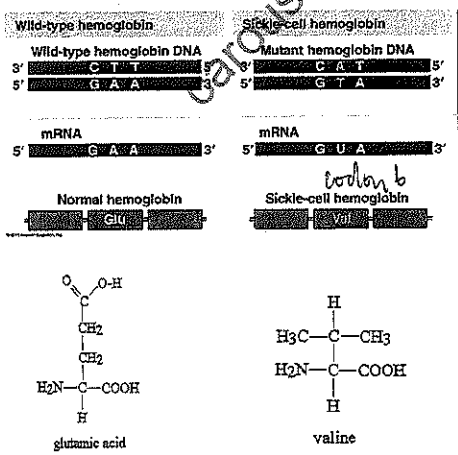


Fig. 25

Left: Sickled cells and normal cells. **Right:** Sickled cells can block blood flow to organs and damage them

- There are two beta globin alleles important for the inheritance of sickle cell anaemia: HbA and HbS. Individuals with two normal HbA alleles (HbAHbA) have normal haemoglobin, and therefore normal RBCs. Those with two mutant HbS alleles (HbSHbS) develop sickle cell anaemia. *Refer to previous notes for details.*



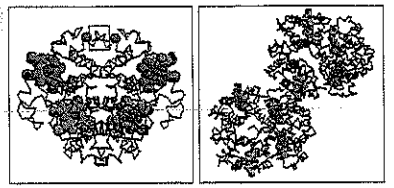


Fig. 26 Left: Mutant adult haemoglobin sequence (HbS) and normal sequence. *Note: the normal codon can also be GAG due to the degeneracy of the code.*
Top left: A normal HbA protein, the red box shows where the glutamic acid is located. **Top right:** 2 HbS protein molecules clumping together.
The R-group of glutamic acid contains a carboxylic acid and hence makes its R-group polar while the R-group of valine contains a methyl group and hence makes its R-group non-polar and hence hydrophobic

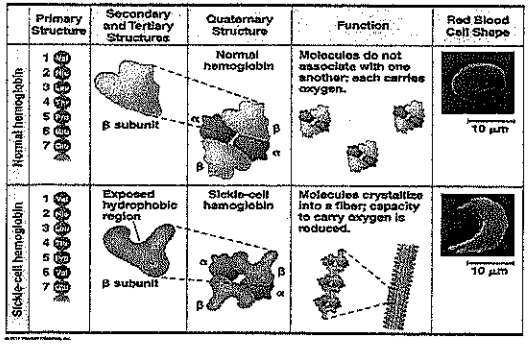


Fig. 27. Showing the formation of fibers.
The mutation results in a conformational change in the molecule such that the hydrophobic patch and groove are exposed. This conformational change makes it possible for the molecules to crystallise to form fibers as the hydrophobic patch of one molecule fits into the hydrophobic groove of another. These fibers distort the shape of the red blood cell resulting in the characteristics sickle shape

- Those who are **heterozygous** for the sickle cell allele (HbAHbS) produce both normal and abnormal haemoglobin. Heterozygous individuals are **usually healthy**, but they may suffer some symptoms of sickle cell anaemia under conditions of low blood oxygen, such as high elevation. Heterozygous (HbAHbS) individuals are said to be "**carriers**" or exhibit **sickle cell trait**.
- **Heterozygote advantage occurs in regions of endemic malaria**. Malaria is characterized by chills and fever, vomiting, and severe headaches. Anaemia and death may result. Malaria is caused by a protozoan parasite (*Plasmodium falciparum*) that is transmitted to humans by the *Anopheles* mosquito.
- The precise mechanism in which heterozygous genotype confers protection/survival advantage against malaria is unknown. A number of factors are likely involved. When malarial parasites invade the red blood cell, their metabolism lowers the oxygen tension within the red blood cells that contain defective haemoglobin causing it to sickle and be destroyed together with the parasites. The crystallization process of HbS also damages the parasites in the cell.
- In a region where malaria is prevalent, individuals with the HbAHbS genotype do not develop sickle cell anaemia and at the same time have **less chance of contracting malaria**. They are able to survive and reproduce in malaria-infected regions. Therefore, **BOTH the HbA and HbS alleles of these people remain in the population**. Thus, the HbS allele confers a survival advantage on people who have one copy of the allele, and the otherwise harmful HbS allele is therefore maintained in the population at a relatively high frequency.
- HbSHbS homozygotes have sickle cell anaemia, which usually results in early death.
- Compared to HbAHbS heterozygotes, people with the HbAHbA genotype (normal haemoglobin) have a greater risk of dying from malaria.

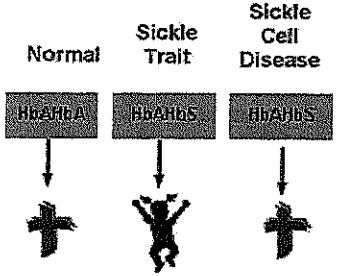


Fig. 28 Schematic representation of the effect of the sickle cell haemoglobin gene on survival in endemic malarial areas.
People with normal haemoglobin (left of the diagram) are susceptible to death from malaria.
People with sickle cell disease (right of the diagram) are susceptible to death from the complications of sickle cell disease.
People with sickle cell trait, who have one allele for HbA and one allele for HbS, have a greater chance of surviving malaria and do not suffer adverse consequences from the HbS allele.

- The frequency of the HbS allele in malaria-infected regions of Africa is 16%. The sickle cell allele is also widespread in the Mediterranean and other areas where malaria is or used to be

a major threat to life. In contrast, the HbS allele frequency is only 4% in the United States, where malaria has been virtually eliminated.

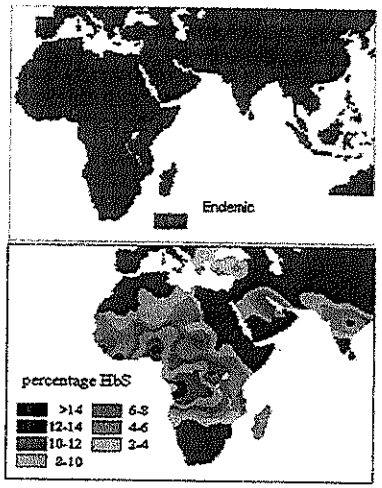


Fig. 29 showing distribution of malaria in Southern Europe, Asia and Africa.

Fig. 30 showing distribution of sickle-cell allele within the same area. The darker the shaded area, the greater the percentage of people carrying the allele. Note the correlation to the map in Fig. 34

- A common misstatement is that malaria selects for sickle cell disease. This is not true. In areas where there is no endemic malaria, a person with sickle cell disease is at an extreme survival disadvantage because of the ravages of the disease process. This means that a negative selection exists for sickle cell disease. Instead we should state that **sickle cell trait (i.e., the heterozygous condition) is selected for in regions of endemic malaria.**

(ii) Frequency-dependent selection

- In frequency dependent selection, the **fitness** (hence selective advantage) of the phenotype **depends on how common it is.**
- In Lake Tanganyika in Africa, the scale-eating fish (*Perissodus microlepis*) exhibits such frequency-dependent selection. These fish attack other fish from behind, darting in to remove a few scales from the flank of their prey. These fish are either "left-mouthed" or "right-mouthed". The **right mouthed allele is dominant to the left-mouthed allele.** The left-mouthed fish always attacks the prey's right side because its mouth twists to the left and vice-versa for the right-mouthed fish.
- The prey guards itself against attack from whatever phenotype of scale-eating fish is most common in the lake. So from year to year, **selection favours whichever mouth phenotype is least common.** As a result the frequency of left-mouthed and right-mouthed fish **oscillates over time** and frequency-dependent selection **keeps the frequency** of each phenotype **close to 50%.**

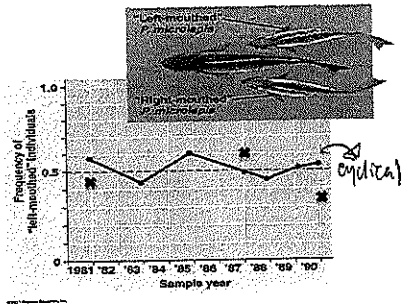


Fig. 31 Frequency dependent selection in scale-eating fish. A study by Japanese scientists show that the frequency of left-mouthed individuals rises and falls in a regular manner (as shown by the trend line). At each of the three time periods when phenotypes of breeding adults were assessed, adults that reproduced (shown by the crosses ✱) had the opposite phenotype of what was common in the population. Thus it showed that the right-mouthed individuals were favoured by selection when left-mouthed individuals were more common, and vice versa.

EXTENSION TOPIC A:

INFECTIOUS DISEASES

Learning outcome:

a. Describe the specific (adaptive) and non-specific (innate) immune systems including active and passive, natural and acquired immunity.

b. Outline the roles of B lymphocytes, T lymphocytes, antigen-presenting cells and memory cells in specific primary and secondary immune responses.

c. Explain the relationship of the molecular structure of antibodies to their functions, using IgG as an example.

d. Explain how genetic recombination during development results in millions of different antibody molecules (including somatic recombination, hypermutation and class switching).

e. Discuss how vaccination can control disease (e.g. in the eradication of small pox), limited to vaccination stimulates immunity without causing the disease and vaccination of a high enough proportion of the population can break the disease transmission cycle.

f. Discuss the benefits and risks of vaccination.

g. Explain how viruses, including influenza and HIV, cause diseases in humans through the disruption of host tissue and functions (e.g. HIV and T helper cells, influenza and epithelial cells of the respiratory tract).

h. Explain the mode of transmission and infection of bacterial pathogens, using *Mycobacterium tuberculosis* as an example.

i. Describe the modes of action of antibiotics, including penicillin, on bacteria.

References:

1. Murphy KM, P Travers, M Walport (Eds.) Janeway's Immunobiology. 8th Edition.
2. Judith Owen, Jenni Punt, Sharon Stranford. Kuby Immunology, 7th Edition.
3. Pommerville, Jeffrey C. Alcamo's Fundamentals of Microbiology: Body Systems.

Table of Contents

1. Introduction

- Infection is the process where a pathogen invades and multiplies in its host, as well as the reaction of the cells in the host to the pathogen.
- **Pathogens** are microorganisms **invading the body to cause diseases.** The 5 classes of pathogens are viruses, bacteria, fungi, protozoa and worms.
- Intracellular pathogens (e.g. viruses, certain bacteria) invade cells, where they utilise the metabolic machinery (e.g. protein synthesis) of the host cells to replicate within it.
- Extracellular pathogens (e.g. certain bacteria) divide outside cells in the tissue fluid or blood.
- **Antigens** are substances that **induce an immune response** as they are **recognised by cells** of the immune system and **antibodies.** An antigen can be a foreign protein, carbohydrate, lipid or nucleic acid.
- One pathogen has many different components that can be recognised by cells of the immune system i.e. many different antigens. E.g. a bacterium's antigens can be found on its cell wall and flagellum, a virus's antigens can be found on its viral glycoprotein.
- Cells of the immune system and antibodies usually do not bind to the entire antigen molecule. **Epitopes** are the **parts on a single antigen** that binds to the antigen-binding site of an antibody or T cell receptor. An epitope is also known as an antigenic determinant. (*See Figure 1*)

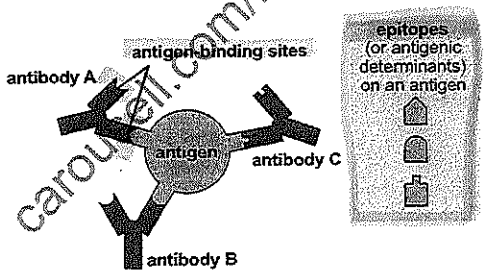


Figure 1. An antigen has many epitopes. (Source: Campbell's Biology, Chapter 43, 8th Edition)

- Immunology is the study of the body's defense against infection at cellular and molecular levels. The following are the questions we would address in this topic:
  - How does the body prevent pathogens from entering the body?
  - When infection occurs, how does the body eliminate the pathogen?
  - How does the immune system protect the body by developing long-lasting immunity to many pathogens encountered?

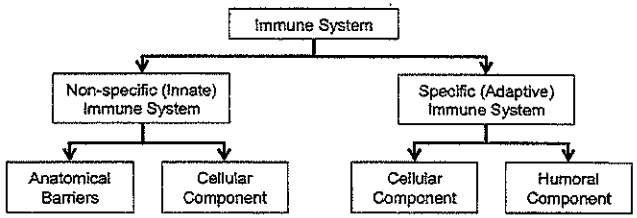


Figure 2. Immune system is typically divided into two categories, innate and adaptive. Both have distinctions but are not mutually exclusive.

- The non-specific (innate) immune system has anatomical features that function as barriers to infection.
- "Humoral" comes from the term, humour, which means body fluid. Thus, "humoral" refers to molecules found in extracellular fluid such as secreted antibodies.
- Although the innate and adaptive immune systems have distinct characteristics, there is interplay between these two systems. Components of innate immune system influence the adaptive immune system and vice versa. Section 2 below introduces some of these components.

2. Components of the Immune System

- Most cells of the immune system are derived from **haemopoietic stem cells** and develop through the process of **differentiation** into functionally mature blood cells of different lineages. (*See Figure 3a*)

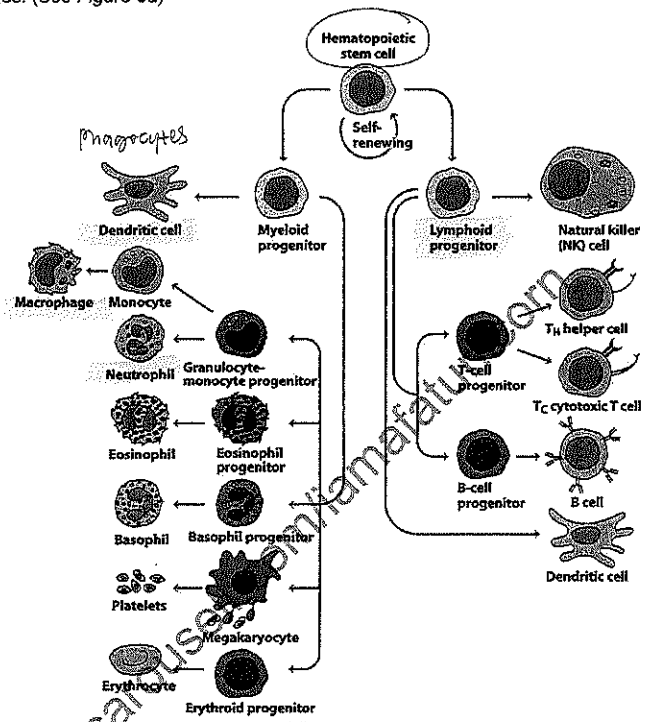


Figure 3a. Haematopoietic stem cell differentiates to either myeloid progenitor cell or lymphoid progenitor cell. Progenitor cells cannot self-renew and are committed to a particular cell lineage. (Source: Kuby Immunology 7th Edition)

- Cellular components of innate immunity
  - ➢ Comprises of **phagocytes,** cells capable of engulfing pathogens via phagocytosis *(See section 3)*
    - **Macrophages and monocytes**
      Monocytes circulating in the blood matures into macrophages that resides within the tissues.
    - **Dendritic cells**
      Resides within tissues.
    - **Neutrophils**
      Circulates in the bloodstream, and migrates to sites of infection.

- Cells involved in adaptive immunity
  - ➢ Comprises of **lymphocytes** (*See section 5*)
    - **T lymphocytes (T cells)**
    - and **B lymphocytes (B cells).**
  - ➢ Lymphocytes can be classified into: (*See Figure 3b*)
    1. naïve cells,
    2. effector cells
    3. and memory cells.
    - **Naïve cells** have not encountered the specific antigen that they are programmed to respond to. E.g. naïve T cells and naïve B cells
    - **Effector cells** have been activated by antigen presenting cells (APCs) or antigens and ready to function in an immune response. E.g. Helper T cells, cytotoxic T cells, plasma cells
    - **Memory cells** are generated following primary response to antigen. They are lymphocytes that mediate a secondary response to subsequent encounters with the antigen. E.g. memory B cells and memory T cells

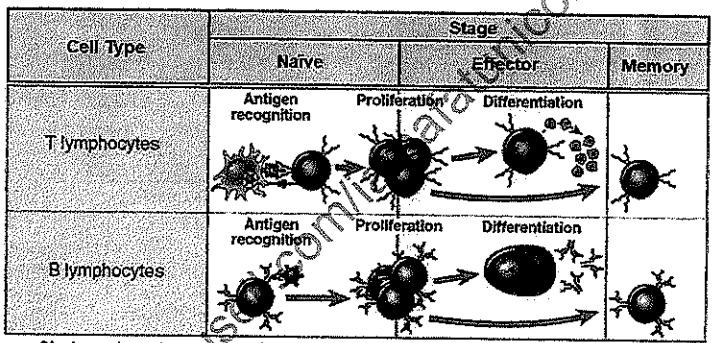


Figure 3b. Lymphocytes can be classified into naïve cells, effector cells and memory cells.

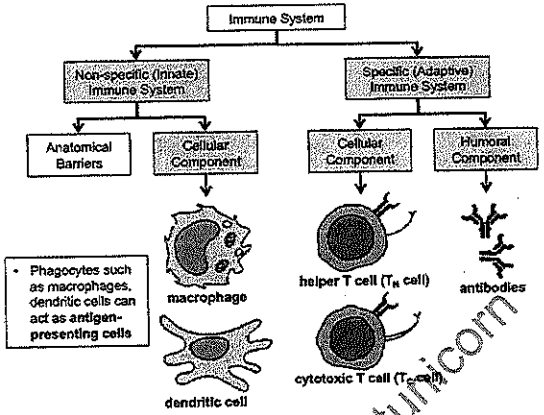
- B cells and T cells are derived from **stem cells** in the **bone marrow.** However, T cells and B cells develop through the process of **differentiation** in different locations.
  - ➢ When these stem cells remain in the **bone marrow**, they differentiate into B cells. (*For additional info about what happens during the development of B cells, see section 6B1.*)
  - ➢ When these stem cells migrate to the **thymus**, they differentiate into T cells. Thymus is an organ located between the lungs. T cells eventually differentiate into two types of T cells:
    - Helper T cells (also known as CD4+ T cells).
    - Cytotoxic T cells (also known as CD8+ T cells).

- B cells and T cells that finally enter the blood circulation have unique or specific cell surface receptors that bind and respond to foreign antigens.

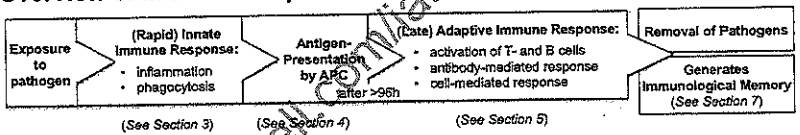
- Humoral components of adaptive immunity (*See section 5B*)
  - ➢ Comprises of **antibodies**, also known as **immunoglobulins.**
  - ➢ Antibodies are proteins are secreted by plasma cells.

Bite-Size Summary of Section 2

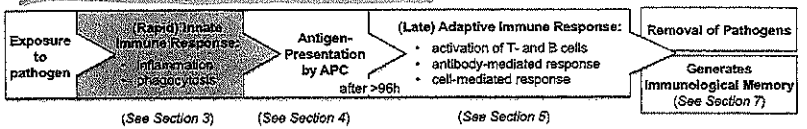
What are the components involved the non-specific and specific immune systems?



Overview of Immune Response



3. Non-Specific (or Innate) Immunity



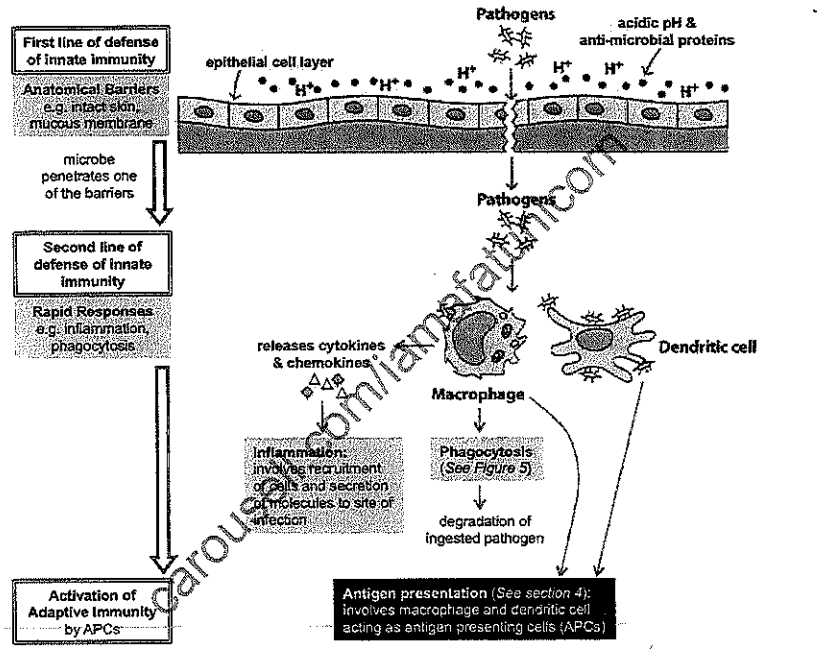


Figure 4. Overview of the innate immune responses. (Modified: Kuby Immunology 7th Edition) Additional information: Cytokine is a small protein secreted by a cell that affects the behavior of other cells. Chemokine is a small chemoattractant protein that stimulates the migration and activation of cells such as phagocytic cells and lymphocytes.

- The non-specific (innate) immune system is the first line of defense upon exposure to a pathogen. Any pathogen that breaches the anatomical barrier (e.g. skin is the first line of defense of innate immunity) will be greeted by cells of innate immunity (e.g. macrophage is the second line of defense of innate immunity).

- This response to an encounter with a pathogen occurs **rapidly**. The first line of host defense is immediate as it consists of mechanisms that are **ready to kill** and resist an invader at any time.
  - ➢ Macrophages are always present under the skin and other tissues to function as "guards" against any pathogen that has penetrated the anatomical barrier.

- Innate immunity is **not specific** to any pathogen and has **no memory.**

- First line of defense of innate immunity (anatomical barriers to infection): *(See Figure 4)*
  - ➢ Includes physical and chemical barriers to **prevent the entry** of pathogens.
  - ➢ Physical barriers include:
    - Epithelial cell layers such as skin.
    - Mucous membranes lining the respiratory tract and gastrointestinal tract.
    - Secretions such as saliva and tears wash away potential invading microorganisms to prevent their attachment.
  - ➢ Chemical barriers include:
    - Antimicrobial substances in secretions to kill these microorganisms.
      For example, lysozyme is an antibacterial protein that cleaves glycosidic bonds of peptidoglycans in cell walls of bacteria, leading to lysis.
    - Acidic pH.
      For example, low pH in stomach causes denaturation of proteins in pathogens.

- Some pathogens may have evolved strategies to penetrate the epithelial cell barrier, or wounds may have disrupted the epithelial layer to allow pathogens to enter the body. The second line of defense occurs to **kill the pathogen**, and thus prevent multiplication of pathogen within the body.

- Second line of defense of innate immunity:
  - ➢ Cellular components of innate immunity comprises of **phagocytes** such as **macrophages, dendritic cells and neutrophils.**
  - ➢ When pathogens breach the epithelial barrier, phagocytes residing in tissues, e.g. macrophages and dendritic cells recognise and engulf these pathogens via **phagocytosis.** *(See Figure 5a)*
    - Macrophages and dendritic cells act as antigen-presenting cells. *(See section 4)*
  - ➢ Macrophages also induce **inflammation** by secreting signaling proteins e.g. cytokines and chemokines.
    - Signaling proteins such as cytokines and chemokines results in increased permeability of blood vessels, to recruit neutrophils from blood to the site of infection. *(See Figure 5b)*
    - Inflammatory response is characterized by heat, pain, redness and swelling.

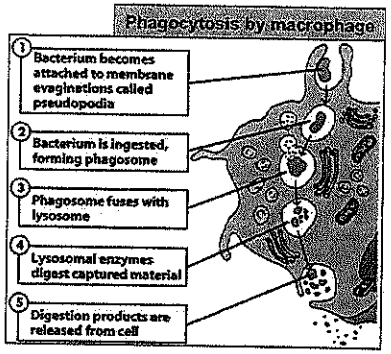


Figure 5a. Phagocytosis and degradation of pathogen

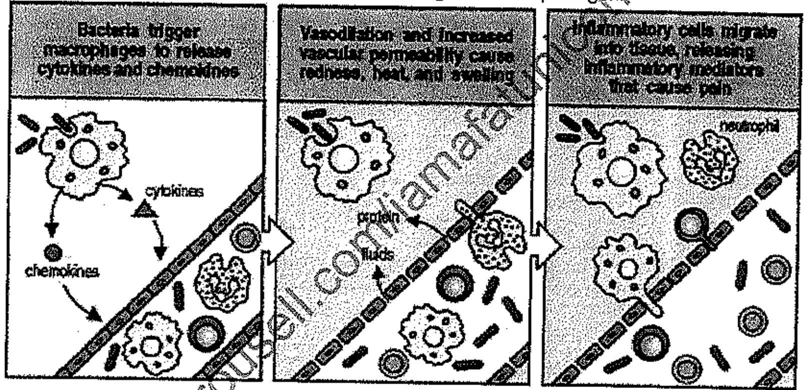
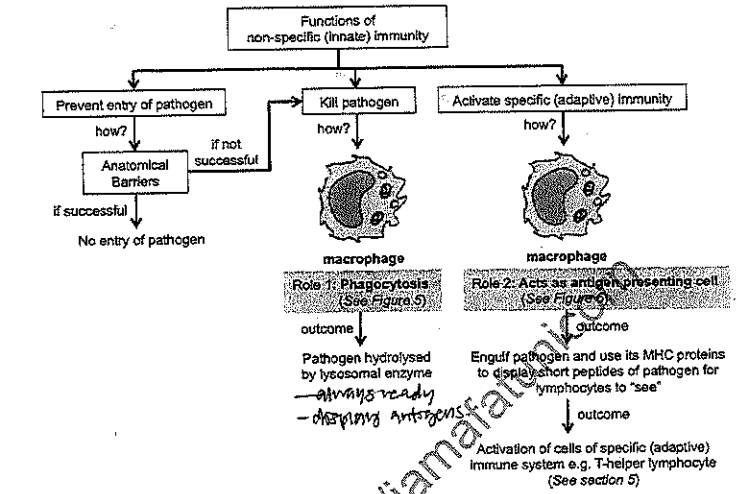


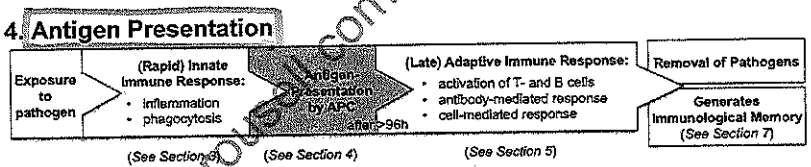
Figure 5b. Inflammatory Response

- The innate immune system also initiates steps to bring the pathogen to the attention of lymphocytes to **activate the adaptive immune system** by **antigen presentation.** *(See section 4)* This is to resolve the infection successfully should the pathogen evades innate immune responses.

Bite-Size Summary of Section 3

Although we have anatomical barriers to prevent entry of pathogens, pathogens may enter the body through wounds. What happens when pathogens penetrate the anatomical barrier?





- **Antigen presentation** is the display of peptides of antigen bound to membrane proteins called MHC (major histocompatibility complex) proteins on the surface of an antigen-presenting cell (APC). This allows specific recognition by T cell receptors (TCRs) on naïve T cells and thus activation of these T cells to effector cells.
  - Naïve cells have not encountered the specific antigen that they are programmed to respond to.
  - Effector cells have been activated by APCs or antigens and ready to function in an immune response

- **Antigen-presenting cells (APCs)** are a specialised group of cells that **take up antigens and process ('cut up') them** into short peptides before **presenting the peptides on MHC to lymphocytes** for recognition.

- APC is a link between the innate and adaptive immune systems. Macrophages and dendritic cells are APCs.

- Role of **macrophages and dendritic cells as antigen-presenting cells** (APCs) in antigen presentation: (*Follow the numbering on Figure 6*)
  (1) After an APC engulf the pathogen by phagocytosis, it can process ('cut up') antigens from the surface of the pathogen into short peptides.
    - Antigens on pathogen are processed into short peptides inside the phagolysosome (formed by fusion of phagosome and lysosome).
      Note: Phagolysosome is not shown in Figure 6.
  (2) Peptides of antigens bind to MHC protein inside endoplasmic reticulum (ER) to form peptide:MHC complex.
  (3) Peptide:MHC complex is transported to the cell surface membrane of APC via vesicles.
  (4) The antigens of pathogen on the cell surface membrane of APC are ready for presentation to naïve T cells. This is possible because the antigen of pathogen on MHC protein is complementary conformation to the TCR. (*See Figure 7*)

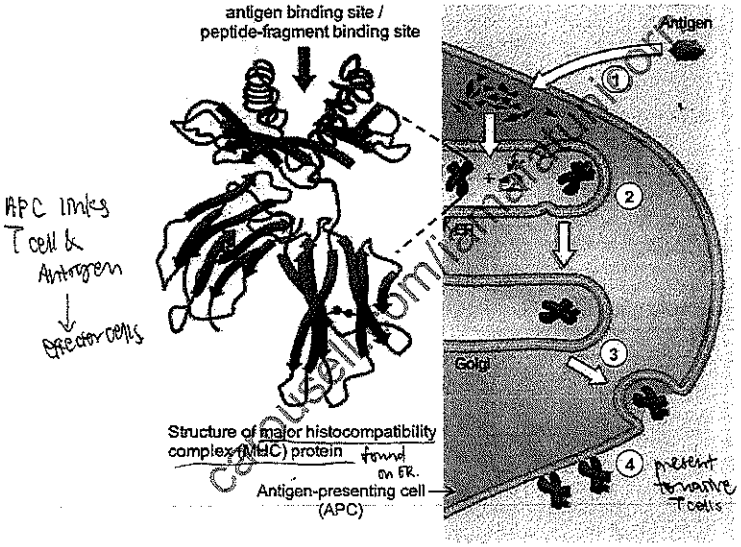


Figure 6. The expression of antigen on MHC protein at the cell surface membrane of APC.

- The peptides of antigens must be bound to MHC proteins in order to be recognised by TCR of naïve T cells. (*See Figures 7 and 9*)

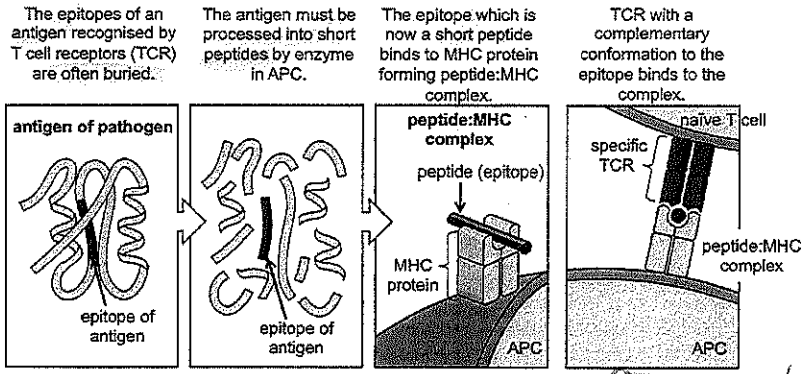


Figure 7. A naïve T cell does not bind to the entire antigen molecule. Epitopes are the parts on a single antigen that contact the antigen-binding site of T cell receptor (TCR). (Modified: Janeway's Immunology 8th Edition)

- Upon naïve T cell activation, the T cell undergoes **clonal expansion** and **differentiation** into effector T cells that are ready to function in the adaptive immune response (*See Figure 8 and section 5A*).
  - Clonal expansion occurs when a single cell stimulated to undergo proliferation via mitosis to produce large numbers of genetically identical daughter cells.
  - **Cytokines** e.g. interleukin (IL) are signaling molecules released by certain cells of the immune system to aid in cell communication. IL is released during T cell activation to stimulate proliferation and differentiation of T cells themselves.

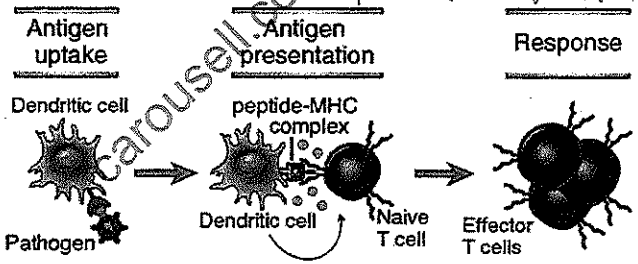
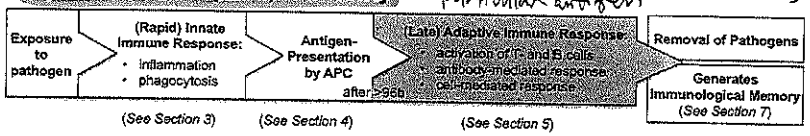


Figure 8. Dendritic cell acts as APC to display antigen on MHC protein to naïve T cell. (Modified: Cellular and Molecular Immunology, Abul K. Abbas)

5 Specific (or Adaptive) Immunity



- When pathogens evade the non-specific (innate) immune response, the specific (adaptive) immune response begins.
- The innate and adaptive immune responses make up an integrated host defense system where cells e.g. APC link the two immune systems. Innate immune response sets the scene for the induction of adaptive immune response which requires a few days to establish.
- Adaptive immune response involves the activation of T cells and activation of B cells with the eventual production of specific cytotoxic T cells and antibodies to clear the infection.
- The specific (adaptive) immunity consists of **cell-mediated immune response** and **humoral response**.
  - Cell-mediated immune response is mediated/brought about by T cells. This response will protect against intracellular pathogens by killing cells that contain these pathogens. (*See section 5A*)
  - Humoral response is mediated/brought about by the antibodies that are secreted by plasma cells. This response will protect against extracellular pathogens and toxins secreted by pathogens into extracellular space. (*See section 5B*)
- Cellular components of adaptive immunity comprises of **lymphocytes**.
  - **T cells**
    - Development from stem cells to T cells occur in the thymus.
    - Each T cell has a specific type of T cell receptors on its cell surface that can recognise and bind to a specific antigen.
  - **B cells**
    - Development from stem cells to B cells occur in the bone marrow.
    - Each B cell has a specific type of B cell receptor on its cell surface that can recognise and bind to specific antigen.
  - B cell receptor is structurally different from T cell receptor. B cell receptor also binds to a different epitope of an antigen from T cell receptor.
- Humoral components of adaptive immunity comprises of **antibodies**. Antibodies are also known as **immunoglobulins (Ig)**.
- **Cytokines** e.g. interleukin (IL) are signaling molecules released by certain cells of the immune system to aid in cell communication. Cytokines produced during adaptive immunity are involved in the **clonal expansion** and **differentiation** of activated lymphocytes.
- Key characteristics of adaptive immunity are:
  - high level of **specificity** for a particular pathogen
  - shows **memory** (i.e. remembers the particular pathogen that previously invaded the body and **responds more quickly** to the re-exposure to the **same pathogen**)

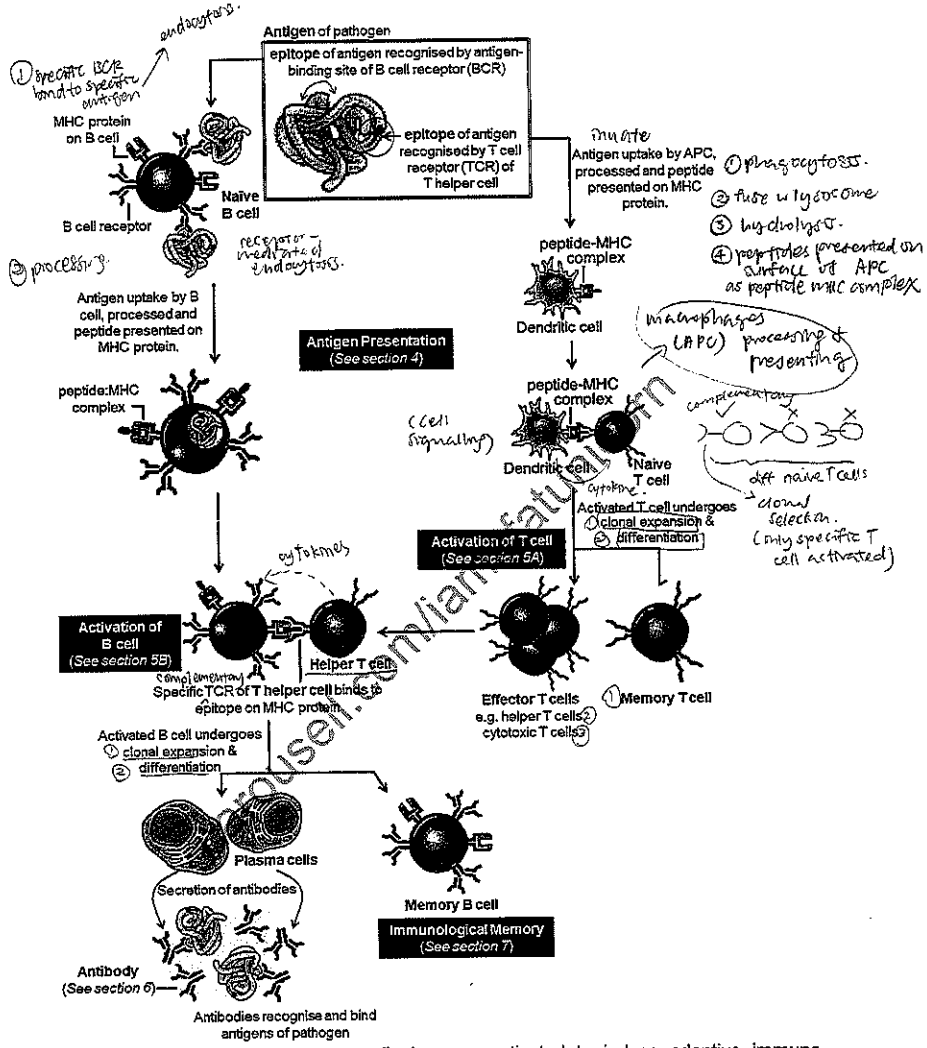


Figure 9. Overview of how naïve cells become activated to induce adaptive immune response.

5A. Role of T Cells in Specific (or Adaptive) Immunity

- Recall from *Section 4* and *Figure 6* that **antigen-presenting cells (APCs)** e.g. macrophages and dendritic cells are phagocytic cells that:
  engulf pathogens and process ('cut up') the **antigens** from the surface of the pathogen into short peptides
  ↓
  peptides of **antigens are bound to MHC proteins** to form peptide:MHC complex
  ↓
  peptide:MHC complex at the cell surface membrane of APC
  ↓
  APC **presenting** the short peptides from **antigen to naïve T cells** for recognition

- Only the naïve T cell with **T cell receptor (TCR)** that is **complementary in conformation** to the peptide displayed on MHC protein on APC can bind.

- After antigen presentation, the specific naïve T cell becomes **activated** to undergo **clonal expansion** (i.e. a single cell stimulated to undergo proliferation to produce large numbers of genetically identical daughter cells) and **differentiation** into **effector cells:** (*See Figure 10*)
  - Cytotoxic T cells (cell mediated response)
  - Helper T cells (bridge to humoral response)
  - Memory T cells (immunological memory. *See section 7*)

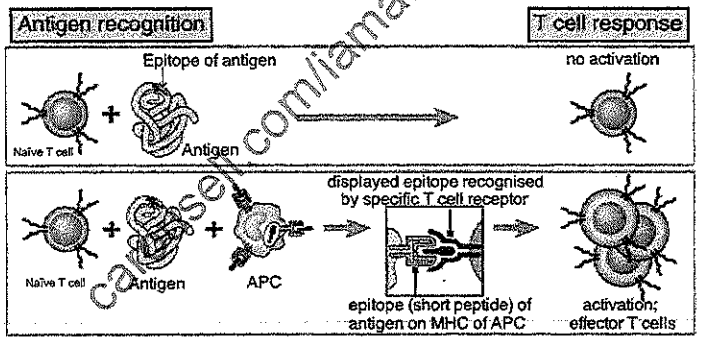


Figure 10. APCs are required for T cell activation to form effector T cells. The antigen has to be processed into short peptides and the epitope displayed on MHC protein, before the complementary T cell receptor on the surface of T cells can recognise and bind. (Modified: Cellular and Molecular Immunology, Abul K. Abbas)

Role of Cytotoxic T cells in Cell-mediated Immune Response

- Cytotoxic T cells **kill infected target cells** i.e. the cells infected by the intracellular pathogens e.g. viruses and some bacteria that multiply in the cytoplasm of host cells. This **prevents the reproduction of the intracellular pathogens.**
  - Cytotoxic T cells can **recognise** these infected target cells because these target cells display the short peptides from antigen of pathogen presented on MHC.

- The T cell receptor is complementary in shape to the peptide:MHC complex found on the target cells. (*See Figure 11*). Specific T cell receptor binds to the peptide:MHC complex
- In contrast, defense against the virus that is still in the circulation (i.e. has not penetrated its host cell) will be mediated by antibodies.

- Cytotoxic T cells can also **kill tumour cells.**

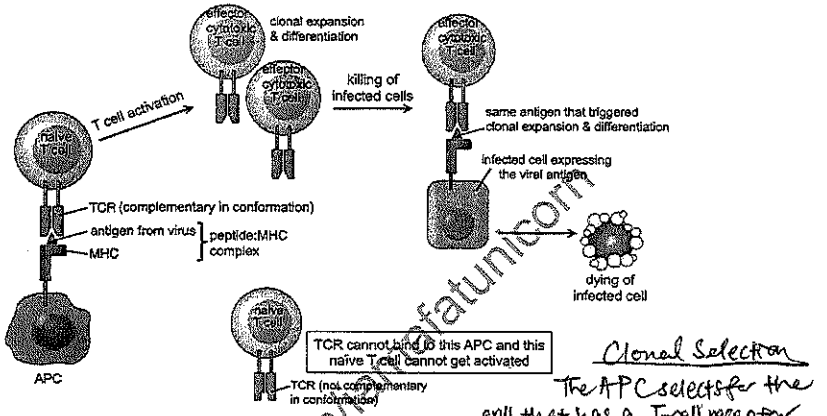


Figure 11

Clonal Selection
The APC selects for the cell that has a T-cell receptor that is complementary to its peptide:MHC complex

- **Cytotoxic T cells** kill infected target cells by releasing: (*See Figure 12*)
  - **perforins** – molecules which makes pores in the infected cell's cell membrane
  - **granzymes** – diffuses in via the pores and activate enzymes involved in **apoptosis**

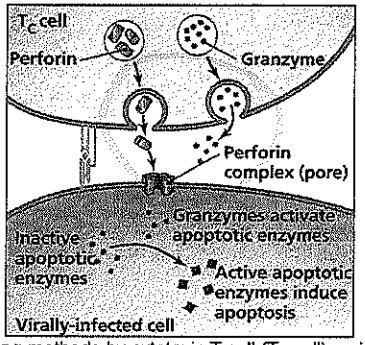


Figure 12. Two killing methods by cytotoxic T cell (T_C cell) on infected target cell.

Role of Helper T cells in Humoral Response

- Helper T cells **activate specific B cells** to make antibodies for **humoral response**. (*See Figure 9 and Figure 13*)
  - Helper T cells do not directly participate in destruction of pathogens.

- How do helper T cells activate specific B cells? (*See Figure 13*)
  - Helper T cell with specific T cell receptor binds to the peptide:MHC complex on cell surface membrane of B cells.
  - This specific Helper T cell secretes **cytokines** that stimulate B cells to undergo clonal expansion and differentiation.

- After carrying out their respective functions, at least 90% of these effector cells die by apoptosis and the rest differentiate to **memory T cells.**
  - Memory cells are generated following primary response to antigen. They are long-lived lymphocytes that mediate a secondary response to subsequent encounters with the antigen. (*See section 7*)

T helper cells secrete cytokines, stimulate/activate macrophages to attack infected cells

5B. Role of B Cells in Specific (or Adaptive) Immunity

- 2 processes are required to activate B cells:
  1) antigen that binds to the specific B cell receptor
  2) Helper T cell with specific T cell receptor that binds to the peptide:MHC complex on cell surface membrane of B cells

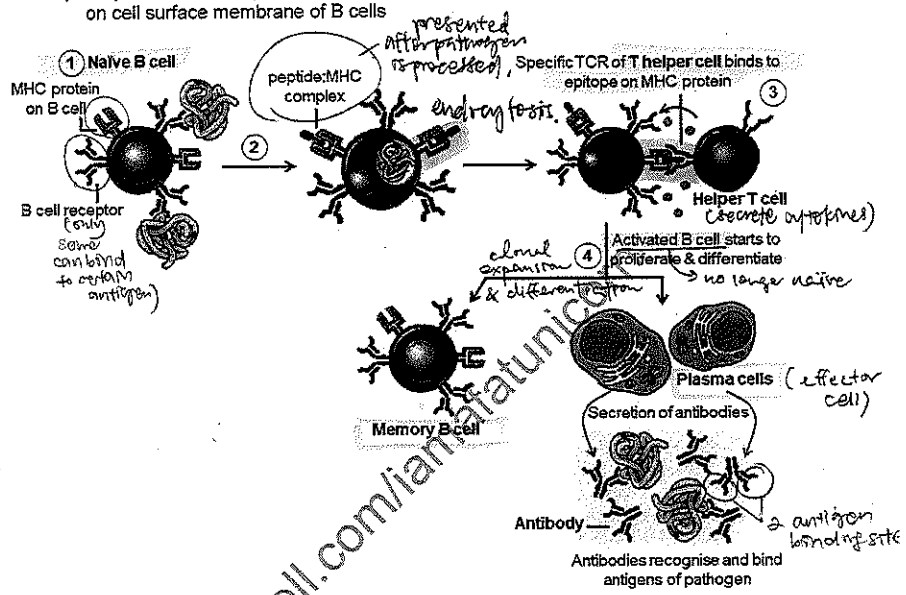


Figure 13. Helper T cells are required to stimulate activation of B cells to proliferate and differentiate into antibody-secreting plasma cells. (Part of Figure 9)

- B cell activation: *(See Figure 13)*
  (1) Each B cell has one specific type of B cell receptor (BCR) on its cell surface that can recognise an epitope and bind to the specific antigen. When a B cell encounters the antigen with epitope that is complementary to its BCR, antigen binding occurs.
  (2) The BCR together with the bound antigen are endocytosed into the B cell. The antigen is processed into short peptides and attached to MHC proteins.
  (3) The peptide:MHC complexes are transported to the cell surface membrane of B cell where the peptide is recognised by antigen-specific helper T cell. (Recall that antigen-specific helper T cells are derived from activation of naïve T cells through antigen presentation discussed in *section 5A*.)
  (4) The interaction between helper T cell and B cell stimulate cytokine secretion from helper T cell. In turn, these cytokines activates the antigen-specific B cell to undergo clonal expansion and differentiation into effector cells:
    - antibody-secreting **plasma cells**
    - **memory B cells.**

- Each B cell has one specific type of B cell receptor (BCR) that can recognise specific antigens of a pathogen. The BCR has antigen-binding sites that can only bind complementary epitope of the antigen. *(See left of Figure 14)*

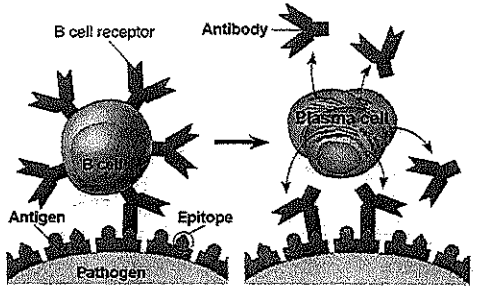


Figure 14. Upon B cell activation with the help from Helper T cell, B cell differentiates to antibody-secreting plasma cell.

- In **humoral response**, the antibodies secreted by plasma cells protect the body against extracellular pathogens and toxins secreted by pathogens into extracellular space.

- The antigen-binding sites of the BCR and the antigen-binding sites of the antibody secreted from plasma cell are the same. The difference between BCR and the secreted antibody lies in their constant region of heavy chains due to an event, class switching. *(Class switching will be discussed later in section 6B3).*

- Antibodies mediate the clearance and destruction of pathogens in the following ways e.g.
  - **Neutralisation to prevent entry**. *(See Figure 17)*
    - Antibodies recognise and bind to the antigens on the **pathogen or bacterial toxins** to prevent the pathogen or toxin from attaching to specific host cell receptor and gain entry into host cell.
  - **Opsonisation to promote phagocytosis.** *(See Figure 18)*
    - Opsonisation is the process of binding of antibodies to the antigens on pathogen to tag the pathogen for uptake by phagocytes.
    - The Fab portion of the antibody binds to the specific antigen while the Fc portion of the antibody binds to the Fc receptor on the phagocyte to promote phagocytosis. *(See Figure 16 for location of Fab and Fc of an antibody).*

6. Types of Immunity

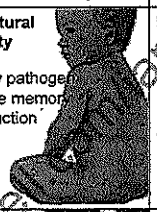
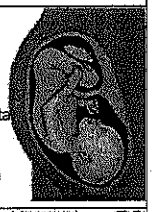
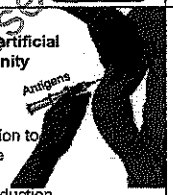
- The type of immunity described so far occurs during the course of an infection when an immune response to antigens activates lymphocytes and antibodies are produced by plasma cells of the infected individual. This is known as **active immunity**.

- Antibodies can also be produced by another individual and transferred to the recipient without the participation of the recipient's immune system. E.g. transfer of IgG antibodies from mother to fetus across the placenta. This is known as **passive immunity.**

- The table below shows the differences between active immunity and passive immunity.

	Active Immunity	Passive Immunity
Antibody production	Antibodies are **produced by the individual's own immune system** in response to antigens introduced naturally or artificially	Antibodies are **transferred to the recipient** without the participation of the recipient's immune system. E.g. (1) Fetus receives IgG antibodies from mother through transfer across the placenta. (2) Newborn receives antibodies such as IgA from mother through the breast milk.
Duration	Immunity conferred is long-lasting since memory cells are formed in the individual after primary immune response to antigen.	Immunity conferred is short-lived since there is no formation of memory cells.

- Immunity is also classified into **natural** and **artificial immunity**.
- The table below shows an overview of the 4 different types of immunity and their corresponding examples.

	Active immunity	Passive immunity
Natural immunity	**Active, natural immunity** infected by pathogen to stimulate memory cells production 	**Passive, natural immunity** • fetus receives antibodies through placenta • newborn receives antibodies from breast milk 
Artificial immunity	**Active, artificial immunity** receive vaccination to stimulate memory cells production 	**Passive, artificial immunity** e.g. kitten bit by venemous snake & doctor gave anti-venom receive anti-serum with antibodies from another host 

7. Antibodies (or Immunoglobulins)

- Antibodies belong to a family of globular proteins called immunoglobulins (Ig). They are secreted by plasma cells to mediate the clearance and destruction of pathogens in a variety of ways (*Discussed in section 5B*).
- There are 5 different types (or classes) of antibodies, IgG, IgM, IgA, IgD and IgE. (*See Figure 15*) IgG is the predominant antibody in blood serum.

	IgG	IgM	IgA	IgD	IgE
Secreted forms of Ig					
Heavy chain	gamma chain	mu chain	alpha chain	delta chain	epsilon chain
Light chain	kappa chain or lambda chain				
No. of antigen binding sites	2	10	4	2	2

Figure 15. Different types (or classes) of immunoglobulins.

- Recall: each B cell has one specific type of B cell receptor (BCR) on its cell surface that can recognise an epitope and bind to the specific antigen.
  - ➢ This means that each B cell produces only one type of heavy chain and one type of light chain.

7A. Structure of IgG (or Immunoglobulin G)

- IgG is a globular protein with **quaternary structure.** (*See Figure 16*)
  - ➢ Consists of **4 polypeptide chains** (2 identical heavy chains and 2 identical light chains).
  - ➢ Each light chain pairs with a heavy chain, held together by disulfide bonds and non-covalent interactions e.g. hydrogen bonds, hydrophobic interactions, ionic bonds.
  - ➢ The 2 heavy chains are linked by disulfide bonds and non-covalent interactions.
- Each light chain is divided into 2 regions, the variable (V_L) region and constant (C_L) region.
  - ➢ Each light chain is folded into its specific tertiary structure to form part of the antigen-binding site of antibody.
  - ➢ Tertiary structure is maintained by disulfide bonds and non-covalent interactions.
  - ➢ The 3D conformation of the light chain is determined by its primary structure.

- Each heavy chain is divided into 2 regions, the variable (V_H) region and constant (C_H) region.
  - Each heavy chain is folded into its specific tertiary structure to form part of the antigen-binding site of antibody.
  - Tertiary structure is maintained by disulfide bonds and non-covalent interactions.
  - The 3D conformation of the heavy chain is determined by its primary structure.

- In the quaternary structure, one V_H and one V_L are brought together to form an **antigen-binding site**. (*See Figure 16*)
  - Heavy chain and light chain are covalently linked by disulfide bonds at the constant regions of both chains.
  - The antigen-binding site is specific to bind to a complementary epitope of an antigen.
  - Each antibody has **2 identical antigen-binding sites.**

- Each IgG has 2 Fab (fragment of antigen-binding) portions and 1 Fc (fragment crystallisable) portion. Fab and Fc portions are joined at the **hinge region**, which is a short sequence of amino acid residues. (*See Figure 16*)
  - This hinge region enables the antibody molecule to be **flexible**. Flexibility at the hinge region allows Fab portion to adopt a wide range of angle, thus allowing antibody to **bind to antigens** that are spaced at variable distances apart.

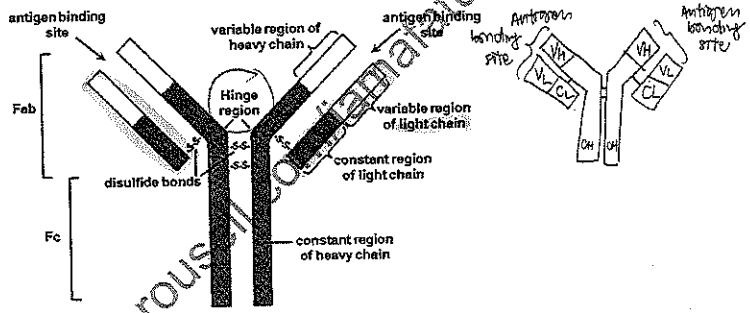


Figure 16. Structure of IgG. Fab (for fragments of antigen-binding), Fc (for fragment crystallisable)

- Each IgG has 2 Fab (fragment of antigen-binding) portions and 1 Fc (fragment crystallisable) portion. (*See Figure 16*)
  - **Fab** portion of the antibody contains antigen-binding site to **bind to epitope of antigen**. Thus, Fab allows antibody to bind to and prevent the pathogen or toxin from attaching to host cell receptor. This is known as **neutralisation** of pathogen or toxin. (*See Figure 17*)
  - After antibodies bind to a pathogen, **Fc** portion of these antibodies **binds to the Fc receptor** on the phagocyte to **promote phagocytosis**. This is known as **opsonisation**.

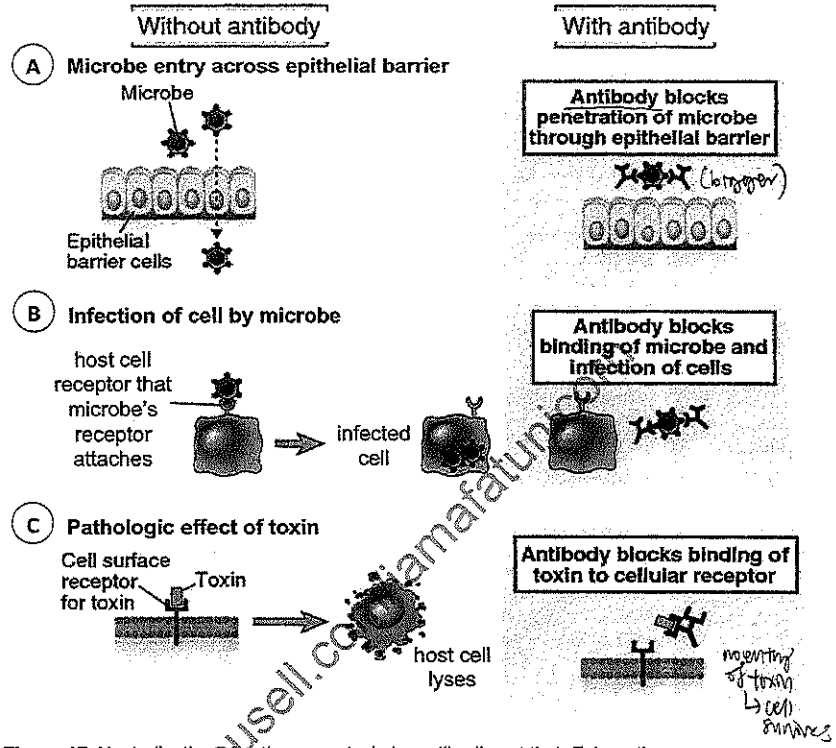


Figure 17. Neutralisation of pathogen or toxin by antibodies at their Fab portions.

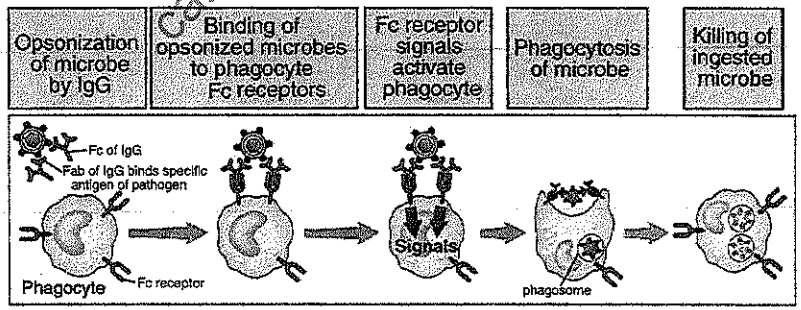


Figure 18. Opsonisation by antibodies to promote phagocytosis to destroy the pathogen.

7B. Generation of Vast Repertoire of Antibodies

- Chromosomal locations of Ig genes in human:
  - ➢ Heavy chain gene locus is on chromosome 14;
  - ➢ Kappa (κ) light chain gene locus is on chromosome 2;
  - ➢ Lambda (λ) light chain gene locus is on chromosome 22.

- A **heavy chain gene** contains:
  - ➢ **V** (variable) **gene segments,**
  - ➢ **D** (diversity) **gene segments,**
  - ➢ **J** (joining) **gene segments,**
  - ➢ and **C** (constant) **gene segments.**
- A **light chain gene** contains:
  - ➢ **V** (variable) **gene segments,**
  - ➢ **J** (joining) **gene segments,**
  - ➢ and **C** (constant) **gene segments.**

- The **multiple gene segments** contribute to the vast number of heavy chain gene and light chain gene diversities. (*See mechanism in section 6B1*)

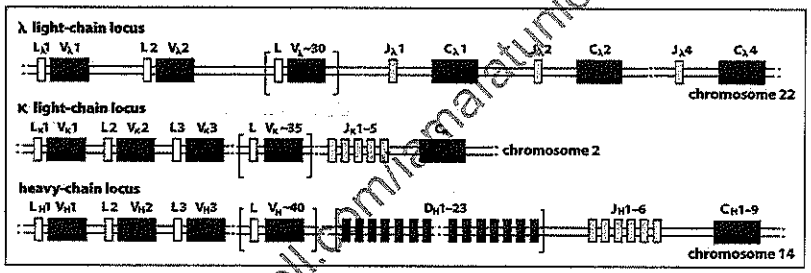


Figure 19. Immunoglobulin (Ig) heavy and light chain gene loci

- Given that the number of heavy and light chains that can be produced by human is more than the number of genes in the entire human genome, what mechanism(s) could lead to this? The mechanisms for generating antibody diversity are:
  - ➢ somatic recombination (or VDJ recombination) (*See section 6B1*)
  - ➢ somatic hypermutation (*See section 6B2*)
  - ➢ combinatorial pairing of heavy and light chains
  - ➢ class switching (*See section 6B3*)

7B1. Somatic Recombination (or V(D)J Recombination)

- **Somatic recombination** is a type of **DNA rearrangement** where DNA sequences that are initially separated from one another are brought together by enzymatic removal of intervening sequences followed by rejoining of sequences.
  - ➢ The somatic recombination is more commonly known as V(D)J recombination.
  - ➢ Enzymes are required for somatic recombination.
    - ↳ recombinase enzymes

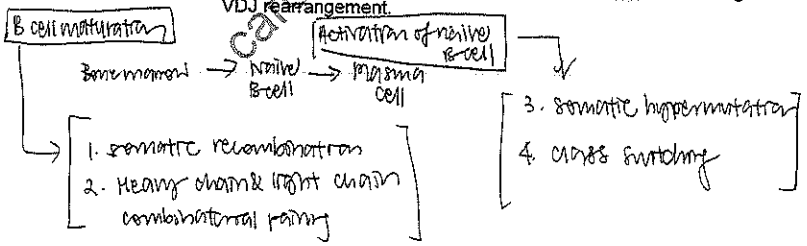
- The multiple V, D and J gene segments allow for the generation of many possible distinct antibody molecules, each with unique antigen binding sites. The table below illustrates the estimated number of possibilities:

	Estimated no. of heavy chain segments	Estimated no. of kappa (κ) light chain segments	Estimated no. lambda (λ) light chain segments
V	40	40	30
D	24	-	-
J	6	5	4
No. of possible combinations	5760 (40 x 24 x 6)	200 (40 x 5)	120 (30 x 4)

As a result, the estimated number of possible heavy-light chains that can be generated is (5760 x 200) + (5760 x 120) = 1.84×10^6

- Somatic recombination occurs in developing B cells and T cells. In this lecture notes, the focus is on **somatic recombination during B cell development in the bone marrow**.

- The process of **VDJ recombination at Ig heavy chain gene locus** involves **random rearrangement** of *one* V gene segment, *one* D gene segment and *one* J gene segment to form a single VDJ exon.
  - ➢ The rearranged VDJ exon will **code for the variable region of heavy chain (V_H).**
  - ➢ How are different variable region sequences generated at heavy chain gene locus? (*See Figure 20*)
    - (1) **Rearrangement of one D gene segment to one J gene segment** forms DJ rearrangement.
    - (2) **Rearrangement of one V gene segment to the DJ rearrangement** forms VDJ rearrangement.



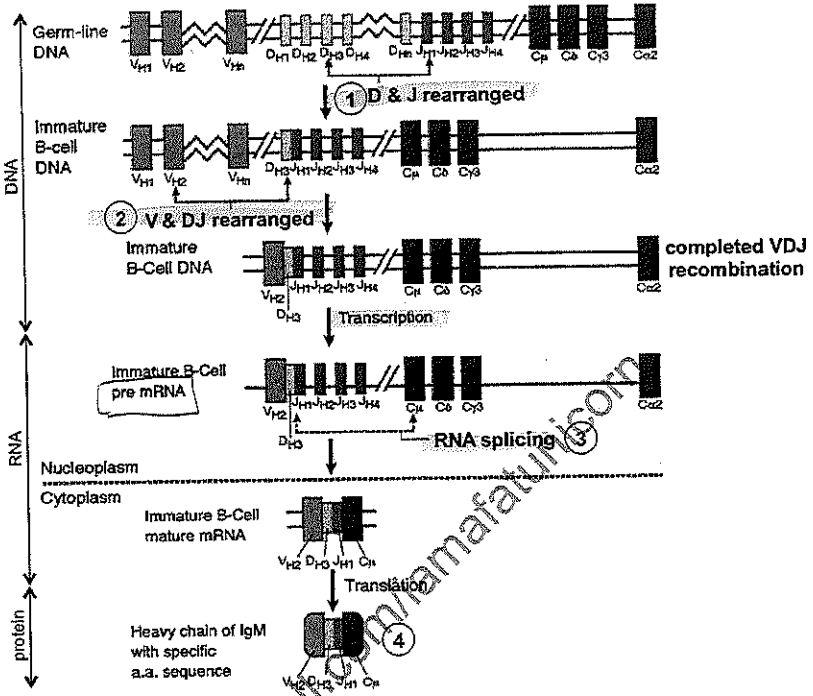


Figure 20. Production of heavy chain of immature B cell

- After VDJ recombination, transcription occurs. (*See Figure 20*):
  - (3) The pre-mRNA contains the VDJ exon and the constant segments. The pre-mRNA undergoes **RNA splicing** so that VDJ exon and Cμ are joined.
  - (4) The mature mRNA codes for a μ heavy chain that has a specific V_H. (*See Figure 21*)

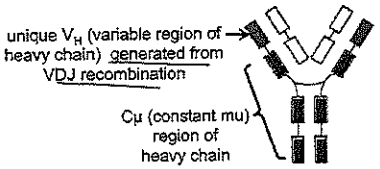


Figure 21. Shaded parts show heavy chain of IgM

- The process of **VJ recombination at Ig light chain gene locus** involves **random rearrangement** of *one* V gene segment and *one* J gene segment to form a single VJ exon.
  - ➢ The rearranged VJ exon will code for the **variable region of light chain (V_L)**
  - ➢ Unlike the heavy chain gene locus, light chain gene loci does not have D gene segments.
  - ➢ How are different variable-region sequences generated at heavy chain gene locus? (*See Figure 22*):
    - (1) **Rearrangement of one V gene segment to one J gene segment** forms VJ rearrangement.
      Note: Figure 22 shows the κ light chain gene locus at chromosome 2.

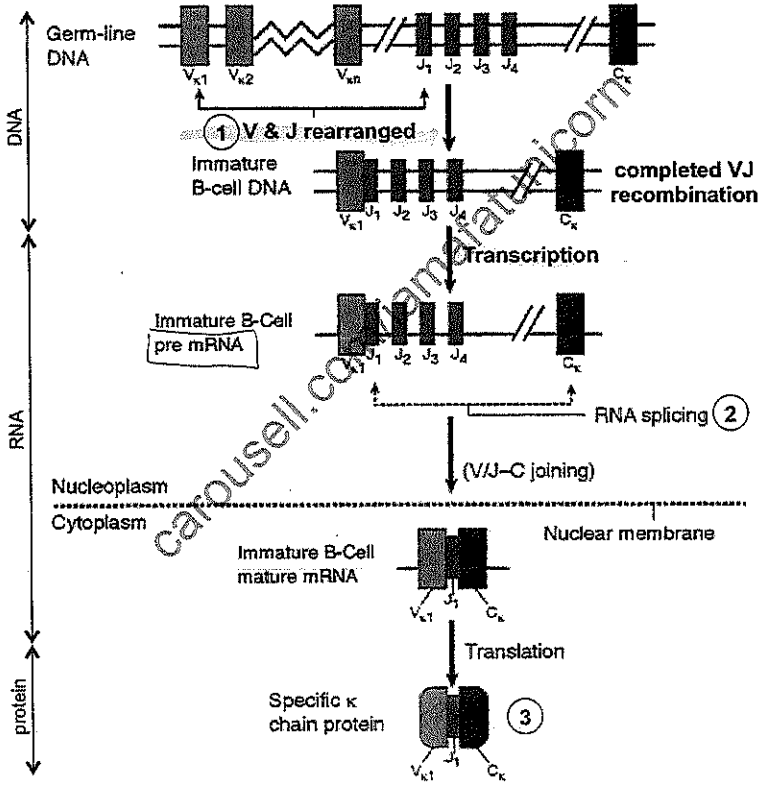


Figure 22. Production of κ (kappa) light chain of immature B cell

- After VJ recombination, transcription occurs. (*See Figure 22*):
  (2) The pre-mRNA undergoes **RNA splicing** so that the VJ exon and the Cκ light chain constant segment are joined.
  (3) The mature mRNA codes for a κ light chain that has a specific V_L. (*See Figure 23*)

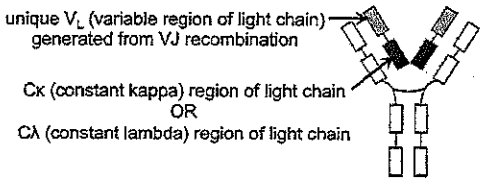


Figure 23. Shaded parts show light chain of an immunoglobulin.

- B cell development occurs in the **bone marrow** where the hematopoietic **stem cell** differentiates to form a B cell.
- The process is as follows:
  Rearrangement of the **heavy** chain gene locus by somatic recombination
  ↓
  Rearrangement of the **light** chain gene locus by somatic recombination
  ↓
  Formation of B cell with specific B cell receptor

Additional Information:

Somatic recombination during B cell development in bone marrow

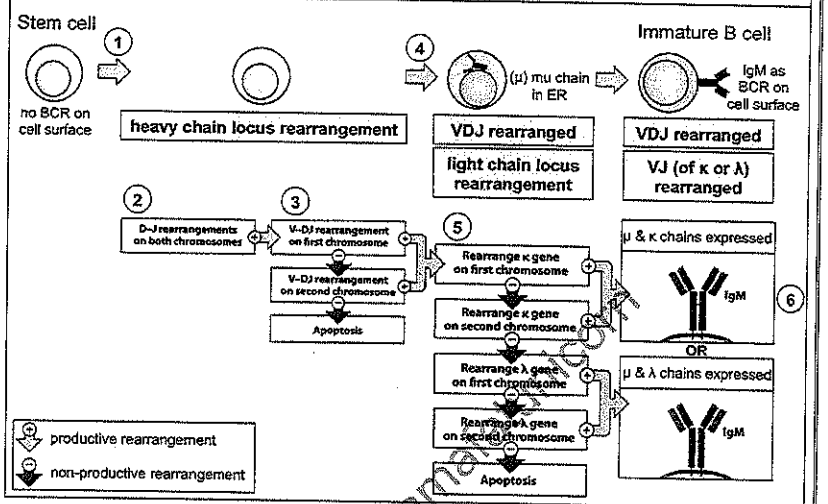


Figure 24. The sequence of events of somatic recombination during B cell development in the bone marrow. (Modified: Janeway's Immunobiology 8th Edition)

- The sequence of events of somatic recombination during B cell development that begins in the **bone marrow** with **stem cell** to form immature B cell (*See Figure 24*):
  (1) Stem cell that is for B cell development will begin with heavy chain gene locus (on chromosome 14) rearrangement by somatic recombination.
  (2) **Rearrangement of a D gene segment to a J gene segment** forms DJ rearrangement for heavy chain gene locus. This occurs on both maternal and paternal chromosomes 14.
  (3) **Rearrangement of a V gene segment to the DJ rearrangement** forms VDJ rearrangement for heavy chain gene locus.
    - If a particular rearrangement on a chromosome 14 fails to produce a functional VDJ exon (i.e. known as a non-productive rearrangement), another attempt at rearrangement on the other homologous chromosome occurs.
    - A cell is only allowed to progress to the next stage when a productive rearrangement of the heavy chain gene locus has been achieved.
    - If both attempts are non-productive rearrangements, the cell undergoes apoptosis.
  (4) After successful VDJ rearrangement for heavy chain gene locus, B cell development continues with light chain gene locus rearrangement by somatic recombination.

(5) **Rearrangement of a V gene segment to the J gene segment** forms VJ rearrangement for light chain gene locus.
- If rearrangement of κ light chain gene locus is can produce a functional VJ exon (i.e. known as a productive rearrangement), rearrangement of λ light chain gene locus is prevented.
- If rearrangement of κ light chain gene locus is a non-productive rearrangement, rearrangement of λ light chain gene locus begins.
- If both attempts of κ and λ light chain gene loci are non-productive rearrangements, the cell undergoes apoptosis.

(6) **Both successful VDJ and VJ rearrangements** will lead to expression of IgM on the cell surface membrane. This cell is now known as **immature B cell.**

- Naïve mature B cells (i.e. has not encountered the particular antigen that is complementary to its binding site) continues to express IgM on the cell surface membrane. Upon activation, the naïve B cell becomes effector (or activated) B cell and **class switching** occurs (*See section 6B3*). The effector B cell expresses IgG that has the same variable regions but different C_H regions.

7B2. Somatic Hypermutation

- Somatic hypermutation is a **random point mutation** in the **rearranged VDJ region** of heavy chain gene locus and the **rearranged VJ region** of light chain gene locus.
  - ➢ Thus, somatic hypermutation **further diversifies** the variable regions (V_H and V_L) of the antibody for antigen binding.
  - ➢ It is called "hypermutation" because it occurs at a much higher rate compared to the normal rate of mutation.

- Somatic hypermutation occurs only on **activated B cells** (i.e. after mature B cells have been activated by the specific antigen that their B cell receptors (BCRs) can recognise and bind). The process therefore takes place outside the bone marrow.

- Activated B cells divide rapidly by mitosis during clonal expansion, and some of these B cells can undergo somatic hypermutation. As a result, each of these B cells with a mutation will acquire slight amino acid difference in the **variable regions** of the Ig chains on the cell surface membrane (i.e. changes in antigen-binding sites of BCR on the cell surface membrane).
  - ➢ Some point mutations result in the B cells expressing **low affinity** Ig on their cell surface membrane.
  - ➢ Some point mutations result in the B cells expressing **higher affinity** Ig on their cell surface membrane.

- By using **somatic hypermutation** to make changes in the antigen-binding sites of the BCR, BCR can be "fine-tuned" to have **higher affinity for its specific antigen**. These B cells that express higher affinity BCR on their cell surface membrane are selected for **clonal expansion** and **differentiation**. This is known as **affinity maturation.**
  - ➢ These B cells differentiate into **antibody-secreting plasma cells**. The antibodies secreted by these plasma cells will have high binding affinities for the specific antigen.
  - ➢ The antigen-binding sites of the BCR and the antigen-binding sites of the antibody secreted from plasma cell are the same.
  - ➢ The difference between BCR and the secreted antibody lies in their constant region of heavy chains due to an event, class switching.

7B3. Class Switching

- There are 5 different types or classes of antibodies, IgG, IgM, IgA, IgD and IgE. (*See Figure 15*) IgG is the predominant antibody in blood serum.
  - ➢ Different class of antibody contains **different constant-region of heavy chain.**

- Class switching occurs only on **activated B cells**. Unlike somatic recombination (or V(D)J recombination), class switching occurs in the presence of antigen.
  - ➢ Class switching is the process of an individual B cell switching to synthesise different class of antibody e.g. from IgM to IgG. The process is induced by cytokines (small signaling molecules) released from helper T cells during B cell activation.

- Definition of class switching – **DNA rearrangement** at the **constant gene segment** of the **Ig heavy chain gene locus.**
  - ➢ The **variable region of the heavy chain (V_H)** that was previously generated during VDJ recombination in B cell development **remains unchanged.**
  - ➢ Before class switching, a B cell with V_H DNA linked to C_μ (mu chain of constant region of heavy chain), produced **IgM** antibodies.
  - ➢ During class switching, DNA rearrangement occurs: the V_H DNA becomes linked to *another* **constant gene segment** (e.g. C_γ gamma chain) of the heavy chain (C_H) gene locus.
  - ➢ Resulting in a B cell that synthesizes **IgG** antibodies.
  - ➢ Only the **constant region of the heavy chain (C_H)** of antibody **changes**, from IgM to IgG, due to the change of C gene segment from C_μ to C_γ.

- Therefore, class switching allows for expression of antibodies that have the same antigen specificity (as V_H remains unchanged) but have different function (*See Figure 15*) (due to different type of C_H).

8. Immunological Memory

- Apart from activating the lymphocytes to rid the host of pathogens, the establishment of a population of lymphocytes i.e. **memory cells** to protect against reinfection is also a consequence of adaptive immunity.

- Immunological memory is established to ensure a rapid re-induction of antigen-specific antibody on subsequent encounters with the same pathogen, thus providing long-lasting protection against it.

- Immune responses are classified as **primary immune response** and **secondary immune response.**

- **Primary Immune Response** occurs on the first occasion the body encounters a new antigen.
  - ➢ The characteristics are : (*See Figure 25*)
    - **Presence of lag period** (~3 to 6 days) between encountering antigen and production of antibody. Lag period reflects the time required for clonal expansion (via cell division) and differentiation of naïve T cells to effector T cells and naïve B cells to plasma cells.
    - **Gradual increase** in antibody concentration.
    - **Lower peak concentration** of antibodies → Weaker response.

- **Secondary immune response** occurs following re-exposure to the **same antigen.**
  - ➢ The characteristics are : (*See Figure 25*)
    - **Faster increase** in antibody concentration as there is no need to activate naïve B and T cells. Memory B and T cells can be reactivated easily, thus respond quickly to the same antigen.
    - **Higher peak concentration** of antibodies → **Stronger response.**
    - **High concentration** of antibodies **persists for a longer period of time.**
  - ➢ The faster increase and higher peak concentration is due to the rapid proliferation of the memory T and B cells and their differentiation into effector T cells and plasma cells respectively.

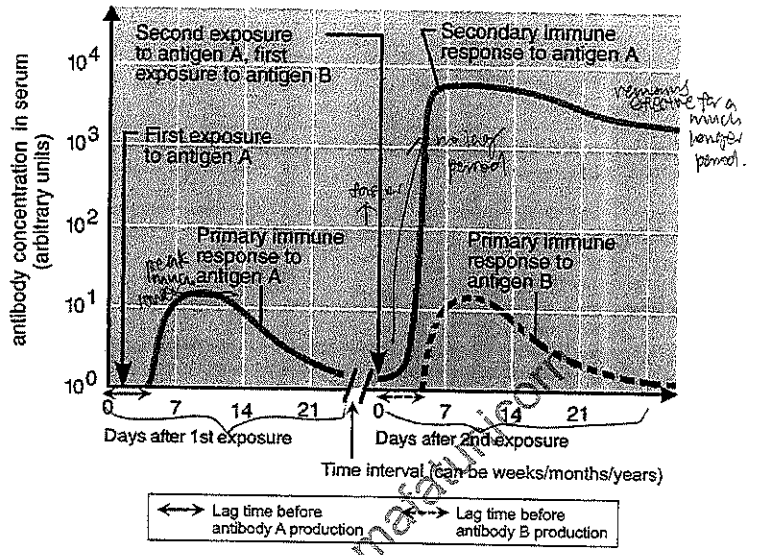


Figure 25. Concentration of serum antibody following primary and secondary exposure to antigens. (Modified: Human Anatomy and Physiology, Elaine Marieb)

9. Vaccination

- Vaccination is the intentional **administration of an antigen,** usually a harmless form of a pathogen in order to **induce a specific adaptive immune response** that protects the individual against later exposure to the pathogen due to production of memory cells.
  - ➢ Vaccination is an **artificial active immunity** since the immune response is activated by artificially introducing antigens into the body to initiate primary immune response. (*See Figure 26*)
  - ➢ Vaccination uses the property of **immunological memory** to provide long-lasting protection against infectious diseases.
  - ➢ When exposed to the actual pathogen, the memory cells trigger a secondary immune response that is faster and stronger. (*See Figures 27 & 28*)

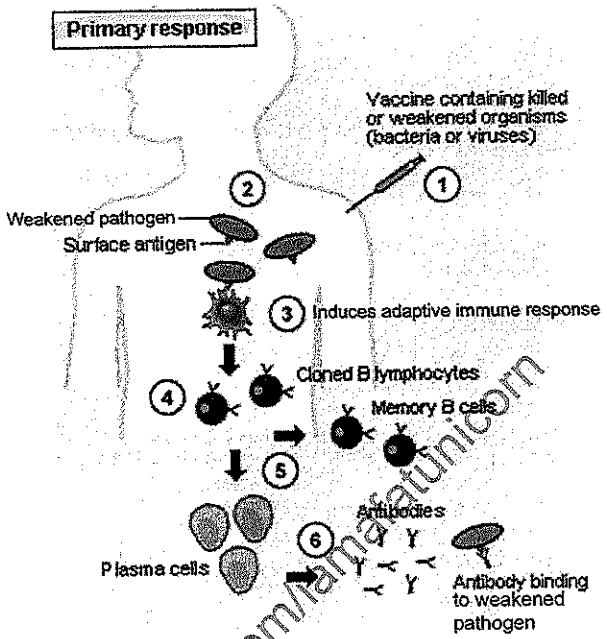


Figure 26. Events that happen after vaccination.

- The events that happen after vaccination (*See Figure 26*):
  (1) A person is vaccinated against a specific disease. The vaccine contains antigens from the pathogen. (refer to pg 37 for types of vaccines)
  (2) The modified pathogen is no longer able to cause disease, but it can still retain immunogenic effect (i.e. ability to elicit immune response) because a characteristic surface antigen of the pathogen is still retained and can be recognised by antigen presenting cells.
  (3) Adaptive immune response occurs. Naïve B and T lymphocytes are activated to become effector lymphocytes.
  (4) The B lymphocyte with the specific BCR that is complementary in shape to the antigen undergoes clonal expansion by dividing repeatedly by mitosis to form genetically identical B lymphocytes which differentiate to become either
    - memory B cells or
    - antibody-secreting plasma cells.

- Vaccination results in generation of immunological memory. Subsequent exposure to the pathogen results in a faster and stronger immune response. (*See Figure 27*)

- The following events occurs after subsequent exposure to the pathogen :
  (1) The virulent pathogen enters the vaccinated individual,
  (2) memory T and B cells will quickly recognize the antigen of the pathogen.
  (3) memory T and B cells rapidly undergo clonal expansion and
  (4) Differentiate into effector T cells and antibody-secreting plasma cells.
  (5) These plasma cells produce a high concentration of antibodies which prevent further infection by :
    - opsonisation/agglutination of the pathogen
    - neutralising the virus or toxins

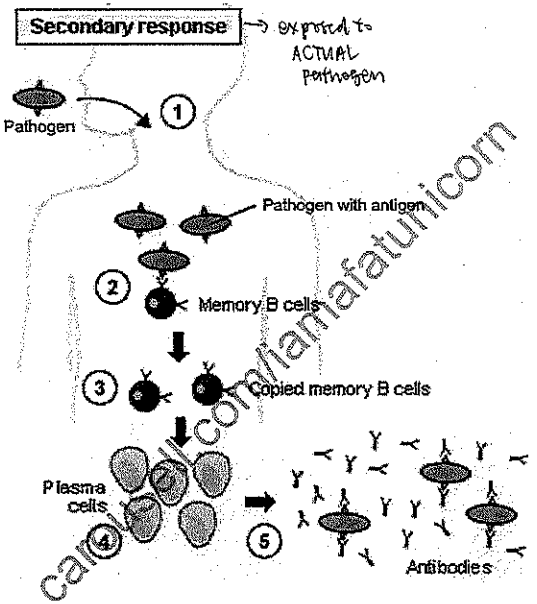


Figure 27. Vaccination can prevent disease by generating a rapid and more intense immune response.

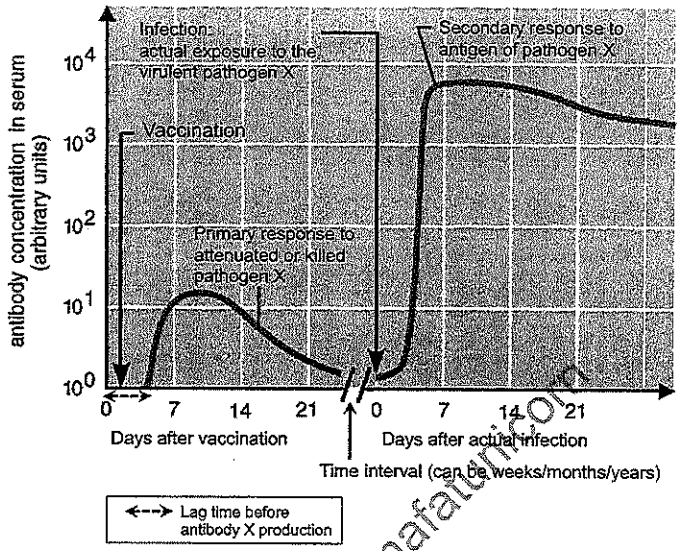


Figure 28. Concentration of serum antibody following vaccination (primary response) and infection by virulent pathogen (secondary exposure). (Modified: Human Anatomy and Physiology, Elaine Marieb)

- A vaccine is a preparation of immunogenic material used to induce immunity against pathogenic organisms. Three types of vaccines exist, they are :
  i. Live, attenuated vaccine
  ii. Inactivated vaccine
  iii. Toxoid vaccine

(i) Live, attenuated vaccine
- ➢ Attenuation refers to weakening of the pathogenic bacteria or virus by making it less virulent, i.e. a modified pathogen that is not capable of causing disease.
- ➢ Examples include BCG (against tuberculosis), measles and typhoid vaccines.
- ➢ Advantages:
  - A live, attenuated vaccine is the closest thing to a pathogen encountered in a natural infection. It contains a range of antigens that will elicit a strong immune response with just a low dosage.
  - It is live and is able to replicate in the host, thus it will persist and continuously stimulate the immune system thus conferring longer-term protection.
- ➢ Disadvantages:
  - Possibility of their reversion to the virulent form by mutation and can cause disease.
  - Need to be refrigerated to stay viable and potent.

(ii) Inactivated vaccine
- ➢ Inactivation involves killing the pathogenic bacteria or virus with chemicals, heat or radiation.
- ➢ Examples include rabies and hepatitis vaccines.
- ➢ Advantages:
  - Will not revert to the virulent form and cause disease.
  - Usually do not require refrigeration, and it can be easily stored and transported in a freeze-dried form. This makes inactivated vaccine accessible to people in less accessible locations.
- ➢ Disadvantages:
  - May elicit weaker immune response, thus may need several doses (i.e. booster shots) to maintain protection.

(iii) Toxoid vaccine
- ➢ Toxoid is a chemically modified toxin from pathogenic bacteria such that it is no longer toxic but is still to elicit immune response.
- ➢ Used when bacterial toxin is the main cause of illness. Vaccination with toxoid induces production of anti-toxoid antibodies which can bind the bacterial toxins and neutralise the toxins.
- ➢ Examples include tetanus and cholera toxoid vaccines.
- ➢ Advantage:
  - Does not contain any pathogens thus there is no risk of reverting to virulent form.
- ➢ Disadvantage:
  - May elicit weaker immune response, thus may need several doses (i.e. booster shots) to maintain protection.
  - Limited use when bacterial toxin is the main cause of illness but not all pathogenic bacteria produce toxins.

9A. Benefits and Risks of Vaccination

Benefits

- Vaccination protects individuals against diseases thus,
  - ➢ preventing deaths due to diseases.
  - ➢ preventing long-term disabilities such as infertility, deafness and blindness due to the diseases
  - ➢ reducing human suffering
  - ➢ reducing health care cost due to costs of treatments

- Vaccine can **increase herd immunity**. If a large number individuals in a population are vaccinated and are immune to an infectious agent, fewer susceptible individual will contract the disease. Transmission of the disease within the population is prevented.

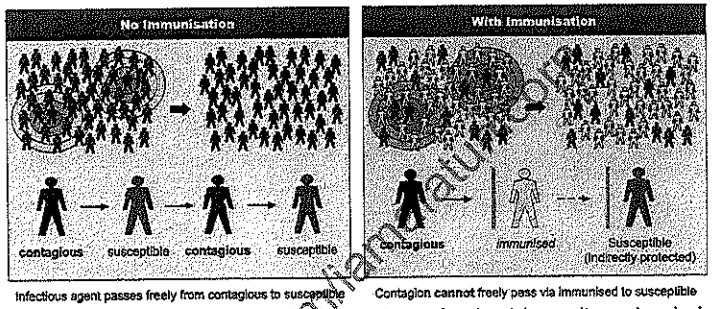


Figure 29 showing the effects of vaccination on improving herd immunity and reducing infection in the population.

- Vaccination enables complete **eradication** of some diseases. E.g. Smallpox.

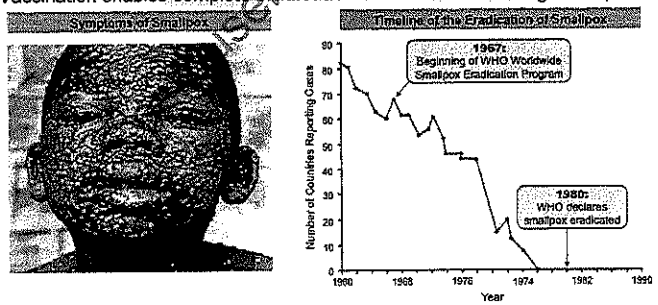


Figure 30 showing the effects of vaccination on the number of smallpox cases reported.

Risks

Risks associated with the use of vaccines include :

- Live, attenuated vaccines may revert to virulent forms and cause disease.
- Antigens used in some vaccines may cause allergy reactions.
- Some vaccines may cause undesirable side effects. ~~For example, the vaccine for mumps, measles and rubella (MMR) has been associated with autism in children.~~
- Excessive vaccination may reduce the effectiveness of the immune system to respond to new infections.

10. Pathogens and Diseases

- Pathogens are microorganisms that invade the body to cause diseases. The 5 classes of pathogens are viruses, bacteria, fungi, protozoa and worms.

- Not all microorganisms are pathogenic, i.e. disease-causing. Only a small proportion of microorganisms cause diseases.

- The table below shows some examples of pathogen and the disease the pathogen causes.

Name of pathogen	Type of pathogen	Name of disease
influenza virus	virus	influenza
human immunodeficiency virus (HIV)	virus	acquired immunodeficiency syndrome (AIDS)
dengue fever virus	virus	dengue fever
Mycobacterium tuberculosis	bacterium	tuberculosis
Plasmodium falciparum	protozoan	malaria

- Pathogens of all classes must have specialised mechanisms for crossing cellular and biochemical barriers of their host organisms and for evading destruction by the host innate and adaptive immune responses. Pathogens can then replicate using host resources.

- In a disease, the presence of the pathogen results in the impairment to the normal function of host.

- A disease is termed as an **infectious**, when it is caused by a pathogen, and able to be transmitted from one host to another.

- Many of the symptoms and signs that are associated with infectious diseases are direct manifestations of the host's immune responses in action. E.g. Swelling and redness at the site of infection and the production of pus (mainly dead white blood cells) in bacterial infection are due to inflammation and recruitment of phagocytes to destroy the bacteria.

10A. Pathogenicity of Influenza Virus

- Target cells:
  - epithelial cells of the respiratory tract
  - Virus binds to the sialic acid receptor found on the epithelial cell membrane.

- Mode of transmission:
  - droplets of moisture from lungs of infected persons or from infected bird droppings

- Mode of replication:
  - *refer to Virus lecture notes*

- The disease:
  - Virus settles on the mucous membrane lining the nose, pharynx, trachea and bronchi.
  - Neuraminidase enzyme on the surface of the viruses helps them to penetrate the mucoproteins in the mucus layer. The mucoproteins are glycoproteins in nature.
  - Haemagglutinin, a glycoprotein on the viral envelope, bind to sialic acid receptors on cell surface membrane of the epithelial cell lining the respiratory tract.
  - Virus penetrates into these host cells. Once inside, the virus replicates within them. (*for details, refer to Virus lecture notes*)
  - Infected epithelial cells are destroyed. This leads to inflammation and the buildup of dead epithelial cells in the airways. These cause the symptoms e.g. running nose and scratchy throat.
  - Weakening of the epithelial layer caused by viral replication can make the respiratory passage more susceptible to secondary bacterial infections leading to diseases like pneumonia which can be fatal.

- Treatment:
  - Treatment usually involves bed rest and the administration of aspirin or paracetamol to alleviate symptoms such as headaches and fever.
  - Antibiotics are sometimes administered to treat secondary bacterial infection like pneumonia.
  - In highly susceptible individuals such as elderly and young children, anti-viral drugs such as
    - oseltamivir (trade name Tamiflu) and zanamivir (trade name Relenza) are recommended. These drugs are neuraminidase inhibitors that are designed to prevent release of virions from the infected cells and thus prevent subsequent spread of the virus in the body.
    - amantadine and rimantadine are designed to block a viral ion channel (M2 protein) and prevent the virus from infecting cells.
- Vaccination
  - Vaccinations against influenza are also sometimes administered. The influenza vaccine contains purified and inactivated virus from the three most common influenza viral strains.
  - The virus strains used to make the vaccine varies from year to year, and between the northern and southern hemisphere.

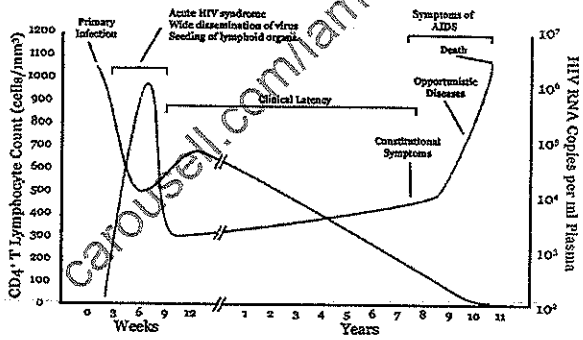
10B. Pathogenicity of HIV

- Target cells:
  - Helper T cells and macrophages of the immune system
  - Virus binds to the CD4 receptor found on the host cell membrane.

- Mode of transmission:
  - unprotected sexual contact with infected individuals
  - by exposure to infected blood and blood products.
  - vertical transmission from mother to child either via the placenta, childbirth or during breastfeeding.

- Mode of replication:
  - *refer to Virus lecture notes*

- The disease:
  - Primary targets are macrophages and helper T cells.
  - The HIV virus contain gp120 which has a very strong affinity for and binds to CD4, a surface protein of helper T cells.
  - HIV then replicates in the helper T cells
  - As the number of HIV virus increases (shown by HIV RNA copies per ml of plasma), virus infects more and more helper T cells.
  - Helper T cells count (shown by CD4+ T lymphocyte count) decreases as they are destroyed.

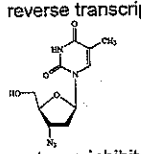


Time course of HIV infection: CD4 T cell count in cells per μL blood (in blue) vs copies of HIV RNA per ml blood plasma (in red)

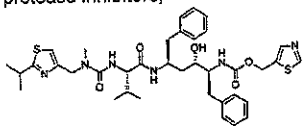
Figure 31 showing the HIV viral load and CD4+ T lymphocyte over time.

  - Loss of helper T cells leads to impaired immune responses and the individual becomes increasingly susceptible to opportunistic diseases.
  - Death usually results from secondary infections.
  - The macrophages may survive HIV infection (because they are not lysed by the virus) and may thus act as reservoirs.
  - The virus mutates at a very high rate during replication resulting in altered proteins on the surface of the virus. In this way, the virus prevents recognition and elimination by the immune system allowing it to evolve rapidly within the body.

- Treatment:
  - ➢ 24 approved retroviral drugs which can be used to treat HIV infection. These include :
    - reverse transcriptase inhibitors,



    - protease inhibitors,



    - integrase inhibitors and
    - entry inhibitors.

  - ➢ The first three enzyme inhibitors work by inhibiting their respective enzymes thus preventing replication of the virus and subsequence integration of the HIV genome as a provirus.
  - ➢ Entry inhibitors work by blocking interaction between the gp120 and CD4 or the co-receptor, or by preventing fusion of the viral and host cell membranes, thus blocking entry of HIV into the cells.
  - ➢ Due to the high rates of virus productions and mutation rate of the virus, treatment of HIV infection generally includes administration of 3 agents in combination (also known a 'cocktail' of drugs). Sustained treatment results in suppression of viral replication, dramatically increasing life expectancy of HIV-infected individuals.

- Vaccination
  - ➢ No effective vaccine currently available

10C. Pathogenicity of *Mycobacterium tuberculosis*

- Pathogen:
  - ➢ *Mycobacterium tuberculosis* (*M. tuberculosis*) which is an obligate aerobe. Obligate aerobe is a microorganism that requires oxygen for survival and growth. Without oxygen, aerobic respiration cannot occur and obligate aerobe cannot survive.
  - ➢ *M. tuberculosis* has cell walls that contain mycolic acids that
    - enable the bacterium to grow inside macrophages.
    - make the bacterium more resistant to chemical damage and dehydration
    - limit the effectiveness of common hydrophilic antibiotics.

- Target organ:
  - ➢ Primary infection is usually in the lungs to cause pulmonary tuberculosis (TB).
  - ➢ Other parts of the body e.g. kidneys, spine can also be infected to cause extrapulmonary tuberculosis (TB).

- Mode of transmission:
  - ➢ The bacteria are transmitted from person to person in fine aerosol droplets when an infected person with the active TB disease sneezes or coughs and an uninfected person inhales the droplets.
  - ➢ Thus, tuberculosis is an airborne disease.
  - ➢ TB spreads rapidly among people living in overcrowded conditions.

- The disease:
  - ➢ The minimum infectious dose for lung infection is around 10 bacterial cells.
  - ➢ Once inside the lungs, alveolar macrophages phagocytose the bacteria and form phagosomes containing *M. tuberculosis*. (*See Figure 32*)

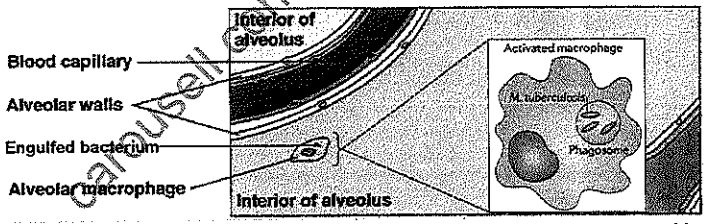


Figure 32. Alveoli of the lung contain macrophages that phagocytose *M. tuberculosis* that reach the alveoli. (Modified: Microbiology An Introduction 8th Edition)

  - ➢ Inside the phagosome, *M. tuberculosis* inhibits the fusion of the phagosome with lysosomes. No phagolysosome is formed and no lysosomal enzymes are available to kill the bacteria.
  - ➢ *M. tuberculosis* survives and continues to multiply inside the macrophages. (*See Figure 33*)

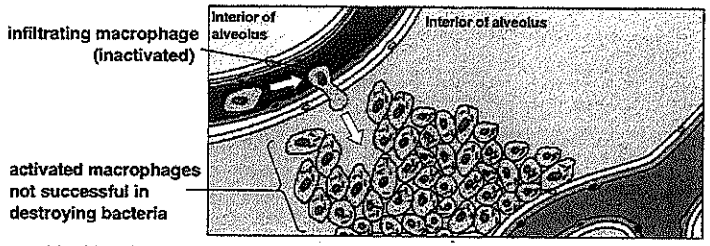


Figure 33. *M. tuberculosis* can survive and multiply in macrophages and more macrophages are brought to the infected alveolus.

- Phagosome containing *M. tuberculosis* may fuse with lysosome to form phagolysosome. The thick waxy mycolic acid cell wall protect it from the reactive oxygen specifies and acids from the lysosome. The bacterium reproduce inside the macrophage and eventually kill it.
- Macrophages, dendritic cells and lymphocytes form a granuloma to 'wall off' and isolate the *M. tuberculosis* and infected macrophages. This eventually leads to the formation of a tubercle.
- A **tubercle**, a tight ball-like formation by clustering cells such as macrophages, is formed. (*See Figure 34*) At the center of the tubercle, cell death by necrosis occurs. Cell death by necrosis, unlike apoptosis, involves the rupturing of cell membrane and releasing of cell content to the surrounding.

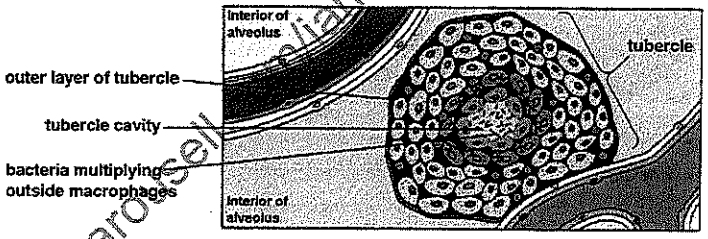


Figure 34. At the center of tubercle macrophages die, releasing *M. tuberculosis* into the cavity.

- The disease may be arrested at this stage and remain latent for several years. People with this latent infection do not spread the disease to others.
- Some people become infected and develop TB after a few weeks. The tubercle cavity enlarges to form an air-filled cavity for the aerobic *M. tuberculosis* to multiply outside the macrophages.
- The tubercle ruptures, allowing *M. tuberculosis* to spill into a bronchiole and spread throughout the lungs. This results in development of a productive cough that facilitates aerosol spread of infectious bacteria. (*See Figure 35*)

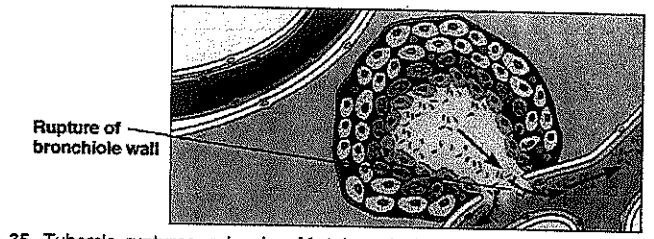


Figure 35. Tubercle ruptures, releasing *M. tuberculosis* into the bronchiole and thus disseminating throughout the lungs and into the circulatory and lymphatic systems.

- The lungs will be progressively destroyed by the formation of cavities due to the rupture of tubercles.
- TB is often the first opportunistic infection to strike HIV-positive people. HIV infection may reactivate dormant infections of *M. tuberculosis* which may have been present in the person from childhood. If the HIV-positive person is previously uninfected by *M. tuberculosis*, he/she will be more susceptible to infection by the bacteria.

- Treatment
  - About 6 months of daily treatment with a combination of at least two antibiotics. If the patient stops taking the prescribed antibiotics before the complete course, or if a dose is skipped, the TB infection may become resistant to the antibiotics. This is potentially serious as it can be difficult to treat and will require a longer course of treatment.
    - A combination of antibiotics is used during treatment to minimise the risk of developing resistance to the antibiotics, and also to achieve an additive effect against the bacteria.
  - Mechanism of action of antibiotics used for TB treatment:
    - Isoniazid - inhibits synthesis of mycolic acids that is used to form mycobacterial cell wall.
    - Rifampin - inhibits RNA synthesis during transcription e.g. antibiotic interferes with prokaryotic RNA polymerase.
- Vaccination
  - Bacillus Calmette-Guérin (BCG) vaccine, is prepared from a strain of attenuated live *Mycobacterium bovis*, a bovine tuberculosis bacillus. It is often used to prevent the spread of TB among children.

11. Mode of action of antibiotics

- Antibiotics are natural substances obtained from microorganisms such as certain fungi and bacteria. They inhibit the growth of bacteria or kill the bacteria by disrupting the metabolism of the prokaryotic cells.
  - A bacteriostatic antibiotic is one that is able to inhibit the growth or binary fission of bacteria.
  - A bactericidal antibiotic is one that is able to kill bacteria when the bacteria are in the process of undergoing cell division by binary fission.
  - Question: Why antibiotics can inhibit or kill bacterial cells but have little or no effect on human cells?

➢ Answer: effective antibiotic specifically disrupts the metabolism of the disease-causing bacteria but not the metabolism of mammalian host tissues because prokaryotic and eukaryotic cells use different metabolic machineries e.g. different ribosomal subunits.

➢

➢ Question: Why antibiotics cannot be used to treat viral diseases?

➢ Answer: Antibiotics do not affect viruses as a virus lacks cell structure and has no metabolism of its own to be disrupted by antibiotics. Viruses use metabolic machineries of host cells that are not affected by antibiotics and can continue their viral reproductive cycles.

- 3 major prokaryotic metabolic pathways that antibiotics usually disrupt:
  i. Cell wall synthesis
  ii. Protein synthesis (translation)
  iii. Transcription

(i) Antibiotics that disrupt peptidoglycan cell wall synthesis:

➢ Most bacterial cell walls contain peptidoglycan. Peptidoglycan, a structure unique to bacterial cells, is essential for maintaining the structural integrity of the bacterial cells. The bacterial cell has a high internal osmotic pressure, and the rigid cell wall maintains the shape of the bacterium.

➢ Peptidoglycan cell wall is best target for antibiotics as peptidoglycan is absent in human cells.

➢ β-lactam antibiotics, such as Penicillin (*See Figure 36*) inhibits bacterial cell wall synthesis i.e. disrupts peptidoglycan synthesis.

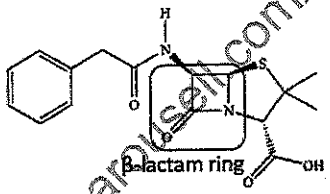
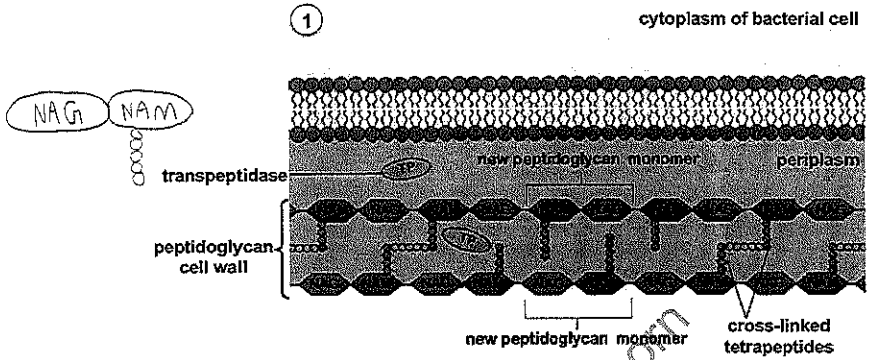


Figure 36. Structure of penicillin, a β-lactam antibiotics. Members of β-lactam antibiotics are characterised by their four-membered, nitrogen containing β-lactam ring structure.

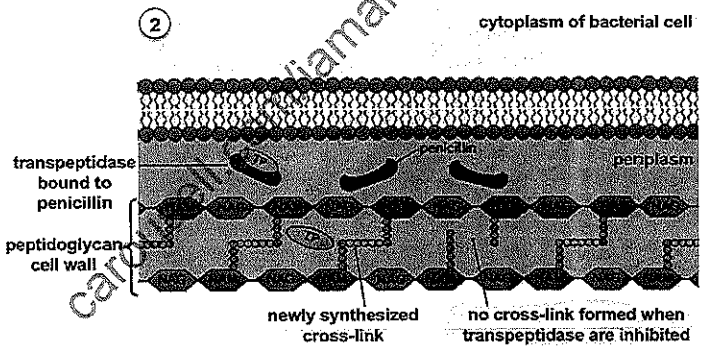
➢ What is the action of penicillin on bacteria? (*See Figure 37*)

- During growth of bacteria, the bacterium remodels the peptidoglycan cell wall by breaking down the cross-links between peptidoglycan chains. An enzyme, transpeptidase reforms the cross-links between new peptidoglycan chains and existing peptidoglycan chains.
- Penicillin acts as a competitive inhibitor and binds to the active site of transpeptidase (*See Figure 37 (1) for transpeptidase*).
- As a result, there is inhibition to the formation of cross-links between adjacent chains.
- Bacterial cell wall becomes weakened. When bacteria takes in water by osmosis, the increased turgor pressure against the weakened cell wall causes the bacteria to swell and lyse.

➢ Penicillin is only effective when bacterium is growing or making new cell wall.



(1) As new monomers are linked to existing rows of peptidoglycan during bacterial cell wall synthesis, transpeptidase enzymes catalyse the formation of cross-links between peptides that are attached to N-acetylmuramic acid (NAM) residues of adjacent chain. These cross-links gives peptidoglycan its high tensile strength.



(2) Penicillin binds to transpeptidase enzymes to inhibit the formation of cross-links between adjacent chains, weakening bacterial cell wall of dividing bacterial cells.

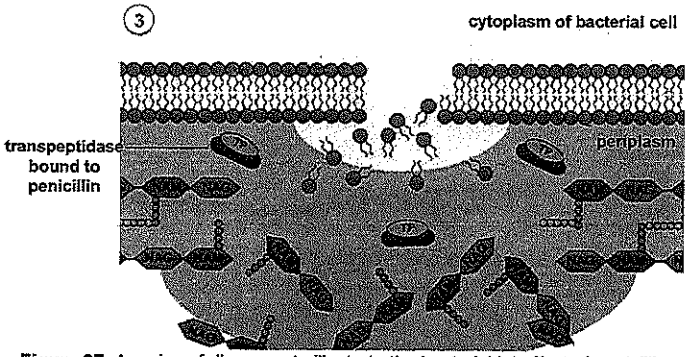


Figure 37. A series of diagrams to illustrate the bactericidal effect of penicillin.

(3) Osmotic lysis happens to dividing bacterial cells since their cell wall synthesis is inhibited.

(ii) Antibiotics that disrupt protein synthesis:

- Ribosomes of prokaryotes are 70S while ribosomes of eukaryotes are 80S.
- 70S ribosomes are thus excellent targets for antibiotics.
- Streptomycin - binds to the small subunit (30S) of the bacterial ribosome such that the initiator tRNA cannot bind to the small subunit.
- Tetracycline - blocks aminoacyl-tRNA from attaching to the A site of bacterial ribosome.

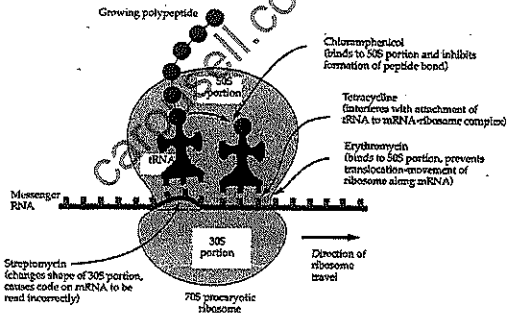


Figure 38. showing the mechanism of actions of various antibiotics that disrupt protein synthesis.

(iii) Antibiotics that disrupt transcription:

- Rifampin inhibits RNA synthesis by binding to bacterial RNA polymerase thus preventing transcription.

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EXTENSION TOPIC B

IMPACT OF CLIMATE CHANGE ON ANIMALS AND PLANTS

Learning Outcomes

Candidates should be able to:

(a) Identify and explain the human activities over the last few centuries that have contributed to climate change through increased emission of greenhouse gases (limited to CO_2 and methane) including burning of fossil fuels linked to increasing energy usage, clearing of forests and food choices (increasing consumption of meat).

(b) Explain the effect of climate change as a result of greenhouse gas emissions, including the melting of polar ice caps, rising sea levels, stress on fresh water supplies, heat waves and heavy rains, death of coral reefs, migration of fishes and insects and release of greenhouse gases in frozen organic matter.

(c) Explain how climate change affects plant distribution (vertical and latitude) and plant adaptations, including morphology and physiology.

(d) Discuss the consequences of global food supply of increased environmental stress resulting from climate change, including the effects on plants and animals of increased temperature and more extreme weather conditions.

(e) Explain how temperature changes impact insects, including increased temperature leading to increased metabolism and the narrow temperature tolerance of insects.

(f) Outline the life cycle of *Aedes aegypti* as an example of a typical mosquito vector.

(g) Outline the development of viral dengue disease in humans, including host-pathogen interactions, human susceptibility to the virus; pathogen virulence, transmission and drug resistance.

(h) Explain how global warming affects the spread of mosquito-borne infectious diseases, including malaria and dengue, beyond the tropics.

(i) Discuss the effects of increased environmental stress (including increased temperatures and more extreme weather conditions) as a result of global climate change, on habitats, organisms, food chains and niche occupation.

(j) Discuss how climate change affects the rich biodiversity of the tropics including the potential loss of this rich reservoir for biomedicines and genetic diversity for food.

Use the knowledge gained in this section in new situations or to solve related problems.

*** This handout is the effort of several Biology teachers at RI (Yr 5-6). It has and will continue to be updated.**

****Any information given in a double-lined box is for your information only.**

Table of Contents

climate change
change in long-term averages of daily weather.

what is weather?
- weather is the short-term conditions of the atmosphere: temperature, clouds, precipitation, humidity, wind

what is climate?
climate is how the atmosphere behaves over a long period of time (i.e. long-term averages of daily weather)

1) INTRODUCTION

A. What are greenhouse gases

- A greenhouse gas (GHG) is a gas that both absorbs and emits radiation in the infrared range. When present in the atmosphere, these gases trap radiation in the form of heat, causing a warming process called the greenhouse effect.
- Some concentration of greenhouse gases in the atmosphere is normal, and necessary for life on Earth. It is also normal for the concentrations of these gases to fluctuate over time.
- The greenhouse gases in the Earth's atmosphere keep the average temperature of the Earth at 15°C, whereas without the greenhouse effect the average temperature would be a frosty -18°C.

UV rays → infrared rays
(short wavelength) (longer wavelength)

B. Greenhouse effect

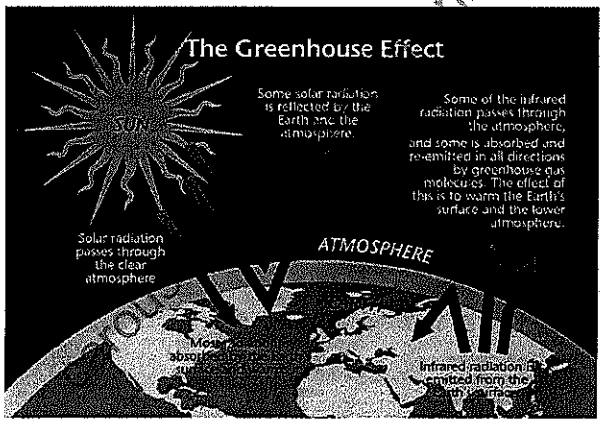


Fig. 1: Diagram showing the Earth's Greenhouse Effect

- The greenhouse effect is a natural process by which heat radiated from Earth's surface is trapped by greenhouse gases.
- Solar radiation is of very short wavelength predominating in the visible or ultraviolet spectrum. Some of this radiation is reflected by the atmosphere back into space while about half of it is absorbed by Earth's surface and warms it.
- The Earth's surface then emits longer wavelength radiation, primarily in the infrared region. Some of this infrared radiation passes through the Earth's atmosphere but most is absorbed and re-emitted in all directions by greenhouse gas molecules and clouds. This results in the warming of the Earth's surface and the lower atmosphere.

- The Earth's greenhouse effect maintains Earth's surface at a life-sustaining temperature and human activities producing greenhouse gases enhances the greenhouse effect, causing global warming.

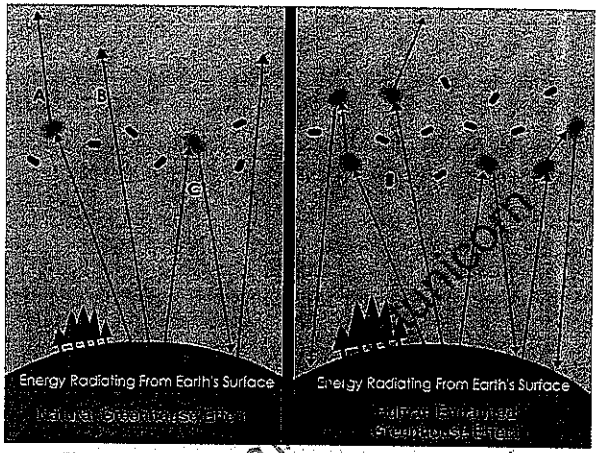


Fig. 2: Diagram showing the natural greenhouse effect and human enhanced (anthropogenic) greenhouse effect

The Earth's surface, warmed by the Sun, radiates heat into the atmosphere. Some heat is absorbed by greenhouse gases like carbon dioxide and then radiated to space (A). Some heat makes its way to space directly (B). Some heat is absorbed by greenhouse gases and then radiated back towards the Earth's surface (C). With more carbon dioxide in the atmosphere due to human activities, more heat will be trapped by greenhouse gases, warming the planet more significantly.

C. Greenhouse gases due to anthropogenic effect (human activity)

- Greenhouse gases include water vapour, carbon dioxide, methane, nitrous oxide, and fluorinated gases.
- Industrialisation and development has increased the amount of greenhouse gases, ie carbon dioxide, methane, nitrous oxide, and fluorinated gases, contributing to the warming of the atmosphere.

Greenhouse Gas	Global Warming Potential (GWP)	How long it stays in the atmosphere
Carbon dioxide (CO_2)	1	Between 50 to thousands of years
Methane	28-36	About 12 years
Nitrous oxide	265-298	About 114 years
Fluorinated gases	Range from a few hundred to 23000	Thousands of years

* **Global Warming Potential (GWP)** is a measure of how much heat a substance can trap in the atmosphere over a period of 100 years, as compared to an equivalent amount of CO_2. (CO_2 has a GWP of 1 by definition.)
* GWP is used to compare the global warming impact of different greenhouse gases. The larger the GWP, the more that a given gas warms the Earth compared to CO_2 over that time period.

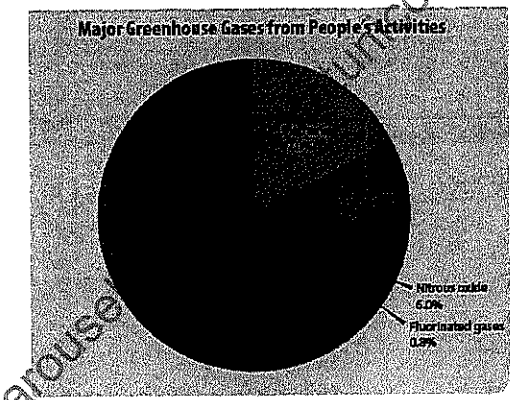


Fig. 3: Diagram showing the amount of warming caused by the major greenhouse gases produced due to human activity

2) INCREASE IN GLOBAL SURFACE TEMPERATURES DUE TO INCREASE IN GREENHOUSE GAS EMISSIONS

- Anthropogenic greenhouse gas emissions have increased since the pre-industrial era, driven largely by economic and population growth. This has led to atmospheric concentrations of carbon dioxide and methane that are unprecedented in at least the last 800,000 years.

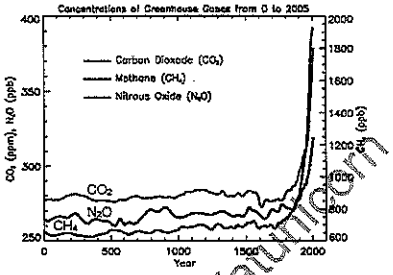


Fig. 4: Diagram showing global greenhouse gas concentrations

Average greenhouse gas concentrations from Year 0 to 2005. Significant rise in greenhouse gas concentrations is seen from around 1850 to current time.

- The Earth's surface temperature follows a similar increasing trend in recent times.

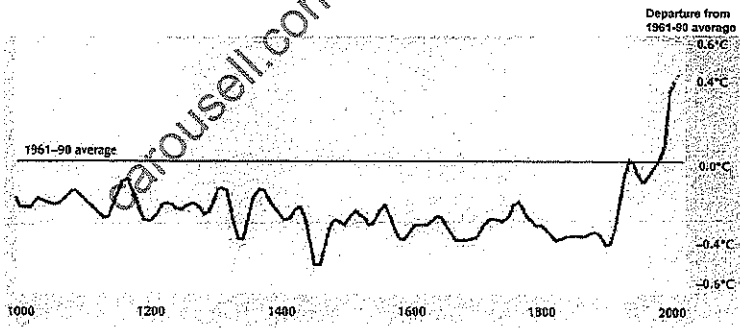


Fig. 5: Diagram showing temperature at Earth's surface

Temperature from 1000 to beyond 2000 in the northern hemisphere. Graph shows a general upward trend in temperature at the Earth's surface in recent times.

- The increase in greenhouse gas emissions that has been detected throughout the climate system is likely to be the dominant cause of the observed warming. This has been attributed to the increase in atmospheric carbon dioxide levels resulting in enhanced (or anthropogenic) greenhouse effect.

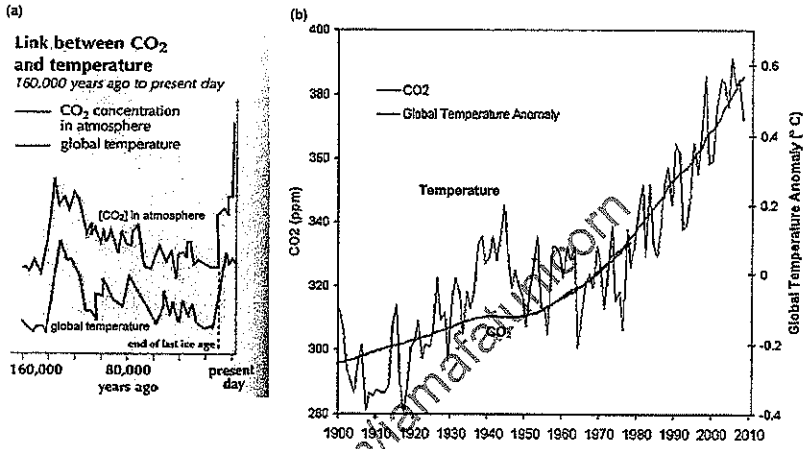


Fig. 6: Diagram showing the correlation between CO_2 levels and temperature

(a) Link between CO_2 levels and temperature from 160 000 years ago to present is evident from the similar trend in both graphs. (b) The relationship between CO_2 levels and the increase in the global temperature anomaly is visible in the increasing trend of both graphs. The CO_2 levels were measured in Antarctica from 1900 to 1980 and in Mauna Loa from 1960 to present day. The global temperature anomaly is calculated as a difference between the combined average temperature over global land and ocean surfaces and the 20th century average of 14.8°C.

3) HUMAN ACTIVITIES AND THE INCREASE IN THE GREENHOUSE GAS EMISSIONS

A. Global Greenhouse Gas Emissions by Various Sectors

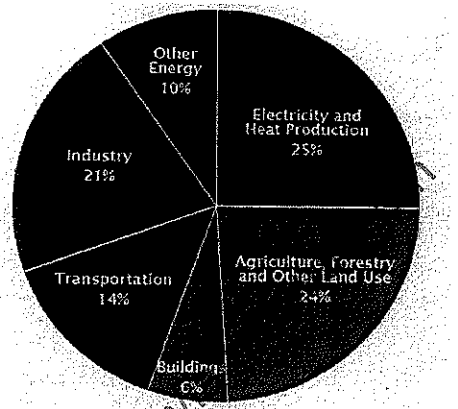


Fig. 7: Diagram showing the greenhouse gas emissions by various sectors

Global greenhouse gas emissions can also be broken down by the economic activities that lead to their production.

- **Electricity and Heat Production** (25% of 2010 global greenhouse gas emissions):

  The burning of coal, natural gas, and oil for electricity and heat is the largest single source of global greenhouse gas emissions.

- **Industry** (21% of 2010 global greenhouse gas emissions):

  Greenhouse gas emissions from industry primarily involve fossil fuels burned on-site at facilities for energy.

  This sector also includes emissions from chemical, metallurgical, and mineral transformation processes not associated with energy consumption and emissions from waste management activities. *(Note: Emissions from industrial electricity use are excluded and are instead covered in the Electricity and Heat Production sector.)*

- **Agriculture, Forestry, and Other Land Use** (24% of 2010 global greenhouse gas emissions):

  Greenhouse gas emissions from this sector come mostly from agriculture (cultivation of crops and livestock) and deforestation.

  This 24% estimate does not include the CO_2 that ecosystems remove from the atmosphere by sequestering carbon in biomass, dead organic matter, and soils, which offset approximately 20% of emissions from this sector.

- **Transportation** (14% of 2010 global greenhouse gas emissions):

  Greenhouse gas emissions from this sector primarily involve fossil fuels burned for road, rail, air, and marine transportation. Almost all (95%) of the world's transportation energy comes from petroleum based fuels, largely gasoline and diesel.

- **Buildings** (6% of 2010 global greenhouse gas emissions):

  Greenhouse gas emissions from this sector arise from onsite energy generation and burning fuels for heat in buildings or cooking in homes.
  (Note: Emissions from electricity use in buildings are excluded and are instead covered in the Electricity and Heat Production sector.)

- **Other Energy** (10% of 2010 global greenhouse gas emissions):

  This source of greenhouse gas emissions refers to all emissions from the Energy sector which are not directly associated with electricity or heat production, such as fuel extraction, refining, processing, and transportation.

Overall, burning of fossil fuels for electricity, transportation and industry, as well as in agriculture result in the bulk of greenhouse gas emissions.

B. Burning of fossil fuels to provide energy

- The main human activity that emits greenhouse gases, especially CO_2, is the combustion of fossil fuels (coal, natural gas, and oil).

- Among the many human activities that produce greenhouse gases, the use of energy represents by far the largest source of emissions.

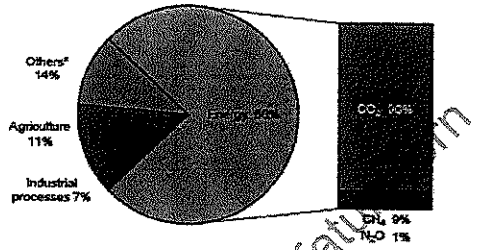


Fig. 8: Diagram showing shares of global anthropogenic greenhouse gas emissions in 2010
Energy emissions, mostly CO_2 account for the largest share of global greenhouse gas emissions.
** Others include large-scale biomass burning, post burn decay, peat decay, indirect N_2O emissions from non-agricultural emissions of NO_x and NH_3, waste and use of solvents.*

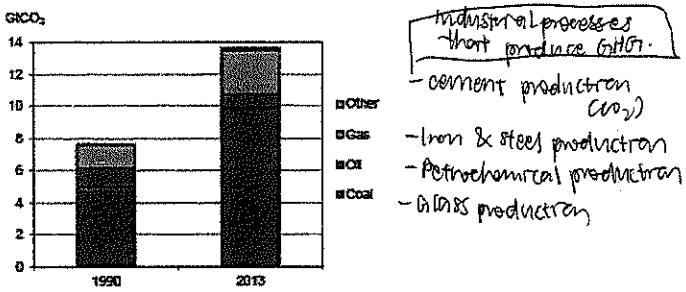
- Fossil fuel is usually burnt for the following purposes:

i. *Electricity and heating* (25 percent of total global greenhouse gas in 2000)

  - The combustion of fossil fuels to generate electricity (for homes, business and industry) is the largest single source of greenhouse gas emissions.

  - The amount of greenhouse gases produced is dependent on the type of fossil fuel used to generate electricity. As coal is the most carbon-intensive fossil fuel, it results in more CO_2 emission compared to oil or natural gas in order to produce a given amount of electricity.

  - Generation of electricity and heat worldwide relies heavily on coal, the most carbon-intensive fossil fuel.



industrial processes that produce GHGs.
- cement production (CO_2)
- Iron & steel production
- Petrochemical production
- Glass production

Fig. 9: Diagram showing CO_2 emissions from electricity and heat generation

CO_2 emissions from electricity and heat almost doubled between 1990 and 2013, driven by the large increase of generation from coal.

ii. Transportation (14% of total global greenhouse gas emissions in 2000)

- The combustion of fossil fuels such as gasoline and diesel to transport people and goods is the fourth largest source of greenhouse gas emissions.
- For transport, the fast emissions growth was driven by emissions from the road sector. (CO_2 emissions increased by 68% since 1990 and accounted for three quarters of transport emissions in 2013.)

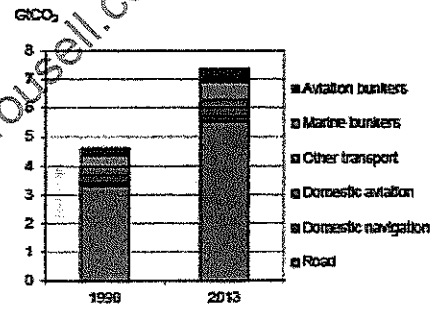


Fig. 10: Diagram showing CO_2 emissions from transport

CO_2 emissions from road are driving the growth of transport emissions.

iii. Industry

- Many industrial processes emit greenhouse gases through fossil fuel combustion.
- Several processes that do not involve combustion can also produce CO_2 emissions through chemical reactions, for e.g.:
  1. production and consumption of mineral products such as cement
  2. production of metals such as iron and steel
  3. production of chemicals
- Increasing demand for energy comes from worldwide economic growth and development.
- Despite the growth of alternative sources of energy (non-fossil), the use of fossil fuels remains unchanged due to the increased demand of energy supply.
- Coal has a heavy carbon content per unit of energy released. Compared to natural gas, coal is nearly twice as emission intensive on average.

(Although coal represented 29% of the world total primary energy supply in 2013, it accounted for 46% of the global CO_2 emissions.)

(Globally, coal combustion generates the largest share of CO_2 emissions, although oil remains the largest energy source.)

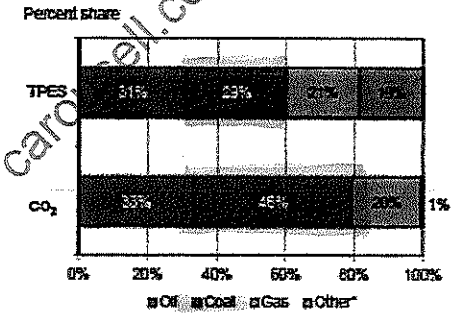


Fig. 11: Diagram the world primary energy supply and CO_2 emissions: shares by fuel in 2013

* *Other includes nuclear, hydro, geothermal, solar, tide, wind, biofuels and waste.*

- Many industrialised nations turning to more efficient fuel use or alternative sources of fuel are to curb their carbon dioxide emissions, emissions have increased markedly in many of the newly industrialising countries.
- While petroleum reserves are limited, there are still hundreds of years of coal reserves which would fill the growing energy demand of developing countries.

C. Clearing of forests

The link between trees, deforestation and global warming

- Trees convert the CO_2 in the air to O_2, through the process of photosynthesis. The more trees, the less CO_2 in the atmosphere and the more O_2.
- Some of the CO_2 absorbed by plants is stored as organic material throughout their lifetime. Soils can also store CO_2, depending on how the soil is managed. This storage of carbon in plants and soils is called biological carbon sequestration. Because biological sequestration takes CO_2 out of the atmosphere, it is also called a greenhouse gas "sink."
- The role of trees is made more important by the increase in CO_2 emissions due to human activities.
  (In the United States overall, since 1990, land use, land-use change, and forestry activities have resulted in more removal of CO_2 from the atmosphere than emissions. Because of this, the land use, land-use change, and forestry sector in the United States is considered a net sink, rather than a source, of CO_2 over this period. In many areas of the world, the opposite is true. In countries where large areas of forest land are cleared, often for agricultural purposes or for settlements, the land use, land-use change, and forestry sector can be a net source of greenhouse gas emissions.)
- Undisturbed waterlogged peatlands (organic soils) store a large amount of carbon and act as small net sinks. Drainage of peatlands for agriculture and other uses results in a rapid increase in decomposition rates and increased risk of peatland fires, leading to increased emissions of CO_2 and N_2O.
- In summary, clearing of forests and mangroves and draining of waterlogged peatlands decrease amount of CO_2 sinks available. The subsequent decomposition and burning of biomass from the cleared forests releases more greenhouse gases. Thus overall, clearing of forests results in significant increase in greenhouse gas emissions.
- Anthropogenic land-use activities and changes in land use (e. g., conversion of forest lands and grasslands to cropland and pasture) result in changes in these natural fluxes.

  For example:

  - Deforestation increases CO_2 emission, while afforestation can reverse the effect of deforestation by creating "sinks of CO_2"
  - Conversion to agriculture can result in production of non-CO_2 emissions such has CH_4 from livestock and rice cultivation and N_2O from manure storage and agricultural soils

preventing deforestation is better than reforestation.

D. Food choices

- Most studies attribute 10 to 35 percent of all global greenhouse gas emissions to agriculture.
- With economic development, industrial style agriculture replaced traditional small-scale farming. The increased efficiency of industrial agriculture has also led to reduced prices for many of our daily products. It helped to reliably nourish large populations, and turned a food that was an occasional meal - meat - into an affordable, every day product for many.
- The growth in per capita meat consumption is strongly linked to increasing levels of income in many countries of the world.

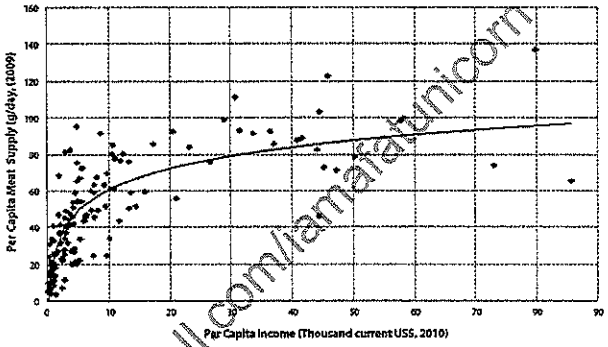


Fig. 12: Diagram showing per capita income versus meat consumption

- Coupled with global human population growth, global meat consumption also increased accordingly.

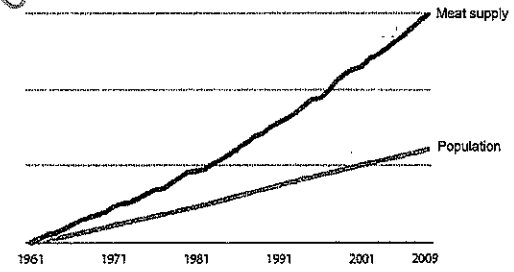


Fig. 13: Diagram showing growth of population and meat supply

- Sources of greenhouse gas emissions from raising livestock

Source of greenhouse gas	How is the greenhouse gas produced?	Which greenhouse gas is produced?
i. Enteric fermentation	• Enteric fermentation is a digestive process by which carbohydrates are broken down by microorganisms into simple molecules for absorption into the bloodstream of an animal	• Methane
ii. Nitrogenous fertilisers	• Application of chemical nitrogenous fertilisers to increase production of animal feed for livestock • Production of chemical nitrogenous fertilisers	• Nitrous oxide • Carbon dioxide and nitrous oxide
iii. Manure	• Stored manure from livestock • Application of manure as fertilisers	• Methane • Nitrous oxide
iv. Deforestation and conversion of grassland into agricultural land	• Decomposition of carbon and nitrogen-rich humus	• Carbon dioxide and nitrous oxide

- Beef produces the highest greenhouse gas emissions in comparison to other products such as pork, poultry and milk. Emissions from beef amounts to 20 times that of wheat. Thus it is more "climate efficient" to produce protein from vegetable sources rather than from animal sources.
  - Changes in human diet may be a practical tool to reduce greenhouse gas emissions.
  - Substantial reductions in meat consumption in developed countries and constrained growth in demand in developing countries would necessary to keep greenhouse emissions from rising.

4) EFFECT OF GREENHOUSE GAS EMISSIONS ON CLIMATE CHANGE

A. Melting of ice sheets, polar ice caps and glaciers resulting in the rise of sea levels

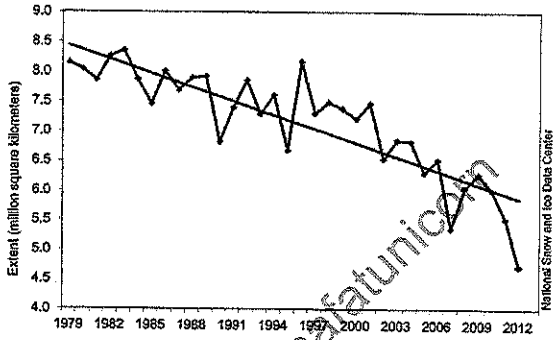


Fig. 14: Average monthly Greenland sea extent from Aug 1979 - 2012

- Global warming has resulted in the rise of sea levels due to:
  - Melting of the Greenland and Antarctic ice sheets, glaciers and polar ice caps (land ice)

    Sea level has slowly been rising, partly due to the addition of water to the oceans through the melting of the polar ice caps.

    The world's two major ice sheets – Greenland and Antarctica – contain about 75% of the world's fresh water, enough to raise the sea level by over 75 metres, if all the ice were returned to the oceans.

land & sea ice helps to reflect radiation from sun back into space

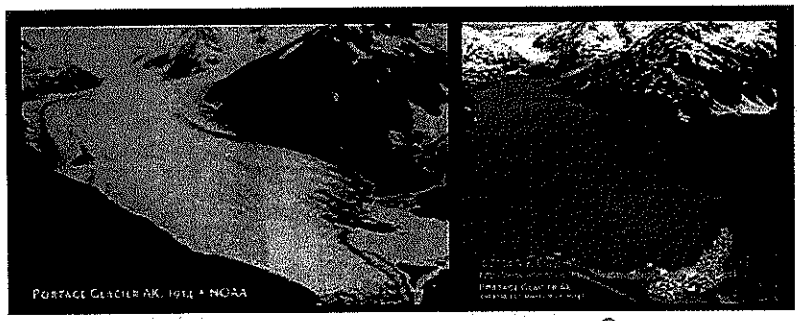


Fig. 15: Melting of ice sheets at Alaska's Portage Glacier in 1914 (left) and 2004 (right)

- Thermal expansion of seawater

  Thermal expansion occurs when the ocean temperature rises and the particles that make up the ocean start to move more vigorously. This causes expansion of water, increasing the overall volume of the ocean, causing a rise in average sea levels.

- Other effects from melting of the Greenland and Antarctic ice sheets, glaciers and polar ice caps (land ice):

  - Loss of fresh water resource:
    Glaciers serve as an important source of fresh water. As glaciers retreat, this resource is lost and floods, avalanches, or landslides may also be triggered by glacial melt.

    Rise in sea levels impact many since almost 50% of the world's population lives close to the seashore.

- Melting of land ice vs sea ice:

  - Melting of sea ice (unlike land ice) does not result in any increase in sea level, as sea ice already displaces water.

  - Melting of sea ice affects marine animals, such as seals, polar bears, and fish, depend on sea ice. With a loss of sea ice, these animals will lose access to their feeding grounds for long periods, which will make it difficult for these populations to be sustained.

B. Heatwaves and heavy rainfall

- How global warming results in change in weather:

Rise in temperature and warmer air
↓
Greater evaporation from land and water into atmosphere
↓
More water vapour in the air
↓
Increased precipitation i.e. more rain and snow.
(Do note that this increase in precipitation is not spread evenly around the globe. Some places will get less precipitation due to shifts in air and ocean currents. This results in change in global weather patterns.)

- Impact of climate change on weather:

  - Overall amount of precipitation increases, but the distribution of precipitation around the world is uneven.

  - Rainfall is likely to increase in the tropics ~~and at high latitudes~~ and decrease in the already dry subtropics.

    - Effect of increase rainfall in the tropics ~~/ at high latitudes:~~

      - Increased frequency and intensity of rainfall produces more pollution such as erosion and sedimentation due to runoff

      - Water-borne diseases and lower water quality likely to increase with heavy precipitation

      - Increased risk of flooding in urban areas.

    - Effect of decrease rainfall in the subtropics:

      - Increase areas of the planet affected by drought

  - Increases in temperature will affect the amount and duration of snow cover which will in turn affect the timing of ice melting and water flowing into waterways, impacting groundwater recharge.

  - The number of frost days is expected to decrease at high latitudes, increasing the growing season, thus increasing food production for the high latitudes.

  - Heatwaves are expected to become more frequent as average temperatures rise

    - Increasingly intense and frequent heat-waves could cause more heat-related deaths, particularly amongst the elderly, chronically ill and very young.

- Conversely, the occurrence of cold-related deaths is expected to fall.

- Stronger storms and hurricanes

  - Hurricanes and other tropical storms get their energy from warm ocean water. As the top layer of the ocean gets warmer, hurricanes and other tropical storms grow stronger, with faster winds and heavier rain.

C. Stress on freshwater supplies

- Freshwater supplies are scarce. The vast majority of the Earth's water resources are salt water, with only 2.5% being fresh water. Approximately 70% of the fresh water available on the planet is frozen in the ice sheets of Antarctica and Greenland leaving the remaining 30% (equal to only 0.7% of total water) available for consumption. From this remaining 0.7%, roughly 87% is allocated to agricultural purposes.

- With climate change, scarcity of freshwater is expected to become an increasing:

1. Groundwater depletion

<affected by change in precipitation pattern>
- Shorter duration of rainfall combined with increased sum of evaporation and plant transpiration from the earth's land surface, affects the replenishment of groundwater negatively.

- In some areas with less rainfall, there will be increased pumping of groundwater for irrigation.

2. Decline in water quality

<affected by change in precipitation pattern>
- In areas with increased precipitation:

  - There will be a corresponding increase in runoff. The water carries larger levels of sediment and contaminants and are flushed into waterways and drinking reservoirs.

- In areas with drought:

  - Groundwater reserves may be depleted.

  - The residual water that remains is often of inferior quality. This is a result of the leakage of saline or contaminated water from land surface, or adjacent water bodies that are contaminated.

  - Decreased precipitation and runoff results in a higher concentration of pollution and nutrients in the water.

  - High level of nutrients tends to an increase load of microbes in waterways and drinking-water reservoirs.

<affected by increase in temperature>
- Increase in water temperatures can lead to:

  - a bloom in microbial populations in freshwater supplies

  - reduce the amount of dissolved oxygen in the water
    The health of a body of water is dependent upon its ability to effectively self-purify through biodegradation, which is hindered when there is a reduced amount of dissolved oxygen.

<affected by increase in sea level>
- For coastal populations, water quality is affected by salinisation, or increased quantities of salt in water supplies. A rise in sea levels will increase salt concentrations in groundwater and estuaries, extending areas of salinity and decreasing freshwater availability.

D. Release of greenhouse gases in frozen organic matter

- Permafrost refers to a layer of soil or rock that is frozen all year round. Plants can still grow in the soil at the surface (active layer), which is not frozen during warmer parts of the year.

Fig. 16: Cross-section of layers of soil

The active layer is the top layer of soil that thaws during the summer and freezes again during the autumn. Beneath that is the permafrost which is a permanently frozen layer of soil. Beneath the permafrost or scattered among the permafrost can be sections of unfrozen soil and rock.

- Permafrost is found throughout much of North America, Scandinavia, Russia and China.

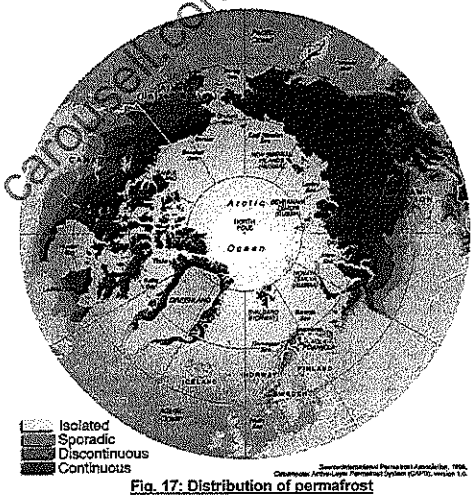


Fig. 17: Distribution of permafrost

- Permafrost stores an immense amount of carbon and methane, twice as much carbon as contained in the atmosphere. In a warming environment, permafrost is expected to degrade, and these gases which have been in storage will be released.

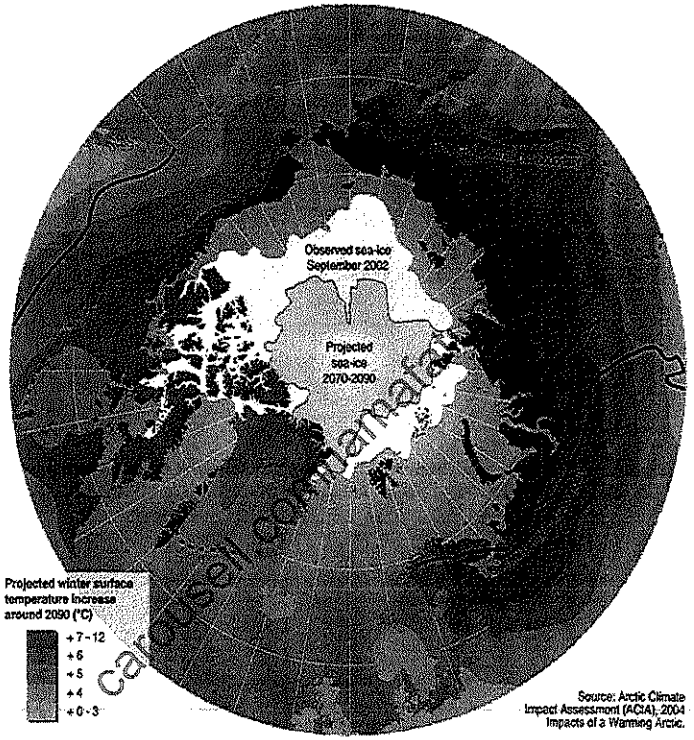


Fig. 18: Projected depletion of permafrost

Expected changes in Artic temperature by 2090 and its effect on the permafrost boundary. Solid lines depict current permafrost boundary. Dashed line is the projected permafrost boundary by 2090.

- Carbon stored in permafrost
  - A third of the Earth's soil carbon is found in the permafrost of the Arctic, where the soil carbon is stored in frozen organic matter.
  - Impact of climate change on carbon in permafrost:
    1. Thawing of permafrost

       If the high northern latitudes warm significantly, permafrost will thaw, allowing the organic matter within the permafrost to decompose. The decomposition will release carbon into the atmosphere.

    2. Faster rate of decomposition of organic matter

       Decomposition of organic matter already happens within the active layer. As the active layer thaws each summer, some organic matter decomposes. Under normal climate conditions (i.e. a cold arctic region), the decomposition is very slow. But as air temperature increases and the ground warms, this process will speed up. $\uparrow CO_2$

- Methane stored in the permafrost
  - Methane exist in the form of methane hydrates which is methane gas frozen into ice structures. They are formed at cold temperatures and under high beneath layers of frozen permafrost.
  - Impact of climate change on methane in permafrost:
    1. Thawing of permafrost to release methane

       When permafrost thaws, methane can be released directly into the atmosphere or it can be broken down into carbon dioxide by bacteria which will then be released.

    2. Increase microbial synthesis of methane

       If areas of thawed permafrost exist between frozen layers of permafrost, microbial activities can continue even during the winter, to create new methane from organic material.

5) IMPACT OF CLIMATE CHANGE ON PLANTS, ANIMALS & MAN

Plants and animals require specific environmental conditions—such as the right temperature, moisture, and light levels—in order to thrive. Even small changes in environmental parameters can affect the reproduction and survival of a species.

A. Effect of Climate Change on Physiology of Plants & Animals

- Alterations of CO_2 concentration, temperature and rain cycles can directly affect the biological processes such as photosynthesis, respiration, growth and composition of plant tissues.
- Water availability is a major rate limiting factor for plant growth. Under drought conditions and/or high temperatures, plants tend to experience **water stress**, and undergo **physiological** and **morphological changes:**
  - reduction of water content and turgor, cell expansion slows down or ceases
  - alteration in level of photosynthetic pigment (reduction or no pigmentation)
  - rapid decrease in the amount of Rubisco which leads to lower amounts of carbon fixation
  - increasing the ratio of roots to shoots; developing more roots in order to access more water, reducing leaf number and leaf area to lower transpiration rates
  - stomatal closure
  - number of stomata per leaf decreases to reduce transpiration

As such, photosynthetic rates decrease and **plant growth is retarded**. Under prolonged drought, many plants **dehydrate and die.**

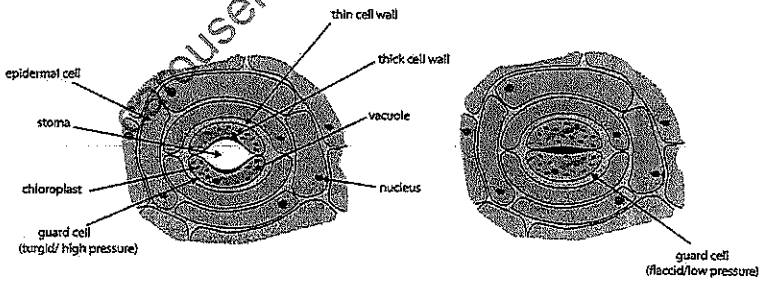


Fig. 19: The opening and closing of stomata controlled by turgidity of guard cells.

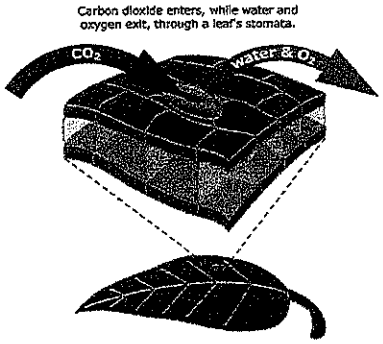


Fig. 20: Stomata allow carbon dioxide in, but they also let precious water escape.

Stomata closure is one of the first events in plants response to water deficiency. This limits gaseous exchange through stomata, reduces transpiration and arrests carbon assimilation that takes place during photosynthesis.

Although CO_2 assimilation and net photosynthesis decreases due to stomatal closure, the attainment of low transpiration rate and prevention of water losses from leaves is a good trade-off for survival in exchange of growth.

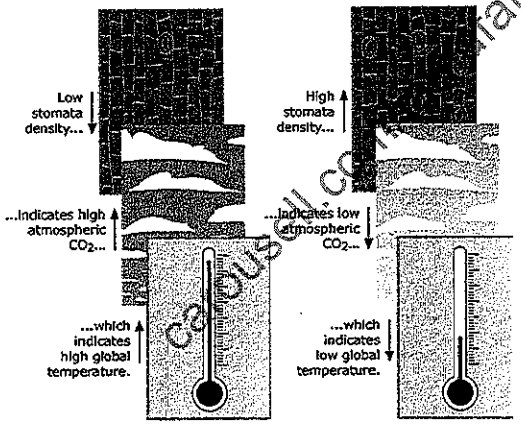


Fig. 21: Correlation between stomata number, carbon dioxide level and temperature.

Typically, plant fossils with many stomata (low carbon dioxide) came from times of low global temperature, and fossils with few stomata (high carbon dioxide) came from times of high global temperatures.

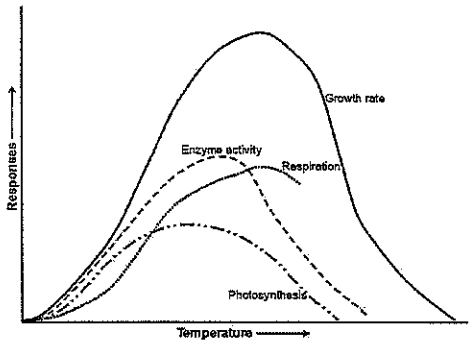


Fig. 22: Effect of temperature on plants.
The average rate of enzymatic reactions increases two-fold with every 10°C increase in temperature up to the optimum temperature (30 – 45°C for most enzymes). Beyond the optimum temperature, enzymes are irreversibly denatured, and plant death results ultimately. The most observed effect of heat stress on plants is the retardation of growth.

- Plants native to dry/hot environments have certain morphological or physiological characteristics that allow them to survive better:
  - **Morphological** characteristics of leaf that helps to reduce loss of water
    - Leaf has small surface area to volume ratio
    - Thick waxy cuticle and epidermis
    - Stomata only present on bottom surface of leaf
    - "Sunken" stomata – Stomata found in grooves and often surrounded by hair: this creates a region of high humidity at the stomata opening, reducing the likelihood of evaporation and water loss through transpiration

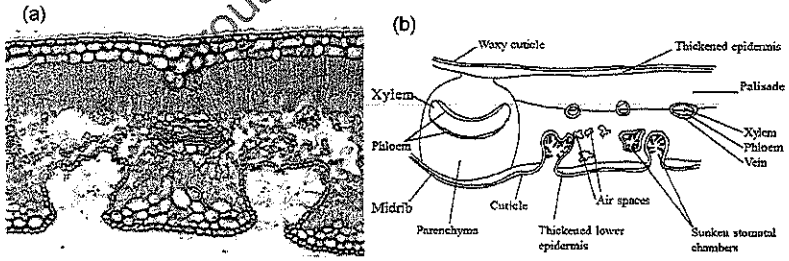


Fig. 23: (a) Transverse section (t.s.) of a xerophyte (plant adapted to live in dry conditions) leaf. (b) Tissue diagram of leaf (t.s)

- Some plants like the marram grass have developed leaves that can curl / roll up in response to the amount of water available. They have "hinge cells" / bulliform cells that allow the leaves to roll up on dry days and to unroll on wet days.

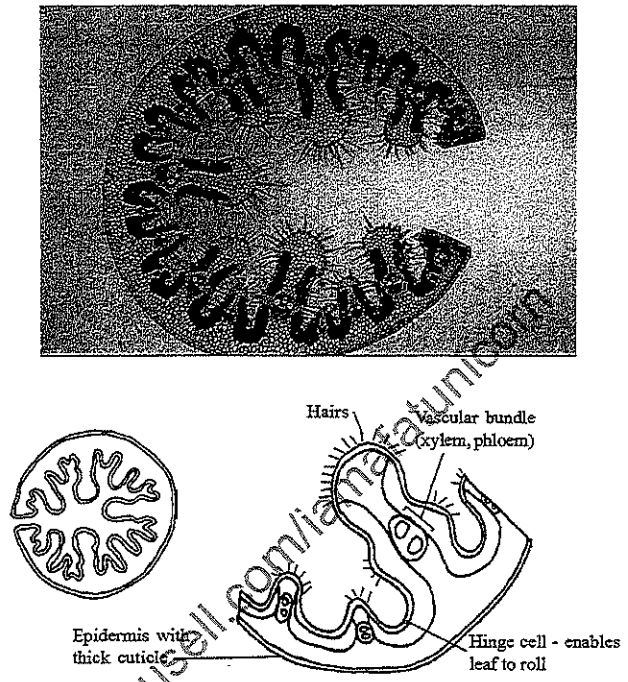


Fig. 24: (a) Transverse section (t.s.) of a marram grass leaf. (b) Tissue diagram of marram grass.

- **Physiological** characteristics of plants in dry environments:

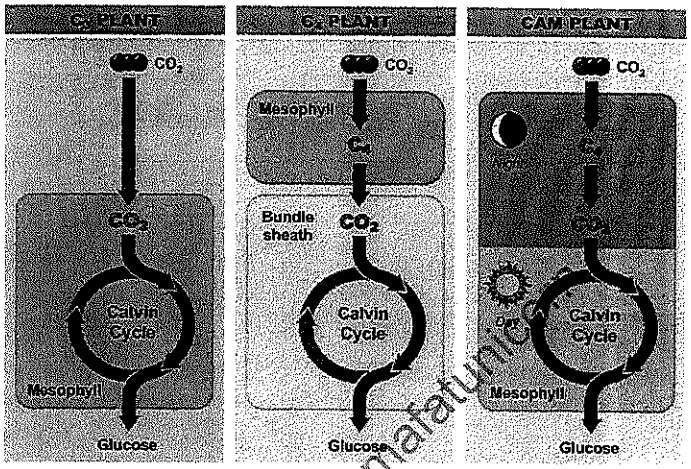


Fig. 25: Different methods of carbon fixation in C_3, C_4 and CAM plants.

- Plants in dry environments close their stomata for most of the day. This reduces the concentration of carbon dioxide in the cell. Oxygen is a competitive inhibitor for RuBisCO. Hence under conditions of low CO_2 and high O_2 concentration, photorespiration occurs where RuBisCO fixes O_2 instead of CO_2. Rate of photosynthesis is lowered as a result.
- Plants overcome this problem by varying their methods of carbon fixation:
  - In C_4 plants, carbon fixation and Calvin cycle takes place in different cells. CO_2 is converted to a 4C compound by an enzyme that does not bind O_2.The 4C compound is then sequestered to a deeper tissue layer where less oxygen is present. CO_2 can then be released and can enter the Calvin cycle without competition from oxygen.
  - In CAM plants, carbon fixation occurs at night. CO_2 is converted to a 4C compound which release CO_2 for Calvin cycle in the day. This allows stomata to remain close in the day and open only at night.

- **Global warming** can also **affect the reproduction** of plants:
  - High temperatures at flowering time **reduce pollen viability** in cereals - it has been found that rising temperatures cause a loss of pollen viability in maize.
  - Deviation from optimal temperature **disrupts flowering process** and **affects seed production** in plants as well (e.g. rice and wheat).

- **Change in body size of animals**
  - Bergmann's rule states that, "in warm-blooded animals, races from warm regions are smaller than races from cold regions".

    →i.e. body size become smaller in response to general warming and larger with cooling.
  - The larger surface areas relative to volume of smaller body sizes serve as efficient heat dissipaters in warm climates, while small body surface area relative to volume may help in heat conservation in cold climates.

- **Changes in temperature affects insects' metabolism**
  - The body temperature of *ectotherms* fluctuates with environmental temperatures, and basic physiological functions such as locomotion, growth, and reproduction are strongly influenced by environmental temperature.
  - Growth and development are fuelled by metabolism. Metabolic rate is the consequence of many different biological reactions; **temperature governs metabolism through its effects on rates of these enzyme-catalysed biochemical reactions.**

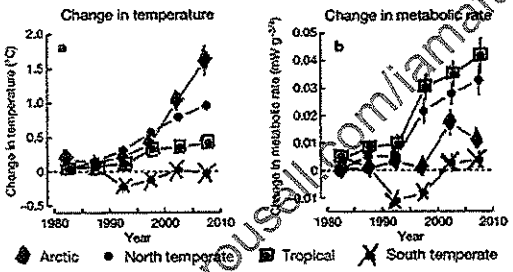


Fig. 26: Global changes in temperature and in metabolic rates of insects since 1980.
a) Changes in mean temperature (5-year averages) for Arctic, North temperate, South temperate and tropical regions.
b) Predicted absolute changes in metabolic rates by geographical region.

  - In ectotherms, **rates of both *somatic growth* and *ontogenetic development* increase with increasing temperature.** Therefore, **the time to maturity is shorter at higher temperatures.**
    (A 10°C increase** in temperature results in approximately **2.5-fold increase in developmental rate)
  - The majority of ectotherms **grow slower** but mature at a **larger body size** in **colder** environments. Cold environments allow ectotherms to reach a relatively large body size by **prolonging growth** and **delaying reproduction.**

Ectotherms: cold-blooded species such as insects and reptiles
Somatic growth: physical development of organism during adolescence.
Ontogenetic development: development of organism from time of fertilisation of the egg to the organism's mature form.

- **Insects have a narrow temperature tolerance**
  - The difference between an organism's thermal optimum and its current climate temperature is known as the **thermal safety margin.**
  - For insect species, **thermal safety margins increase sharply with latitude.**
  - Insects in tropic environments:
    - Species living in **tropic** environments (e.g. *Acyrthosiphon pisum*) that are already close to their physiological optimum have **small thermal safety margins.** Thus, even small amounts of warming will likely decrease performance, as tropical insects will thus approach near-lethal temperatures much faster.
    - Many species also have small dispersal ranges which increase their **risk of extinction** through small changes in habitat or environment.
  - Insects in temperate environments:
    - Species living in **temperate** environments have **large thermal safety margins.** Their environments are on average cooler than their physiological optimal, and thus have broader thermal tolerance.
    - Warming may **enhance the fitness** of insects in temperate environments:
      - Increase in temperature can cause initial increases in population growth rates and performance.
      - With warmer winters, the insects will not all die out like they do now. Since spring starts earlier, these also emerge even earlier. With summer lasting longer, the insects also persist in the environment for longer than before.

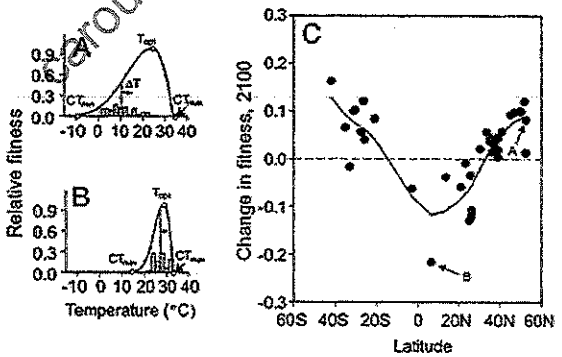


Fig. 27: Fitness curves for representative insect taxa from temperate (A) and tropical (B) locations, and (C) the predicted change in fitness because of climate warming for all insect species studied as a function of latitude. Fitness curves A and B are derived from measured intrinsic population growth rates versus temperature for 38 species. At mid to high latitudes, population growth rates are predicted to increase, indicating enhanced population fitness because of warming. In the tropics (i.e. low latitude), however, rates of population growth are expected to decrease by up to 20%, implying that warming will substantially reduce fitness.

- **Changes in timings of seasonal life cycle events with climate change**
  - Many species take their cues about when to migrate, flower, nest or mate from seasonal changes in temperature, precipitation and daylight.
  - Global warming is confusing those signals – the changes in the climate force wildlife to alter life cycle and seasonal events. For example:
    - American flowering plants like columbines and wild geraniums are blooming earlier than before.
    - North American Fowler's toads are breeding six days earlier than they did a decade ago.
    - Increased warmth also advances the onset of insect life cycles for the many species that use thermal cues to match the timing of life history events with the changing seasons
    - Tree swallows (Tachycineta bicolor) are nesting and laying their eggs earlier.
    - Birds are migrating and arriving at their nesting grounds earlier and in North America, one species of hummingbird has ceased to migrate.
    - Marmots are ending their hibernations about three weeks earlier than they did 30 years ago.
  - Changes like these can lead to mismatches in the timing of migration, breeding and food availability - species might get out of synch with other species in their ecosystem or with other natural events.

B. Effect of Climate Change on Plant Distribution and Animal Migration

As global temperatures rise, both animal and plant populations are projected to **gradually shift upward to higher elevations and toward northern latitudes** where temperatures are cooler in order to stay within their ideal range of environmental conditions.

Shift to higher elevations

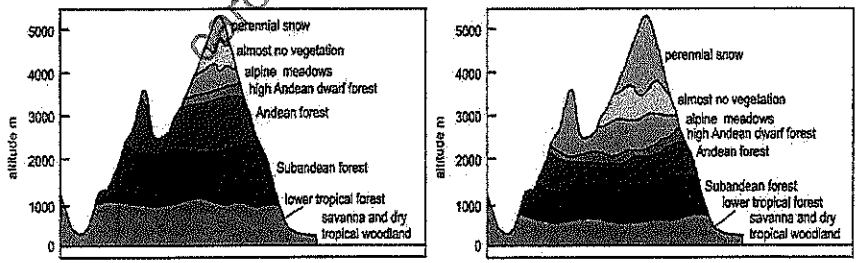


Fig. 28: Change in altitude of the zones of vegetation belts in the Columbian Andes of South America due to global warming, where each zone was about 1200 metres lower during the last glacial period as compared to present day. The average temperature drops as altitude increases, ~ 0.55°C per 100m.

- Eg. The American pika shifting to higher altitudes

Fig. 29: The American pika, a small mammal that lives on Western mountaintops. It has adapted to life in mountainous areas that rarely get above freezing and can die when exposed to temperatures as mild as 25.6°C. As global warming increases average temperatures, the pika is being forced to move to higher and higher altitudes to find the tolerable alpine temperatures it calls home. Unlike many wildlife species that are shifting their ranges north in response to changing climate, the pika and other alpine animals may soon run out of places to go.

- Trapped at the top, alpine wildlife is vulnerable to several of global warming's damaging effects, including vegetation changes, the invasion of new predators and pests, reduced winter snowpack and increases in extreme weather events.

Shift towards northern latitudes

- Forests can be classed into three different types according to latitude – tropical, temperate and boreal forests.

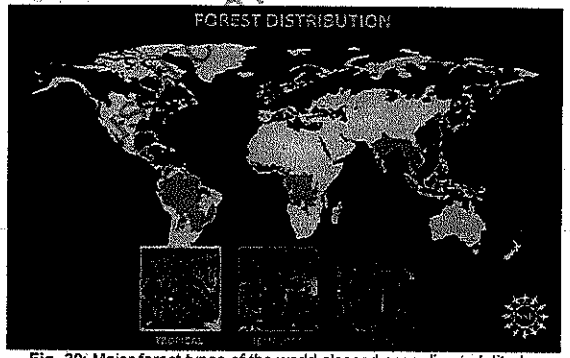


Fig. 30: Major forest types of the world classed according to latitude.

- In terms of migration latitude-wise, boreal forests could shift into the tundra, while being displaced by temperate forests and grasslands. The fate of tropical forests is uncertain, but some future climate scenarios indicate possible large losses.

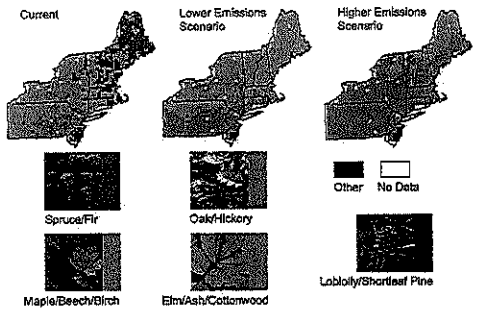


Fig. 31: Tree species in the Northeast USA are shifting northward. The range of spruce/fir, maple, elm/ash/cottonwood forests are shrinking and being replaced by oak/hickory forest in most of the region, and by loblolly/shortleaf pine forest in the southernmost areas. Maps of the Northeast USA show the distribution of several types of trees under the current conditions, a lower emissions scenario, and a higher emissions scenario. Under current conditions, there is a wide variety of trees in forests across the Northeast.

Under the lower emissions scenario, the map becomes predominately Maple, Beech and Birch, with a significant portion of Oak and Hickory. The Spruce and Fir disappear completely from the map. Under the higher emissions scenario, only northern New York, New Hampshire, Vermont and Maine have Maple, Beech and Birch trees. Oak and Hickory dominate the large majority of the region. Under both the lower and higher emissions scenario, the map of tree species shows that there will be significantly less diversity than the current conditions.

- Where forests will gain or lose ground depends on the regional characteristics of their environment, including water availability and temperature, and one other crucial factor – how fast the species can migrate.

  Compared to animals, plants are more sedentary and, thus, may be in greater risk of being affected by changes in the climatic condition. **The rate of climate change is likely to exceed the migration rates of most plant species.**

  The replacement of dominant species by locally rare species may require decades, and **extinctions may occur** when plant species cannot migrate fast enough to escape the consequences of climate change.

- Eg. Birds common in Florida like the cardinal, mockingbird and Carolina wren have steadily moved farther north, and are now found regularly in New England and Canada.

- Many marine species have certain temperature ranges at which they can survive. The warming of the Atlantic Ocean is causing many fish species to shift north towards cooler waters so they can live within their temperature range.

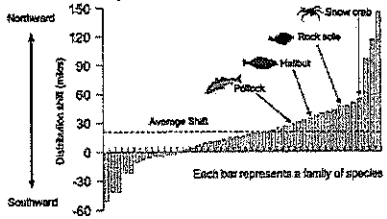


Fig. 32: The ranges of marine species have shifted northward as waters have warmed.

- **Impact of Temperature Changes on Distribution of Insects**
  - With the warmer climate, many species including insects would migrate northward or expand their range so as to remain within their temperature tolerance ranges.
  - Ecologists have already documented a shift towards the north in many species of insects. For instance, two-thirds of 35 butterfly species assessed in Europe shifted their ranges northwards by 35–240 km.

Fig. 33: The northern range of the Sooty Copper butterfly ended in Austria, but it can now be found in Estonia where it had never been seen before.

Therefore, because of global warming, some species of insects would adapt by migrating. Inability of insects to adapt to the warmer temperatures could lead to extinction, or a sharp drop in total population.

Global Warming & Spread of Mosquito-borne Disease beyond the Tropics and altitude

- Temperature is an important factor in the spread of malaria and other mosquito-borne diseases.
  1. With increased temperatures, mosquitoes that carry malaria, dengue fever and other tropical diseases have expanded their ranges towards the northern latitudes.

     → mosquito borne diseases like malaria and dengue fever may become **increasingly common in more northerly regions**

  2. Increased temperatures and precipitation can also increase abundance of mosquitoes and result in faster disease transmission.

     Using dengue fever as an example:

     - Mosquito vector, *Aedes aegypti*, lives and breeds in moist tropical regions;
     - With global warming, the duration for development/life cycle of mosquito from first instar to emergence is shorter;
     - As an increase in temperature increases their metabolic processes by increasing rates of enzyme-catalysed reactions;

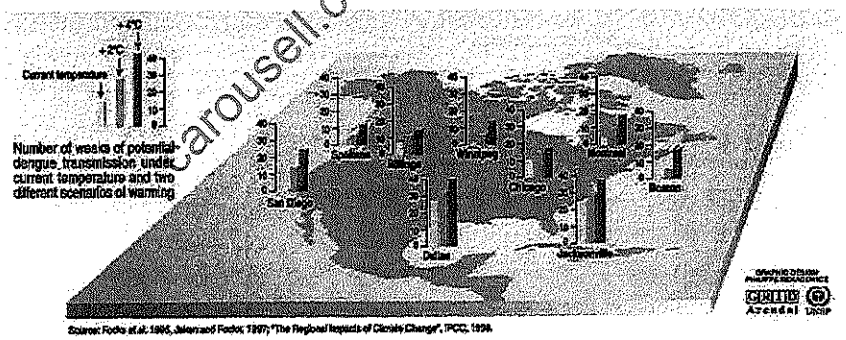
- resulting in faster development rate and decreasing the length of reproductive cycles and stimulating hatching of eggs;
- More mosquitoes result in greater transmission of dengue fever;
- Increased temperature also results in shorter extrinsic incubation period as virus can reproduce faster in mosquito vector;
- Global warming could result in more rainfall in certain areas creating more pools of stagnant water for mosquito breeding (ORA less rainfall in some areas so less standing water for mosquitoes to breed);

Seasonal pattern:

- Hotter summer months results in an increase incidence of dengue fever in summer;

Geographical pattern:

- Global warming results in temperate regions becoming warmer, such that conditions become more suited for the survival of Aedes mosquitoes;
- Mosquitoes will thus move to higher latitude so they spread to new areas expanding their distribution;
- Global warming also results in higher altitudes becoming warmer, thus mosquitoes will be able to colonise altitudes higher up the mountain;



Source: Focks et al. 1995, Jetten and Focks, 1997; "The Regional Impacts of Climate Change", IPCC, 1998.

Fig. 34: Projected dengue transmission in North America with rising temperatures. Although dengue is most common in tropical regions, with warmer climate, mosquito vectors would be present for a larger portion of the year in North America and the incidence of dengue would likely increases there as well. This figure depicts the number of weeks of the year potential dengue transmission in various North America cities with the current temperature (yellow), with 2°C warming (light orange) and with 4°C warming (dark orange).

Another example: malaria

- The mosquito vector of the malaria parasite, *Anopheles* mosquito, thrives in regions with warm temperatures, humid conditions, and high rainfall.
- Warm temperatures are also required for malaria parasites to complete their growth cycle within the *Anopheles* mosquitoes.

→ With global warming, malaria also may expand into new regions.

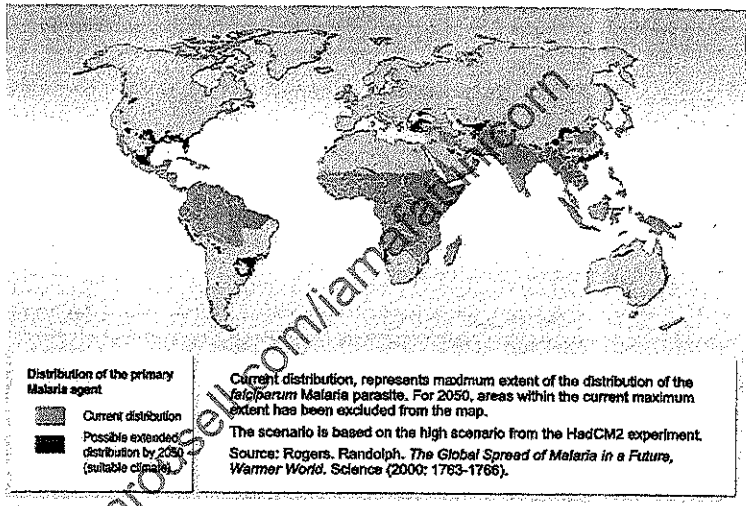


Fig. 35: Projected spread of malaria by 2050 due to climate change. Global warming will allow malaria to spread into new areas.

C. Impact of Climate Change on Biodiversity

Biodiversity is the variability among living organisms from all sources, including terrestrial, marine, and other aquatic ecosystems and the ecological complexes of which they are part.

What if organisms are unable to adapt or migrate in response to climate change?

- **Problems migrating organisms may face:**
  - Some migrating species will find that they must compete with other native species for food and other resources.
  - There may also be new predators the migrating species have to deal with – and will be unable to, since they have not fully adapted to the region.

- Not only predators, but possible exposure to new forms of disease may inhibit the effectiveness of their migration.
- Inability to adapt to these changes could severely impede the species' migration.
- However, moving into new areas may put these species into competition with other species over food and other resources. These shifts can also cause ecological disruptions as predators become separated from their prey.

- Approximately 20 – 30% of plant and animal species assessed so far are likely to be at increased **risk of extinction** if global average temperature increases by more than 2.7 - 4.5°F (IPCC).
- Wildlife that already live at high altitudes or latitudes, such as the American pika or polar bears in the Arctic, may find themselves with nowhere to go.

  - **Eg. Polar bears in the Artics**
    As climate change melts sea ice, the U.S. Geological Survey projects that two thirds of polar bears will disappear by 2050

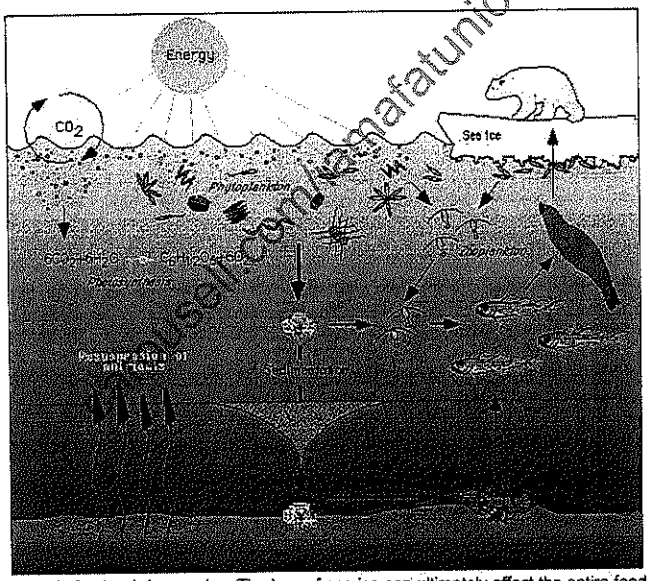


Fig. 36: The Arctic food web is complex. The loss of sea ice can ultimately affect the entire food web, from algae and plankton to fish to mammals. The melting of sea ice due to increased temperatures in the Arctic leads to declines in the abundance of ice algae which thrive in nutrient-rich pockets in the ice. These algae are eaten by zooplankton, which are in turn eaten by Arctic cod, an important food source for many marine animals, including seals. As seals are eaten by polar bears, declines in sea algae can contribute to declines in polar bear populations

- **e.g. death of coral reefs**

  - Reef-building corals are marine invertebrates found in shallow, clear, tropical oceans. These corals have a calcium carbonate skeleton which helps to build up the reef.

  - Zooxanthellae are a group of unicellular algae from the genus *Symbiodinium* that live within corals.

  - The symbiotic relationship between algae and coral:

    - Algae photosynthesise, producing chemical energy / food for the corals.
    - Algae protect absorb light energy, protecting the corals from the harmful effects of sunlight.
    - Nitrogenous waste from corals serve as nutrients for algae.

  - The relationship is described as mutualistic since it is beneficial to both coral and Zooxanthellae.

  - Corals are sensitive to temperature changes and increases in water temperatures can place stress on them.

  - Extended periods of elevated temperatures can disrupt the symbiotic relationship between corals and Zooxanthellae, causing Zooxanthellae to leave the corals. This results in coral bleaching.

    (Without zooxanthellae, the calcium carbonate skeleton of corals appears white because zooxanthellae give corals their color.)

  - With acidification of the ocean due to increased CO_2 emission, calcification rates of corals decrease and the growth of coral reefs are reduced.

  - The loss of coral reefs will reduce habitats for many other marine organisms, disrupting the marine ecosystem.

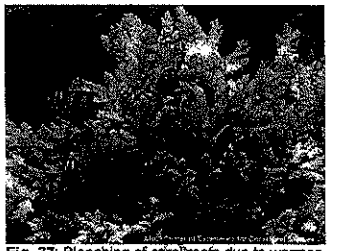


Fig. 37: Bleaching of coral reefs due to warmer temperatures.

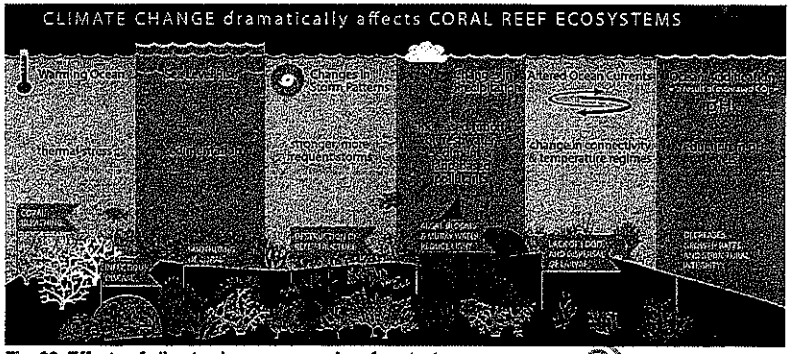


Fig. 38: Effects of climate change on coral reef ecotystems.
1. A warming ocean: causes thermal stress that contributes to coral bleaching. 2. Sea level rise: causes increases in sedimentation that can lead to the smothering of coral. 3. Changes in storm patterns: leads to stronger and more frequent storms that can cause the destruction of coral reefs. 4. Changes in precipitation: increased runoff of freshwater, sediment, and land-based pollutants contribute to algal blooms and cause murky water conditions that reduce light. 5. Altered ocean currents: leads to changes in connectivity and temperature regimes that contribute to lack of food for corals and hampers dispersal of coral larvae. 6. Ocean acidification (a result of increased CO_2): causes a reduction in pH levels which decreases coral growth and structural integrity.

- **Eg. Salmon**
  Warmer water temperatures may affect the lifecycle of salmon and increase the likelihood of disease. Combined with other climate impacts, these effects are projected to lead to large declines in salmon populations.

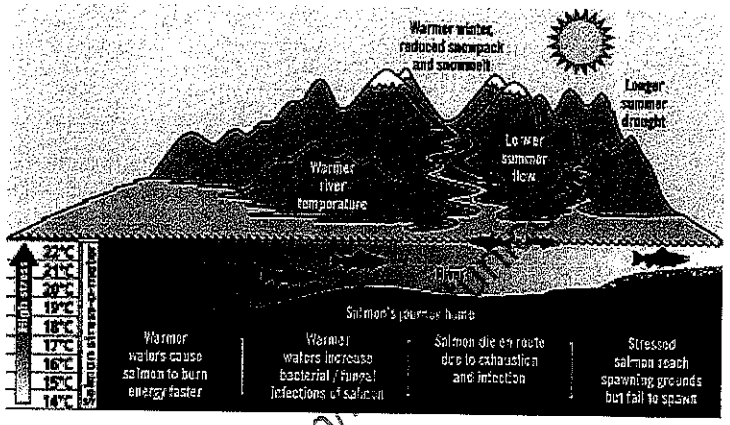


Fig. 39: Higher temperatures are likely to increase water temperatures, which could be harmful to salmon during spawning, incubation and rearing stages of their life cycle.

- Loss of snowpack and shrinking glaciers resulted in reduced stream flows in summer and fall. Survival rates of fish like salmon and trout lowers when water levels in rivers and streams falls, as this allows bears to snap at spawning salmon more easily.
- Lower stream flow in summer makes it more difficult for salmon to return to spawning grounds and for juvenile fish to reach the ocean.
- Warmer stream temperature lowers the cardiovascular capacity of salmon, and the fish may become more vulnerable to disease and predators, causing high numbers of deaths at temperatures at or above 22°C.
- Intense spring floods can also wash away salmon eggs laid in stream beds.
- While other species can migrate to cooler waters, salmons usually return to their native rivers to spawn.

Impacts from loss of biodiversity:

- **e.g. loss of biomedicines:**
  - Over a quarter of all pharmaceutical products come from rainforest produce – they have provided treatments for diseases like leukemia, glaucoma, malaria, Hodgskin's disease, snake bites, along with breast, cervical and testicular cancer, and are presently used in research for a possible treatment for AIDS.

Fig. 40: Cacao tree with cocoa beans. The cocoa tree is a tropical tree native to South America. Cocoa butter obtained can be used in medicine for creams and suppositories. Chocolate is also made from cocoa.

Fig. 41: Curare vines. Curare is used in medicine as a muscle relaxant and in Parkinson's disease.

Fig. 42: *Calophyllum lanigerum var austrocoriaceum*. Calanolide A, a complex natural product, is obtained from the bark and latex of *Calophyllum lanigerum var austrocoriaceum*. It is now undergoing clinical trials for the treatment of HIV infection. When extracts of this plant was discovered to show good anti-viral activity towards HIV, researchers returned to the original site where the tree was found in the island of Borneo in the Malaysian State of Sarawak. However, the tree was gone, cut down for firewood or building purposes. Fortunately, an intense search led to additional samples located in the Singapore Botanic Garden - the British had planted several collected specimens over a century ago. Medical research narrowly escaped a major scientific loss.

- **e.g. loss of genetic diversity of foods:**
  - Approximately 80% of the foods we eat come from the tropical rainforest in some way.

    These include avocados, coconuts, squash, yams, bananas, plantains, mangoes, pineapple, citrus fruits, chocolate, vanilla, ginger, peppercorns and cinnamon. Some other foods grown in the rainforest also include nuts, coffee, tea, rice and corn.

  - Genetic diversity in crop production is vital to the sustainability of a food source. Without consistent mutation, and genetic variation within the population, a species cannot continue to thrive in the face of disease or pestilence.

    (The impact of biodiversity loss on food production is visible in that from 1981 to 2002, nearly 44 million combined tons of wheat, barley and corn had been reduced from the world's food supplies due to climate change.)

D. Impact of Climate Change on Global Food Supply

Climate change affects agriculture in a number of ways - through changes in average temperatures, rainfall, and climate extremes (e.g. heat waves), changes in pests and diseases, atmospheric carbon dioxide, nutritional quality of some foods and changes in sea level.

Impact of Climate Change on Crops

- The **effects of climate change on crops are unevenly distributed across the world.** In mid- to high-latitude regions, moderate warming benefits crop and pasture yields, but even slight warming decreases yields in seasonally dry and low-latitude regions.

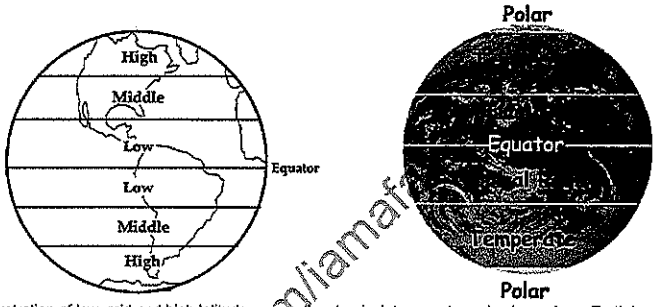


Fig. 43: Illustration of low, mid and high latitudes, as well as tropical, temperate and polar regions. Earth has three main climate zones – tropical, temperate and polar. The tropics are regions found near the equator and the climate is hot and wet all year. The temperate regions have warm summers and cool winters. The polar regions have cold and dry climates, with long and dark winters.

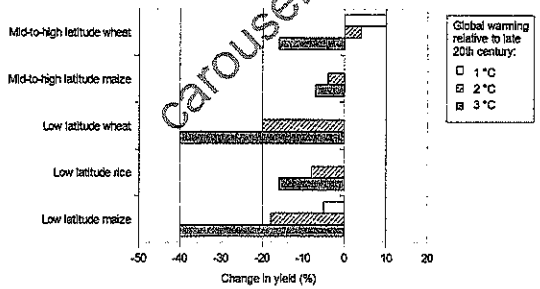


Fig. 44: Projected changes in crop yield at different latitudes with global warming.

Rain-fed crops in **low latitude countries** (e.g. Africa and Latin America) are near their maximum temperature tolerance. As such, their **yields for major cereals are likely to fall sharply** for even small temperature increases.

However, effects on crops in northern/ southern latitudes may be positive or negative. Marginal increases in temperature in **temperate regions or regions of higher altitude may benefit** leafy vegetable production as the temperature moves towards the optimum for seed germination.

- **Global warming can reduce crop yield.**
  - Water is essential for plant growth. Too much or too little water at any particular growth stage reduces yield potential.
  - With droughts occurring more frequently because of global warming and 80% of world agriculture being rain-fed, changes in the amount and pattern of precipitation and evaporation will significantly slow down global crop development and reduce yield.
  - ***e.g. rice & wheat***

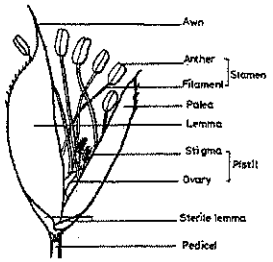


Fig. 45: A rice *spikelet* is the floral unit of rice plants.

- Rice spikelets that are exposed to temperatures of more than 35°C for about 5 days during the flowering period are **sterile and set no seed.** This reduces grain yield.
- Sterility is caused by **poor anther dehiscence** and **low pollen production**, and hence low numbers of germinating pollen grains on the stigma (Matsui *et al.*, 2000, 2001; Prasad *et al.*, 2006).

  - For some temperate cereal crops (e.g. winter wheat), a period of low winter temperature to initiate or accelerate the flowering process is required.
  - With global warming, **low flower bud initiation** results and this ultimately reduces the yield of the crops.

- **Global warming can alter the patterns of weeds, plant pests and pathogens.**

  Because of higher temperatures and humidity, there could be an increased pressure from insects and disease vectors.

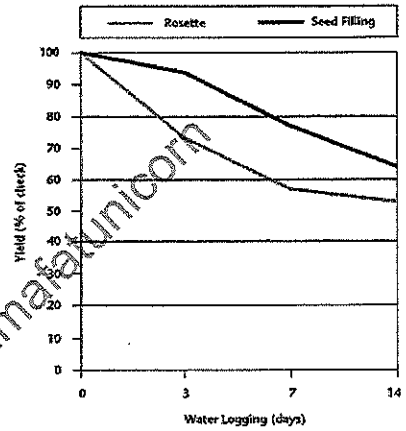
  - Insect pests and weeds may survive or even reproduce more often each year if cold winters no longer keep them in check.
  - New pests may also invade each region as temperature and humidity conditions change. Lower-latitude pests may move to higher latitudes. This would cause new problems for crops previously unexposed to these species.

- **Global warming can lead to loss of arable land.**
  - The warmer atmospheric temperatures are expected to lead to a more vigorous hydrological cycle, including more extreme rainfall events.
  - High precipitation and flooding can result in submergence and soil erosion that destroys vegetable plots, resulting in yield reduction.
  - Eg. High temperatures and water logging can lower yield of canola

Fig. 46: Effect of water logging on yield of canola plants.

The amount of yield loss will depend on the growth stage at the time of water logging, the duration of water logging and the temperature.

Water logging for seven days at the rosette growth stage can reduce plant height, while number of branches and seeds per pod decrease with three days or more of flooding. Water logging for three days or more during flowering reduces the number of pods per branch as well as seeds per pod. Higher temperature during water logging reduces plant growth and dry matter production. Water logging for seven days at seed filling decreases individual seed weight and oil content. High temperatures combined with water logging increases the detrimental effects on canola yield.



Arable land: land capable of being ploughed and used to grow crops.

Hydrological cycle: continuous process by which *water* is circulated throughout the Earth and its atmosphere; water passes from vapor in the atmosphere through precipitation upon land or water surfaces and ultimately back into the atmosphere as a result of evaporation and transpiration.

Impact of Climate Change on Fishery

- Global fisheries catches were increasingly dominated by warm-water species as a result of fish migrating towards the poles in response to rising ocean temperatures. In the tropics, climate change meant fewer marine species and reduced catches, with serious implications for *food security*.
- World's oceans are gradually becoming more acidic due to increases in atmospheric carbon dioxide (CO_2) levels.
- Extreme ocean temperatures and ocean acidification place coral reefs—the foundations of many of the world's fisheries—at risk. Acidity could harm shellfish by weakening their shells.

Impact of Climate Change on Livestock

- **Higher temperatures can cause heat stress in animals.**

  For instance, when cattle are under heat stress, farmers can expect their livestock to have:
  - reduced grazing time (because animals might be seeking shade)
  - reduced feed intake
  - increase in body temperature
  - increased sweating and panting
  - weight loss

  - Over time, heat stress can increase vulnerability to disease, reduce fertility, and reduce milk production.
  - Higher summer temperatures can lead to increase in livestock mortality, especially during transportation.

Fig. 47: Holstein-Friesian cow and calf. A change in average temperature over the hot dry period and a change in the number of extreme days will both likely lead to changes in dairy production. In dairy cows, heat stress reduces the amount of milk produced, reduces milk fat and protein content, and decreases reproduction rates.

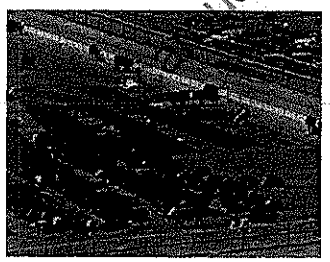


Fig. 48: Shelter from the sun provided for cattle at a feedlot. Beef cattle in feedlots subject to heat stress can experience reduced health and a reduction in growth, influencing the amount of beef product sent to market.

- **Higher temperatures can affect the food availability of livestock.**
  Higher temperatures can cause drought, resulting in the loss of pasture grazing land for livestock. For animals that rely on grain, decline in crop production yields due to drought could also become a problem.

6) MOSQUITO VECTOR & DENGUE

- The main vector of dengue virus is the mosquito *Ae. aegypti*, as dengue virus is transmitted between humans through the bite of infected female *Ae. aegypti* mosquitoes.
- Dengue cannot be spread directly from one person to another directly. Mosquitoes are necessary for the transmission of the dengue virus. The dengue virus is thus spread through a human-to-mosquito-to-human cycle of transmission.
- Infected mosquitoes can continue transmitting the dengue virus to healthy people for the rest of their life spans, generally a two to three week period under natural conditions.

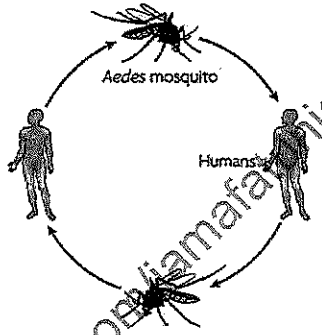


Fig. 49: Dengue transmission.

- Dengue poses the greatest risk in highly populated regions with rainy seasons where there are large populations of *Ae. aegypti* with a high degree of contact between the mosquitoes and humans.

A. Life Cycle of *Aedes aegypti*

Ae. aegypti is a holometabolous insect, meaning that it goes through a complete metamorphosis with an **egg**, **larva**, **pupa**, and **adult stage**. The adult life span can range from two weeks to a month depending on environmental conditions.

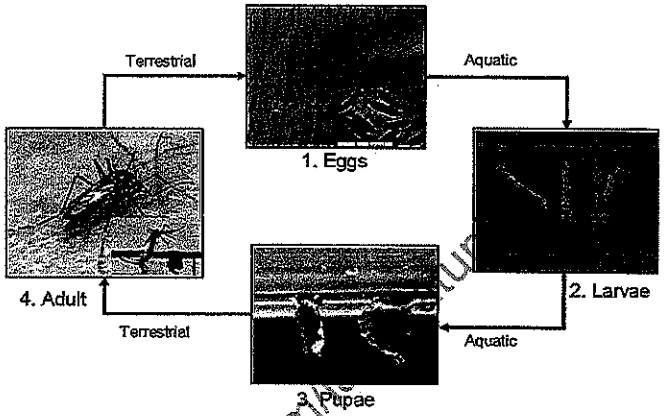


Fig. 50: Life cycle of *Ae. aegypti*.

Female mosquitoes lay their eggs on the inner, wet walls of containers with water. Larvae hatch from eggs (picture 1, inset) when water inundates the eggs as a result of rains or the addition of water by people.

In the following days, the larvae (picture 2) will feed on microorganisms and particulate organic matter, shedding their skins three times to be able to grow from first to fourth instars (not shown in diagram; an instar refers to the developmental stage of an arthropod, e.g. insects, between moults until sexual maturity is reached).

When the larva has acquired enough energy and size and is in the fourth instar, metamorphosis is triggered, changing the larva into a pupa (picture 3).

Pupae do not feed; they just change in form until the body of the adult, flying mosquito is formed. Then, the newly formed adult emerges from the water after breaking the pupal skin (picture 4, inset).

The entire life cycle lasts 8-10 days at room temperature, depending on the level of feeding. Thus, there is an aquatic phase (larvae, pupae) and a terrestrial phase (eggs, adults) in the *Ae. aegypti* life cycle.

Egg stage

- After taking a blood meal, the female *Ae. aegypti* mosquito produce on average 100 to 200 eggs per batch. The female can produce up to five batches on eggs during a lifetime. The number of eggs is dependent on the size of the blood meal.
- **Eggs are laid on damp surfaces** in areas likely to temporarily flood (e.g. tree holes and man-made containers like barrels, drums, jars, pots, buckets, vases, tins, tyres, discarded bottles etc). Eggs will most often be placed at varying distances above the water line.
- Laid eggs can **survive for very long periods in a dry / dessicated state**, often for more than a year. However, they **hatch within one to two days in water**.
  - ➔ This means that even if all larvae, pupae, and adults were eliminated at some point in time, re-population will occur as soon as the eggs in the containers are flooded with water. This makes the control of the *Ae. aegypti* mosquito a very difficult task.

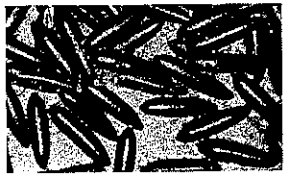


Fig. 51: *Ae. aegypti* eggs. ~1mm long. Smooth, long and ovoid shaped.

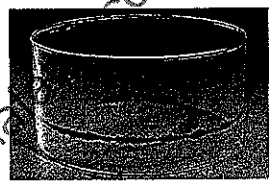


Fig. 52: *Ae. aegypti* eggs in water.

Larval stage

- After the eggs hatch, the larvae feed on organic particulate matter in the water, such as algae and other microscopic organisms.
- Most of the larval stage is spent at the water surface, although they will swim to the bottom of the container if disturbed or when feeding. *Ae. aegypti* larvae **breathe oxygen through** a posteriorly located **siphon**, which is **held above the water surface** while the rest of the body hangs vertically.
- **Larval development is temperature dependent.**
  - The larvae passes through **four instars / larval stages**, spending a short amount of time in the first three, and up to three days in the fourth instar (**Fig.**). Fourth instar larvae are approximately 8mm long.
  - If temperatures are cool, *Ae. aegypti* can remain in the larval stage for months so long as the water supply is sufficient. The larva generally takes about **four days** to develop into a pupa.

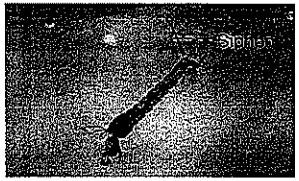


Fig. 53: Larvae of *Ae. aegypti*.

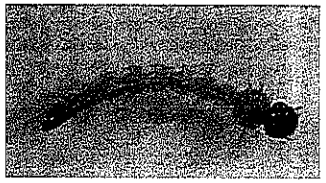


Fig. 54: Fourth instar larvae of *Ae. aegypti*.

Pupal stage

- After the fourth instar, the larvae enters the pupal stage. Like the larval stage, pupal stage is also **aquatic.**
- **Pupae do not feed** and take approximately **two days** to develop into a fully developed adult mosquito.

Adult stage

- Adults emerge from pupal stage after two days by **ingesting air** to **expand the abdomen**, thus **splitting open the pupal case** and **emerging head first.**
- **Three days after** the female mosquito has taken a **blood meal**, it will **lay eggs** and the **cycle begins again.**

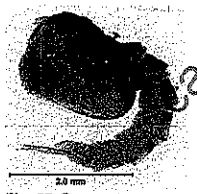


Fig. 55: Pupae stage.

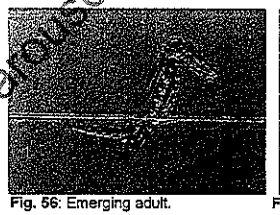


Fig. 56: Emerging adult.

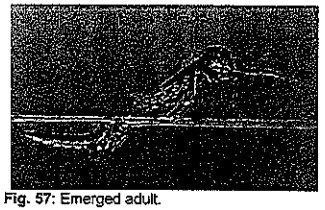


Fig. 57: Emerged adult.

B. Development of Dengue in Humans

- When an infected mosquito feeds on a person, it injects the dengue virus into the bloodstream. The virus infects nearby skin cells called keratinocytes, the most common cell type in the skin. The dengue virus also infects and replicates inside a type of dendritic cell in the skin, called the Langerhans cell.
- Infected Langerhan cells secrete cytokines to help limit the spread of the infection.
- Infected Langerhan cells also travel from the infection site in the skin to the lymph nodes and display dengue viral antigens on their surface. The displayed antigens activate the innate immune response by activating monocytes and macrophages to fight the virus.
- Normally, monocytes and macrophages ingest and destroy pathogens, but instead of destroying the dengue virus, they are now targeted and infected by the virus.
- As the infected monocytes and macrophages travel through the lymphatic system, the dengue virus spreads throughout the body and infects more cells. These include cells in the lymph nodes and bone marrow, macrophages in both the spleen and liver, and monocytes in the blood.
- The spread and increase of the virus results in viremia, a condition in which there is a high level of dengue virus in the bloodstream.

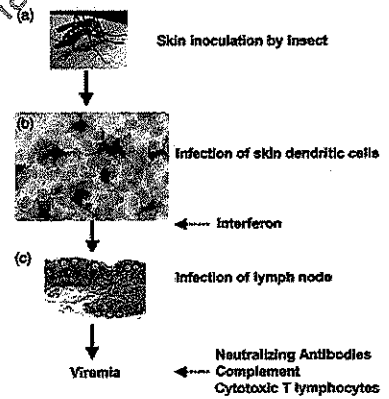


Fig. 58: Dengue Viral Infection
(a) A person is infected with the dengue virus when an infected mosquito bites the person's skin.
(b) The dengue virus infects the Langerhans cells, a type of dendritic cell in the skin.
(c) The infected Langerhans cells produce interferons to help limit the continuous spread of the infection. Other infected Langerhans cells travel to the lymph nodes carrying viruses, which infect more cells. The spread of the dengue virus results in viremia, which is a high level of the virus in the bloodstream. To fight the infection, the immune system produces antibodies to neutralize the dengue viral particles, and the complement system is activated to help the antibodies and white blood cells remove the virus. The immune response also includes cytotoxic T cells (lymphocytes), which recognize and kill infected cells.

Langerhans cell: a type of dendritic cell. Langerhans cells detect invading pathogens and display antigens from the pathogens on their surface. These cells then migrate to the lymph nodes and alert the immune system to trigger the immune response because a pathogen is in the body.

How does the immune system defeat the dengue virus?

- Despite infecting immune cells and spreading throughout the body, the immune system has additional defenses to fight the virus.
- The infected cells produce and secrete small proteins called interferons that are part of a large group of proteins called cytokines. Interferons activate both the innate and adaptive immune system defenses. They help the immune system recognize dengue-infected cells and help protect uninfected cells from infection.
- Under the adaptive immune response:
  - B cells produce antibodies called IgM and IgG that are released in the blood and lymph fluid, where they specifically recognize and neutralise the dengue viral particles.
  - Cytotoxic T cells recognize and kill the cells that are infected with the dengue virus.

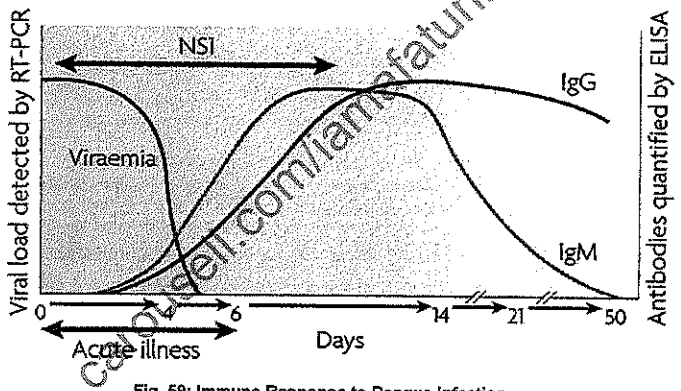


Fig. 59: Immune Response to Dengue Infection
An infected person experiences the acute symptoms of dengue when there is a high level of the virus in the bloodstream. As the immune response fights the dengue infection, the person's B cells begin producing IgM and IgG antibodies that are released in the blood and lymph fluid, where they recognize and neutralize the dengue virus and viral molecules. The immune response eliminates the virus, leading to recovery.

** The simplest and most direct way in which antibodies can protect from pathogens or their toxic products is by binding to them and thereby blocking their access to cells that they might infect or destroy. This is known as neutralisation and is important for protection against bacterial toxins and against pathogens such as viruses, which can thus be prevented from entering cells and replicating.*

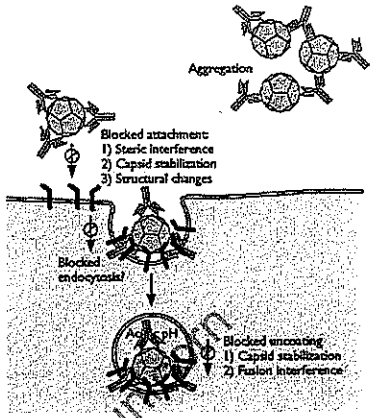


Fig. 60. Antibodies can block virus infection in a number of ways. They may interfere with virion binding to receptors, block uptake into cells, prevent uncoating of the genomes in endosomes, or cause aggregation of virus particles.

Secondary Dengue Infections

The dengue serotypes:

- Dengue infections are caused by four closely related viruses named DEN-1, DEN-2, DEN-3, and DEN-4. These four viruses are called serotypes because each has different interactions with the antibodies in human blood serum.
- The four dengue viruses are similar — they share approximately 65% of their genomes — but even within a single serotype, there is some genetic variation. Despite these variations, infection with each of the dengue serotypes results in the same disease and range of clinical symptoms.

Severe Dengue:

- After recovering from a first dengue infection, a person is still susceptible to future dengue infections. This is because the memory cells only provide immunity from re-infection with the dengue serotype that caused the first infection.
- Infection with the dengue virus can also cause a disease known as severe dengue, such as Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome, which is more serious than dengue fever.

- Severe dengue has been observed to occur frequently with secondary dengue infection. Subsequent infections with a different serotype can put individuals at a greater risk for severe dengue illnesses than those who have not been previously infected.

Fig. 61: Mild and Severe Symptoms of Dengue. DF can sometimes lead to severe dengue. DHF is characterised by increased vascular permeability, decrease in blood volume in the body and abnormal blood clotting mechanisms. In moderate DHF cases, all symptoms abate after fever subsides. However, in severe cases, the patient's condition deteriorates after a few days of fever; blood pressure can drop to dangerous levels, causing shock - DSS. DSS is also characterised by bleeding that may appear as tiny spots of blood on the skin and larger patches of blood under the skin. DSS may cause death in 12 to 24 hours.

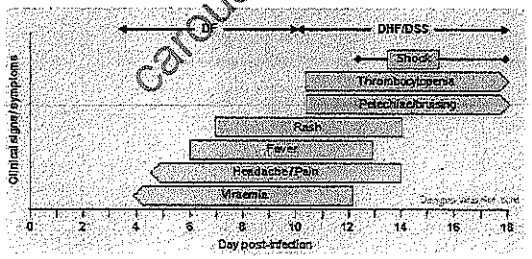


Fig. 62: Generalised time course of events associated with dengue fever, DHF and DSS. The incubation period before the development of signs of infections generally ranges from 4 to 7 days.

- Severe symptoms of dengue are due to a phenomenon called "antibody-dependent enhancement of infection".

  When a person is infected with a second dengue serotype, antibodies from the first infection actually help the virus infect host cells more efficiently, thus increasing viremia.

ANTIBODY-DEPENDENT ENHANCED (ADE) IMMUNITY

Dengue infects by attaching to a cell that then engulfs the virus in a vacuole via endocytosis. The virus rearranges its coat proteins to bind to the vacuole membrane, releasing its capsid and genome into the cytoplasm, where it is replicated and packaged into newly made virions ❶. The infected cell also triggers an immune reaction ❷ that includes the recruitment of T cells that release pro-inflammatory cytokines and B cells that generate antibodies. These antibodies are specific for the infecting virus, and bind and cover dengue's coat ❸. When this happens, macrophages and monocytes clear the virus from the bloodstream by binding to and engulfing the antibody-coated viruses—which can no longer escape the endocytic vacuole—and destroying them ❹.

When a person is infected with a different dengue virus type ❺, memory B cells created during the first infection spring into action ❻, flooding the bloodstream with antibodies specific to the first type. These antibodies, however, may not bind to the new type as well ❼. As a result, when the partially-coated virions are taken up by macrophages and monocytes, they can escape the endocytic vacuole, infecting and replicating within these immune cells ❽.

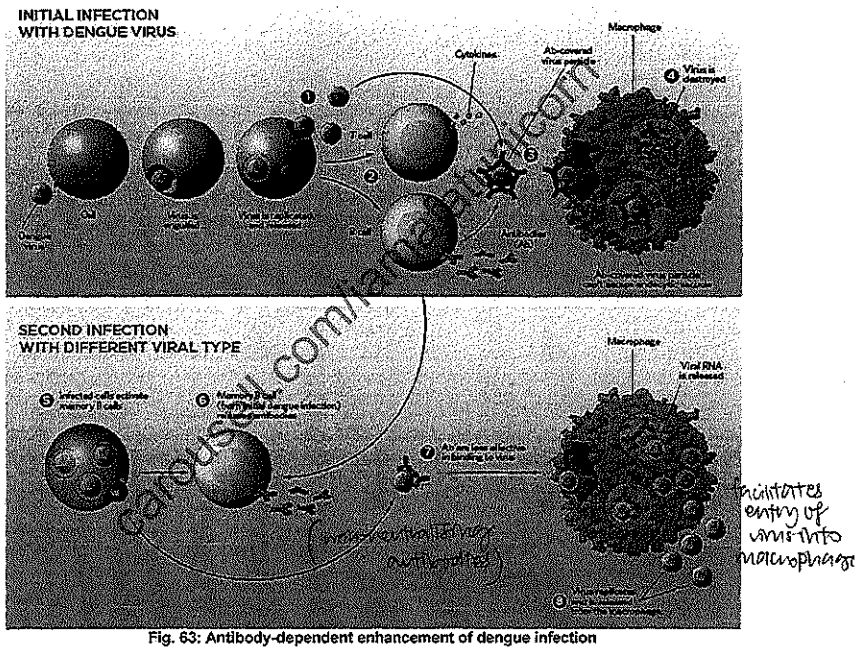


Fig. 63: Antibody-dependent enhancement of dengue infection

- Antibody (Ab)-dependent enhancement of infection occurs when pre-existing antibodies present in the body from a primary (first) dengue virus (DENV) infection bind to an infecting DENV particle during a subsequent infection with a different dengue serotype. The antibodies from the primary infection cannot neutralize the virus. The outcome is an increase in the overall replication of the virus and a higher risk of severe dengue.

- During a second infection with dengue, the cytotoxic T cells produced by the immune system provide only partial immunity against the new dengue serotype.
- The cytotoxic T cells do not effectively clear the virus from the body, and they release excess quantities of molecules called cytokines.

  In normal quantities, cytokines help the immune response; however, in high quantities, cytokines can produce serious inflammation and tissue damage such as leakage from the capillaries, possibly contributing to the development of severe dengue diseases.

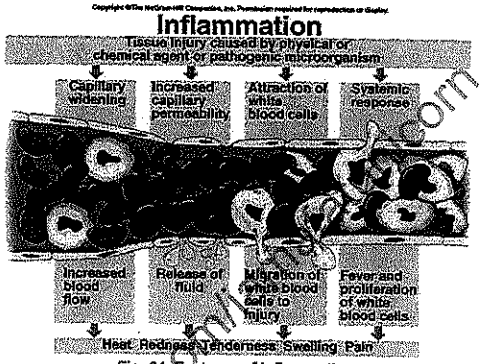


Fig. 64: Features of inflammation.

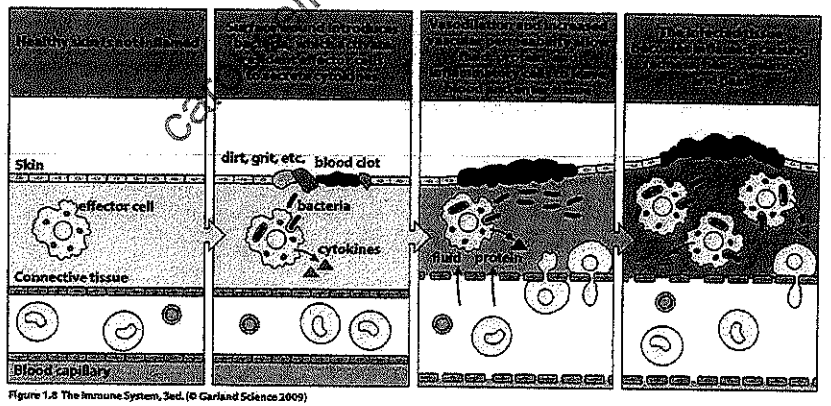


Figure 1.8 The Immune System, 3ed. (© Garland Science 2009)

Fig. 65: Role of inflammation in an immune response.

- Part of the normal inflammatory response is to allow for vasodilation to reduce blood flow and to increase vascular permeability to allow for white blood cells to enter the tissue.
- Serious inflammation is linked to the development of severe dengue.
  - With increased vascular permeability, the leakage of plasma from the circulatory system can cause fluids to collect in body cavities.
  - Another symptom is severe bleeding. In some cases, stomach and intestinal bleeding can cause death. Patients with severe dengue have a tendency to bruise easily
  - The loss of plasma and protein can cause the patient to experience a condition called shock. Patients in shock show signs of circulatory failure. The lack of blood circulation causes the patient to have cold, clammy, bluish skin. Their blood pressure and pulse may also be undetectable.