RIVER VALLEY HIGH SCHOOL

JC 2 PRELIMINARY EXAMINATION

CANDIDATE NAME											
CENTRE NUMBER	S					CLASS	22J	INDEX NUMBER				

BIOLOGY **9744/03**

Paper 3 Long Structured and Free-response Questions **15 September 2023**

2 hours

Candidates answer on the Question Paper.
No Additional Materials are required.

READ THESE INSTRUCTIONS FIRST

Write your Centre number, index number and name in the spaces at the top of this page.
Write in dark blue or black pen.
You may use an HB pencil for any diagrams or graphs.
Do not use staples, paper clips, glue or correction fluid.
DO **NOT** WRITE ON ANY BARCODES.

Section A
Answer **all** questions in the spaces provided on the Question Paper.

Section B
Answer any **one** question in the spaces provided on the Question Paper.

The use of an approved scientific calculator is expected, where appropriate.
You may lose marks if you do not show your working or if you do not use appropriate units.

The number of marks is given in brackets [ ] at the end of each question or part question.

For Examiner's Use	
Section A	
1	
2	
3	
Section B	
Total	

Section A

Answer **all** the questions in this section.

1 The gut microbiota is the community of all micro-organisms (bacteria, archaea, protozoa, fungi, and viruses) present in the digestive tract. Bacteria are the largest component of the microbiota.

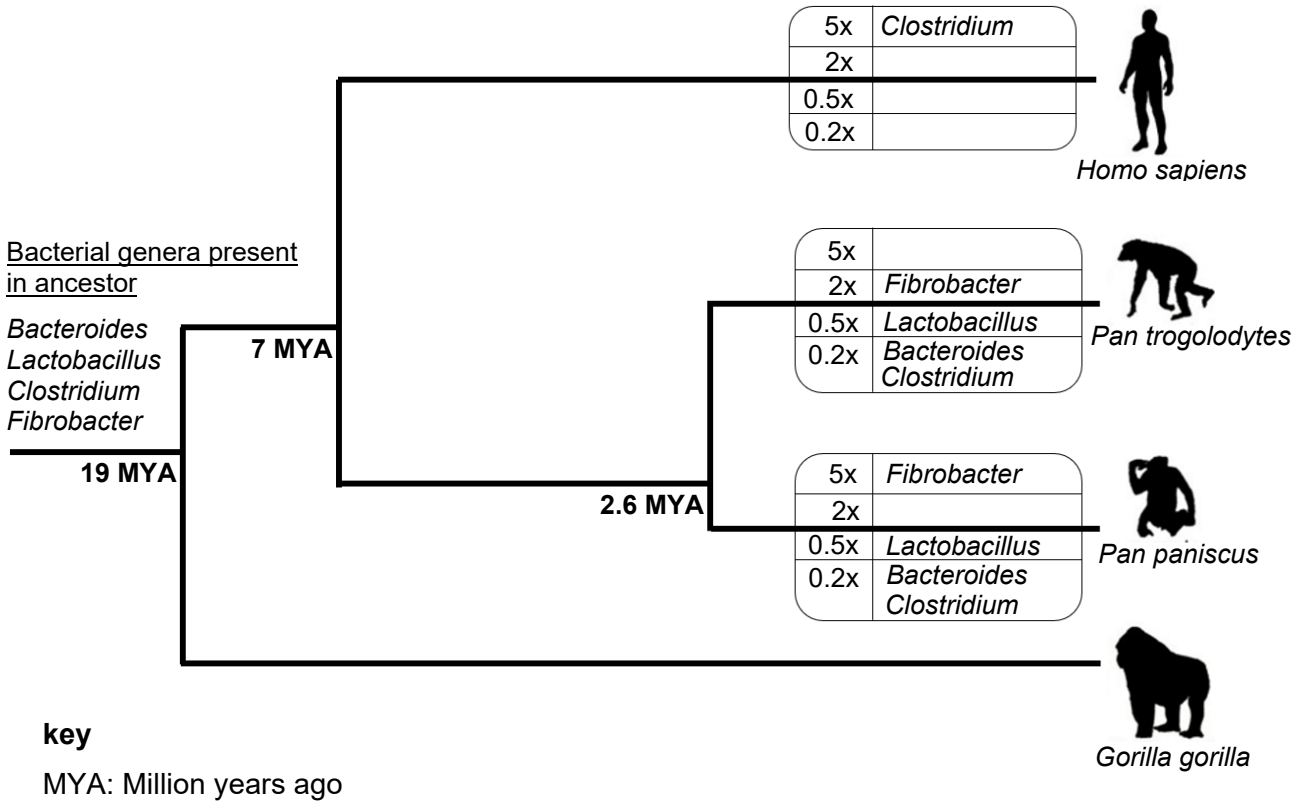
(a) Describe the structure of bacterial genome. [3]

1. Haploid;
2. Double-stranded;
3. Circular DNA;
4. Associated with basic proteins;
5. Presence of plasmid(s);
6. Few million nucleotides in length;

[R: introns]

Gut bacteria are present in a range of species in Kingdom Animalia including insects, fish, and apes. Gut bacteria are passed vertically from mother to offspring. Its composition and abundance have changed in the generations with evolutionary time.

Fig. 1.1 shows the relative change in abundance of selected bacterial taxa in the evolution of humans (*Homo sapiens*). Relative change is shown by the "_x" in the box beside the descendant species, for example "5x" refers to a five times increase in abundance from the ancestor.



key

MYA: Million years ago

Adapted from Moeller et al, PNAS, 111(46), 2014

Fig. 1.1

(b) With reference to the **animals** in Fig. 1.1, explain how phylogeny is related to classification. [2]

1. degree of relatedness in evolutionary relationship of phylogeny;
2. is observed in the hierarchical classification of species;
3. organisms of same genus are more closely related / diverged more recently / have a more recent common ancestor; [A: reverse argument]
4. shown by shorter distance between branches of species *Pan trogolodytes* and *Pan paniscus*;
5. and the longer distance between branches of genus such as *Homo sapiens* and *Gorilla gorilla*;

Max. 2m

Diet has a significant influence on gut bacteria composition. Different diets contribute different nutrients to the digestive tract which affects the presence and proportion of various bacterial genera. Chimpanzees' (genus: *Pan*) diet mainly comprise of plants and plants' produce, with meat contributing only 2% of their diet.

Table 1.1 shows how different nutrients affect selected bacterial genera.

Table 1.1

nutrient		bacterial genus		
		Bacteroides	*Clostridium*	*Fibrobacter*
carbohydrates	digestible (e.g. fruit)	–	–	+
	non-digestible (e.g. plant fibers)		–	++
protein	animal-derived	+	++	–
	plant-derived	–		+
fats	animal-derived	+	+	–
	plant-derived	–	–	

Adapted from Tomova et al, Frontiers in Nutrition, 6(47), 2019

key

\+ nutrient causes an increase in abundance of bacteria
– nutrient causes a decrease in abundance of bacteria
shaded boxes mean that information is unknown

(c) Using your knowledge and information from Fig. 1.1 and Table 1.1,

(i) predict the change in proportion for *Bacteroides* and *Fibrobacter* in the gut of *Homo sapiens* and fill in the boxes in Fig. 1.1 with their genus names, and [2]

Fibrobacter @ 0.5X or 0.2X

Bacteroides @ *2X*

(ii) comment on the validity of the statement "gut bacteria can act as evidence of evolution". [4]

Valid

1. all four bacteria genera (*fibrobacter, lactobacillus, bacteroides and clostridium*) are present in all descendants;;
2. due to descent from common ancestor;
3. female individuals better adapted to environment can survive and reproduce, passing gut bacteria to offspring;

OR

4. different abundance of bacteria genera in descendants;;
5. due to different nutrients in diet;
6. different selection pressure resulting in selection for & against of different phenotypes;

Not valid - Biodiversity of gut bacteria affected by:

7. other than maternal gut microbiota;;
8. other sources include vagina, breast milk, mouth and skin of mothers;;

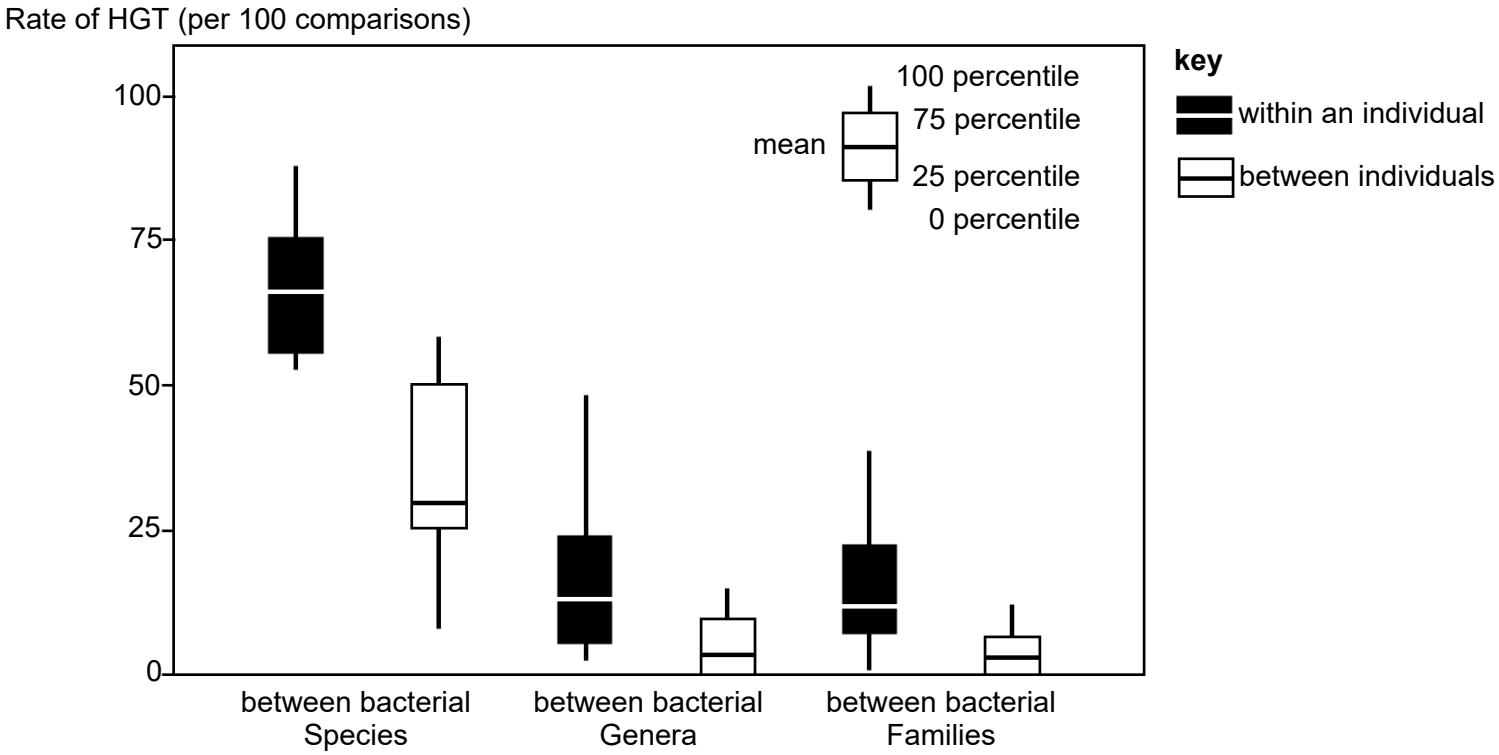
OR

9. external factors of related to individuals;;
10. such as use of antibiotics / lifestyle (including sanitation, caesarean section) / diet;;

Max. 2m

Horizontal gene transfers (HGT) occur frequently in gut bacteria, particularly in response to selection pressures targeting new functions such as antibiotic resistance.

Fig. 1.2 shows the HGT rate of antibiotic resistance genes across taxonomic levels of bacteria, and two sets of data, the HGT rate **within** an individual's gut and the HGT rate **between** two individuals' gut.



Adapted from Kent et al, Nature Communications, 2020

Fig. 1.2

(d) (i) Outline how antibiotic resistance genes are transferred between bacteria. [3]

1. Donor / F+ cell synthesizes a sex pilus;
2. Makes contact with recipient / F- cell;
3. Sex pilus retracts;
4. And forms a temporary mating bridge;
5. One strand with antibiotic resistance gene moves to recipient cell;
6. DNA strand then acts as a template;
7. Plasmid in recipient circularises;

Max. 3m

(ii) Account for **one** similarity and **one** difference in the two data sets in Fig. 1.2. [4]

Similarity

1. Both mean rates of HGT decreases from between species to between genera / between families;;
2. Sex pilus on donor less able to recognise and bind to target receptors on recipient cells;
3. due to decreasing degree of similarity in nucleotides / shared characteristics at higher taxonomic ranks;

Difference

4. Mean HGT rate for within individual is higher than that for between individuals from between species to between families;;
5. Additional step needed for transfer of bacteria;
6. from one individual's gut to the other individual's gut;

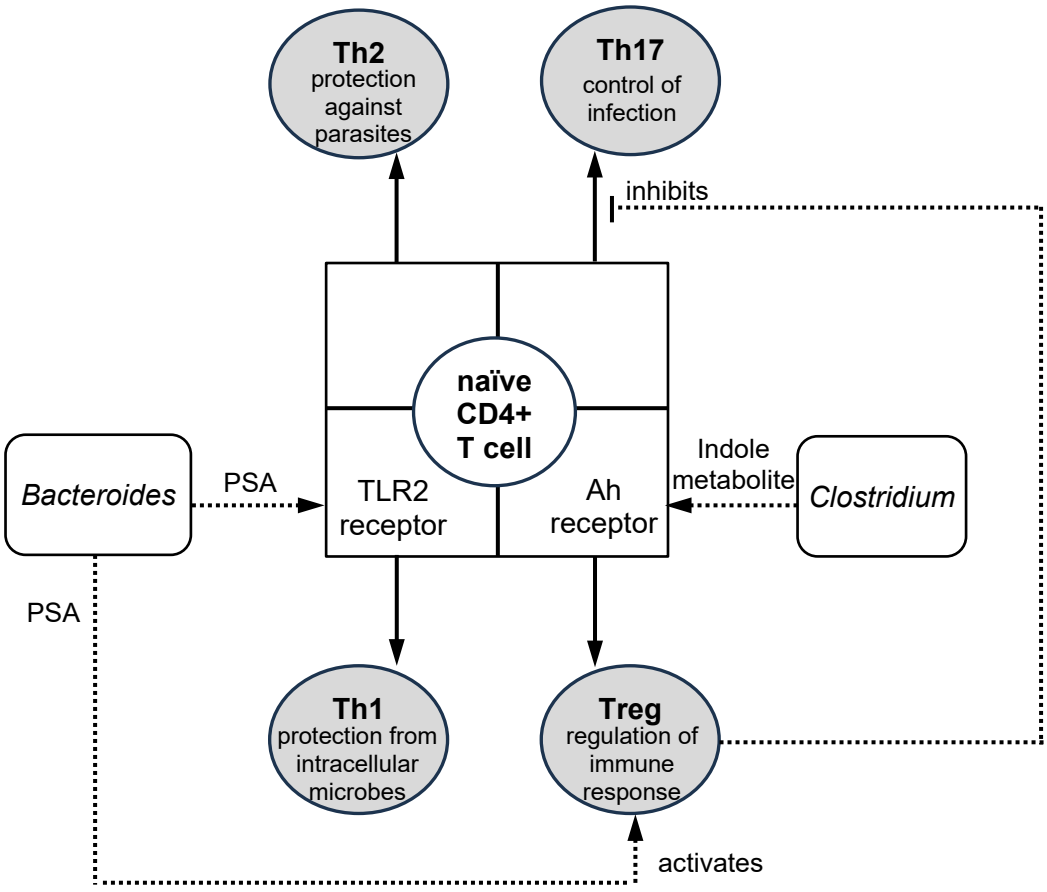
Gut bacteria provide essential health benefits to their hosts. These include nutrient metabolism, drug metabolism and regulating immune homeostasis. Gut bacteria have a role in innate immunity, and more importantly can also induce T lymphocyte differentiation.

Details of how gut bacteria induces T lymphocyte differentiation are provided below:

- Naïve CD4+ T cells can differentiate into four major T cell subtypes: Th1, Th2, Th17 and regulatory T cell (Treg).
- Differentiation into subtypes Th1 and Treg can be triggered by gut bacteria.
- *Bacteroides* and *Clostridium* secrete Polysaccharide A (PSA) and indole metabolite respectively, which bind different receptors on the naïve CD4+ T cell.
- This induces different transcription factors resulting in differentiation into the 4 lineages.

Uncontrolled Th1 and Treg responses result in gastrointestinal-associated autoimmune diseases while uncontrolled Th2 responses result in allergic reactions.

Fig. 1.3 shows the interactions of gut bacteria, *Bacteroides* and *Clostridium*, on naïve CD4+ T cells and their differentiated T cell subtypes.



Adapted from Wu et al, Gut Microbes, 4(14), 2012

Fig. 1.3

(e) Using information provided in this entire question, deduce the outcomes on an individual with a gain-in-function mutation of the promoter of the TLR2 receptor gene, and living in a country of high affluence. [5]

1. GOF mutation results in increased TLR2 receptor expression;
2. Coupled with increased meat consumption due to increased affluence;
3. More *bacteroides* present in gut;
4. Leading to more PSA secreted;
5. More naïve T cells differentiate to form Th1 cells;
6. [Impact 1] Resulting in more intracellular microbe protection;
7. But increased activation of Treg;
8. Also more *clostridium* present in gut;
9. Leading to more indole metabolites;
10. More naïve T cells differentiate to form Treg cells;
11. Resulting in greater inhibition of Th17 differentiation;
12. [Impact 2] Results in increased gastrointestinal auto-immune disease;

Max. 5m

(f) State the advantage of cell signalling shown in Fig. 1.3. [1]

Simultaneous activation different cell types, naïve T cell and Treg cell, by a single ligand, PSA;;

(g) Scientists identified Mg^{2+} ion as a possible second messenger involved in T lymphocyte activation.

Describe the nature of second messengers such as Mg^{2+}. [1]

1. Small, non-protein;
2. Water-soluble ions;

[Total: 25]

2	Apoptosis is an important part of tissue homeostasis. It ensures that cells die quickly and are safely removed when necessary.		
	(a)	Protein kinases play an important role in the cell signalling pathway leading to apoptosis. Explain the role of protein kinases.	[2]
		1. Transfers phosphate group (from ATP) to substrate (other protein kinases); 2. inducing conformational change; 3. of substrate into its active form; 4. initiating phosphorylation cascade;	
	(b)	The BCL-2 protein is involved in the regulation of apoptosis. It is a monomeric protein consisting of a single polypeptide chain. Outline how BCL-2 protein is synthesised from its mRNA.	[5]
		1. BCL-2 mRNA serves as the template; 2. mRNA molecule binds to the small ribosomal subunit (at the 5' end); 3. initiator tRNA binds to the start codon on the mRNA; 4. large ribosomal subunit binds to the small ribosomal subunit (forming a translational complex); 5. anticodon of an incoming tRNA molecule pairs with the mRNA codon at aminoacyl-tRNA site of ribosome (via complementary base pairing); 6. ribozyme/peptidyl transferase catalyse the peptide bond formation between amino acids; 7. ribosome translocates; 8. elongating polypeptide until ribosome reaches stop codon; 9. release factor binds directly to stop codon at A site; 10. polypeptide hydrolysed from the tRNA; 11. polypeptide folds into its three-dimensional shape; max 5	

The *BCL-2* gene is a proto-oncogene found on chromosome 18. In B-cell lymphoma, a cancer involving B-cells, a mutation shifts the *BCL-2* gene to a different chromosome where the gene coding for antibodies (*IgH*) is found.

Fig. 2.1 shows the mutation and how it results in the disruption of the apoptotic pathway.

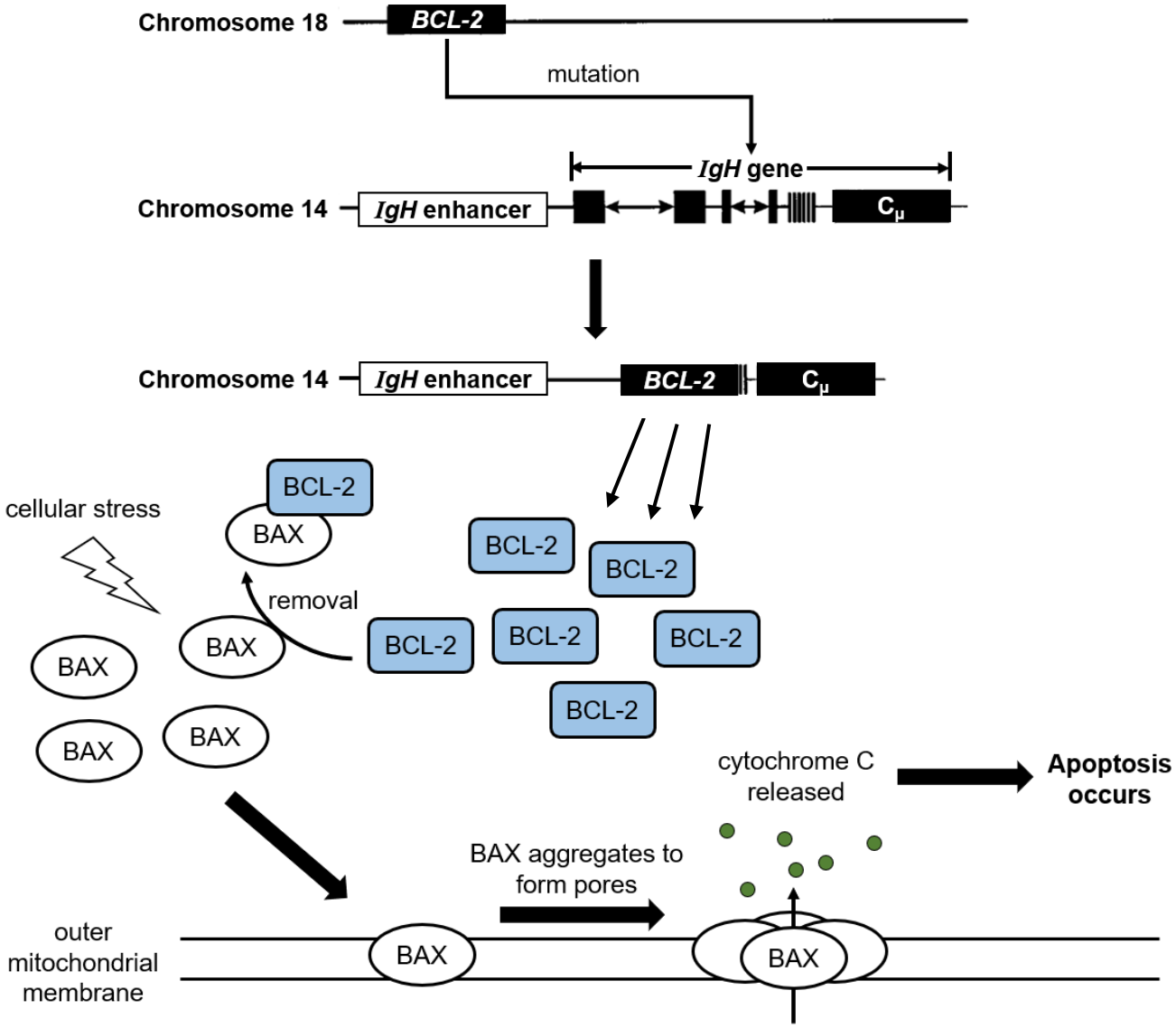


Fig. 2.1

(c) With reference to Fig. 2.1, explain how the mutation of *BCL-2* proto-oncogene results in B-cell lymphoma. [4]

1. Chromosomal translocation (of *BCL-2* gene);
2. from chromosome 18 to chromosome 14;
3. BCL-2 gene brought downstream of enhancer of *IgH* gene;
4. increased BCL-2 gene expression / transcription and translation;
5. excessive amounts of BCL-2 protein produced;
6. BCL-2 protein binds to BAX proteins;
7. prevents BAX from inserting into mitochondrial membrane to form pores;

8. apoptosis does not occur despite unfavourable conditions e.g. DNA damage;
9. leading to uncontrolled cell division;

max 5

In chemotherapy, a prescribed drug is used to kill dividing cancer cells.

Fig. 2.2 shows the number of healthy cells and cancer cells per unit volume of blood in a patient receiving chemotherapy. The arrows labelled **F** to **I** show when the prescribed drug was given to the patient.

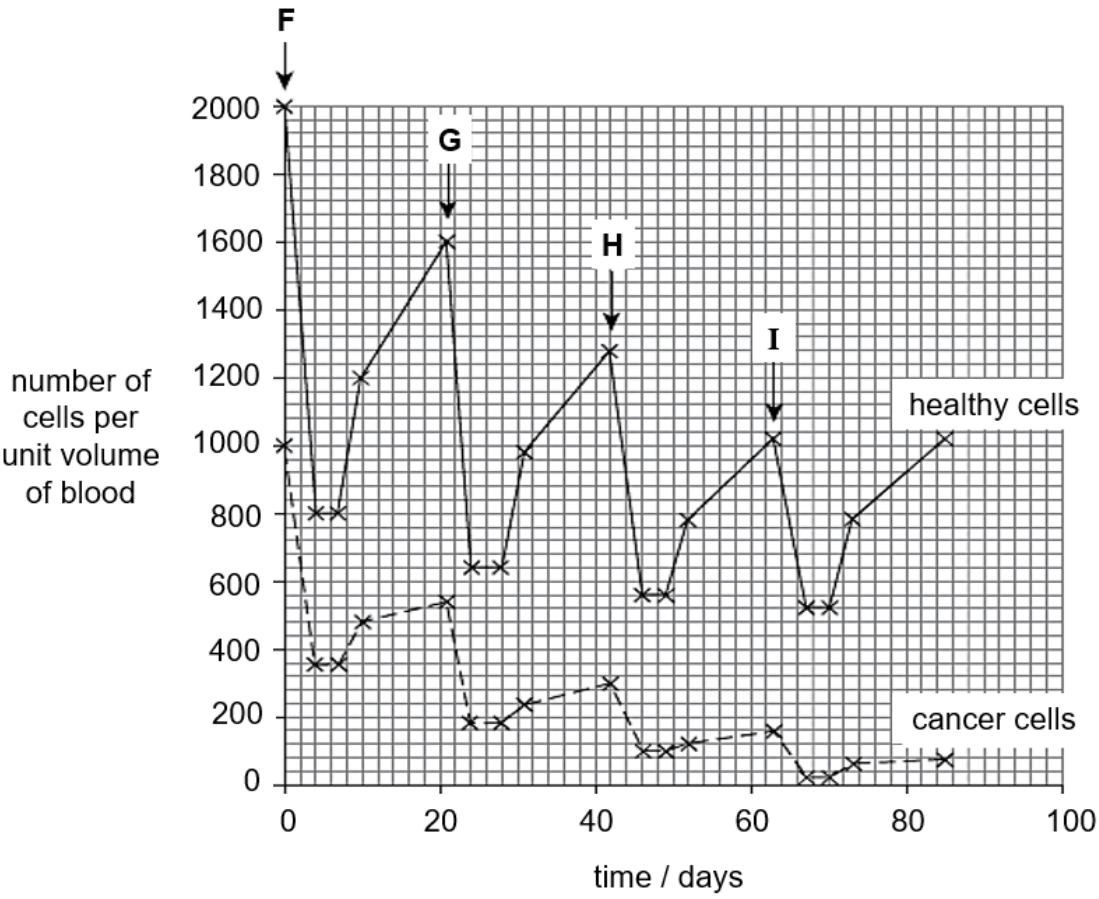


Fig. 2.2

(d) With reference to Fig. 2.2, describe the limitations of using chemotherapy in treating cancer patients. [4]

1. Healthy cells are also removed in chemotherapy / collateral damage to healthy cells;;
2. quote at least 1 data e.g. from day 0 to day 4, steep decrease in number of healthy cells per unit volume of blood by 1200 from 2000 to 800 when drug was administered;;
3. Multiple treatments are required in chemotherapy;;
4. number of cancer cells decreased to a low level e.g. 20 cells per unit volume of blood only after 4 treatments;;

			5. Cancer cells continue to divide / increase in number after each treatment;; 6. quote at least 1 data e.g. from day 4 to day 21, number of cancer cells per unit volume of blood increased from 360 to 540;; 7. Presence of cancer cells after multiple treatments / cancer cells not completely removed after multiple treatments;; 8. after 4 treatments, 80 cancer cells per unit volume of blood still remained;; Any 2 pairs	
				[Total: 15]

3 Maize (*Zea mays*) is a staple food that originated in the Americas. Due to its importance in global food security, much research has been undertaken in particular to understand how maize may be affected by changes in the climate.

In one experiment carried out in 1998 and 2022, scientists investigated the relative effects of genotype and environment on the yield of maize.

Maize seeds with different 'inbreeding coefficients' were used. The greater the inbreeding coefficient, the greater the degree of homozygosity in the maize plants.

Maize seeds with different inbreeding coefficients were planted in two different areas in 1998, and in the same two areas in 2022.

Fig. 3.1 shows the results.

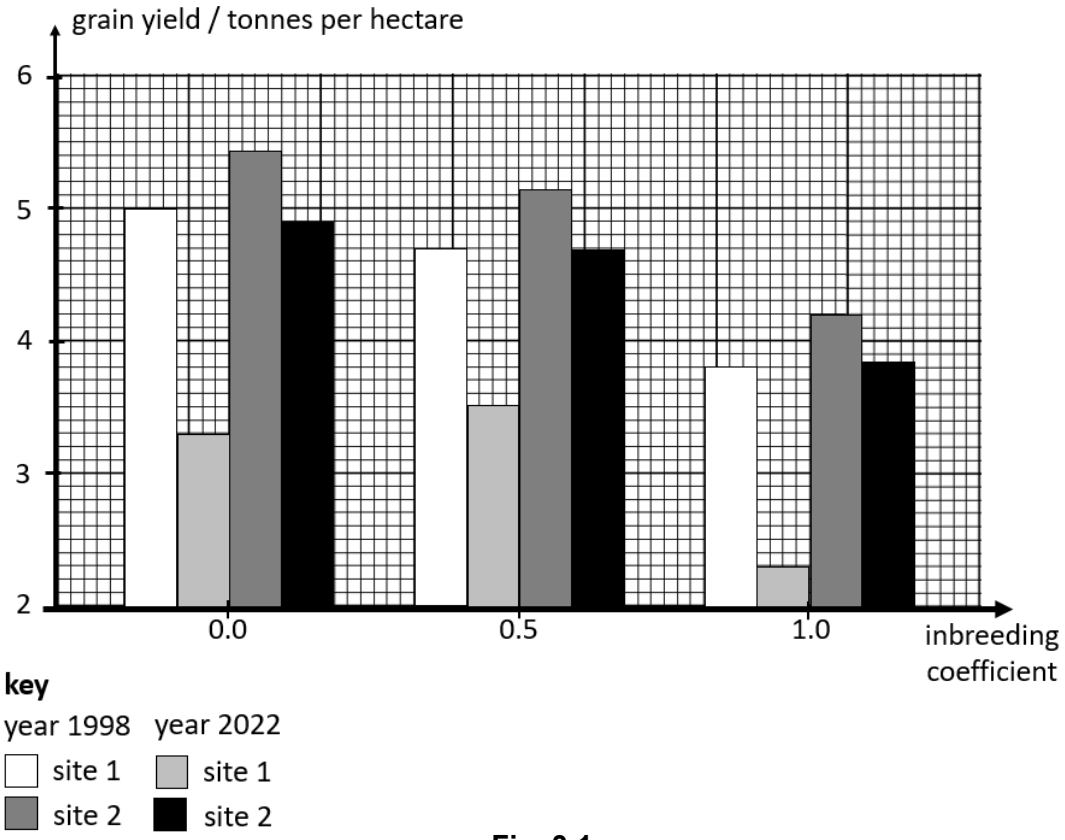


Fig. 3.1

Table. 3.1 shows the average annual environmental conditions at the two areas, where all other variables are kept constant.

Table. 3.1

site	year 1998			year 2022		
	temperature / °C	precipitation / mm	acidity / pH	temperature / °C	precipitation / mm	acidity / pH
site 1	28.3	870	6.8	28.5	420	7.1
site 2	30.7	650	7.2	27.4	880	6.9

The optimal conditions for maize are for annual temperature (28 °C – 32 °C), for annual precipitation (600 mm – 1000 mm) and for soil acidity (pH6 – pH7).

	(a)	Inbreeding depression is a reduction in vigour that results from inbreeding. Using the information provided, evaluate if inbreeding depression in maize is affected by the environment.	[4]
		Yes. Environment affects inbreeding depression. As inbreeding coefficient increases from 0.0 to 1.0, 1. In site 1, as average annual precipitation decreased from 870 mm (in 1998) to 420 mm (in 2022), grain yield increased from 3.3 to 3.5 tonnes per hectare; then decreased to 2.3 tonnes per hectare (in 2022);; 2. but grain yield decreased from 5 tonnes per hectare to 3.8 tonnes per hectare (in 1998); No. Environment does not affect inbreeding depression. As inbreeding coefficient increases from 0.0 to 1.0, 3. In site 2 in 2022 and site 1 in 1998, lower temperature 27.4 °C compared to 28.3 °C; 4. but grain yield per tonne decreases by similar extent;; 5. Showing that lower temperature has no impact on inbreeding depression; OR 6. In site 2, in 1998 and 2022, higher temperature but lower precipitation of 30.7 °C and 650 mm compared to 27.4 °C and 880 mm; 7. but grain yield per tonne decreases by similar extent;; 8. Showing that change in temperature and precipitation have no impact on inbreeding depression; 9. AVP;;	
	The fluctuation of external environmental conditions often has limited effect on internal conditions of living organisms. This is particularly important in cells to maintain optimum conditions for metabolic processes to proceed.		
	(b)	The optimum pH for activity of rubisco is pH8. Explain how the illumination of chloroplasts leads to optimum pH conditions for rubisco.	[3]
		1. energy from photons transferred via resonance; 2. to excite electrons (in pair of special chlorophyll a) to higher energy state; 3. high energy electrons passed along ETC; 4. energy released is used to pump H^+ from stroma to thylakoid lumen; 5. lower H^+ concentration in stroma results in increased pH; 6. Rubisco is present in stroma;	

Due to the more limited migration range as compared to animals, plants often have to adapt to changes in their habitat's environment.

Fig. 3.2 shows the relationship between stress due to high light intensity and temperature, and some of the short-term and long-term adaptations plants may employ.

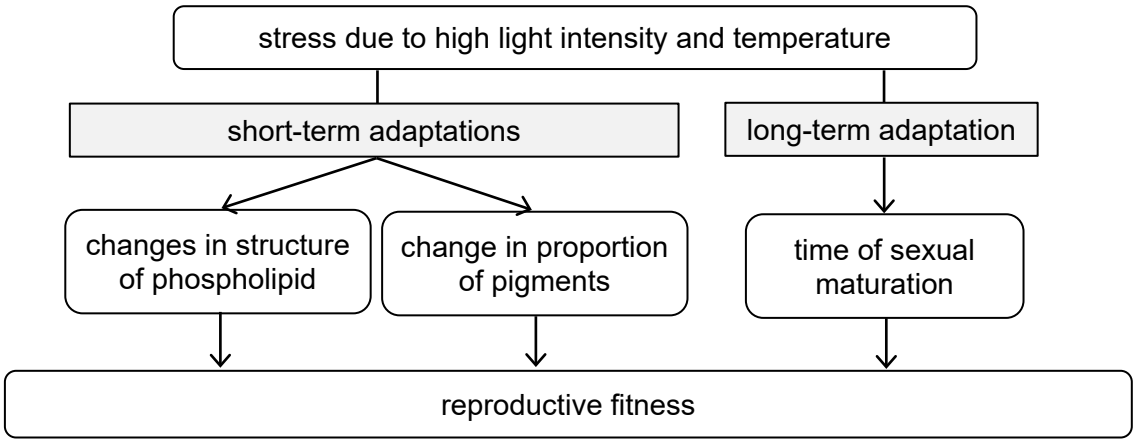


Fig. 3.2

Adapted from Mathur et al., J Photohem Photobiol B., 2014

(c) With reference to Fig. 3.2, explain how plants improve their reproductive fitness in response to stress due to high light intensity and temperature. [3]

Short-term adaptations:

Increased light intensity

1. Increased proportion of carotenoids;
2. Prevent photobleaching of chlorophyll pigments;

Increased temperature

3. Increased hydrocarbon chain length;
4. Increased degree of saturation of hydrocarbon tails;
5. Resulting in decreased membrane fluidity;

Long-term adaptation:

6. Earlier / shorter time to sexual maturation;
7. More plants have chance to survive and reproduce;

Max. 3m

[Total: 10]

Section B

4	**(a)**	Carbohydrates are involved in various functions in a living organism, including energy storage and structural support. Describe the structural significance of **two** named carbohydrates to their different functions and explain how a wide variety of carbohydrate structures is achieved.	[15]
		Starch → energy storage 1. Starch; 2. Amylose / amylopectin are large polysaccharides / thousands of α-glucose residues; 3. Therefore insoluble in water; 4. Does not exert osmotic pressure; 5. And stores large amounts of energy; 6. Glucose residues linked by glycosidic bonds; 7. Which are easily hydrolysed to release respiratory substrates; 8. Amylopectin is highly branched due to α-1,6 glycosidic bonds; 9. Many sites for hydrolysis; 10. Allowing for rapid release of glucose; 11. Amylose is helical; 12. Therefore compact for storage; Cellulose → structural support 13. Cellulose; 14. Large polysaccharide / thousands of β-glucose residues; 15. Therefore insoluble in water; 16. Alternate β-glucose residues are inverted; 17. Linked by β-1,4 glycosidic bonds; 18. Forming straight chains; 19. Hydroxyl groups (OH) project outward from cellulose chains; 20. Forms extensive hydrogen bonds with neighbouring chains; 21. Forms microfibrils which then bundle to form macrofibrils; Max. 10m Wide variety of carbohydrate structures due to 22. Different monosaccharides; 23. Give 2 examples: glucose, fructose; 24. Joined by different glycosidic bonds; 25. Give 2 examples: β-1,4 glycosidic bonds, α-1,4 glycosidic bonds; 26. Different number of monosaccharides joined together; 27. In different combinations / heteropolymer; 28. Or same monosaccharides / homopolymer; 29. To form linear chain(s); 30. Or a ring(s); 31. Can associate with non-carbohydrate moiety; 32. Such as amino acids; Max 4m 33. AVP;	

QWC: Accurately describes structure to function adaptations of 2 carbohydrates (of different functions) and how different structures are formed with use of correct scientific terminology;;

(b) Outline the reproductive cycles of a temperate phage and describe the differences between the release of bacteriophages and the movement of water across membranes. [10]

Lysogenic cycle

1. During attachment, phage tail fibre binds;
2. To specific receptor on host bacterium outer cell surface membrane;
3. Via tail sheath;
4. Phage dsDNA genome is injected into the host cell's cytoplasm;
5. DNA genome circularises;
6. Inserts genome into host's genome as prophage;
7. During spontaneous induction;
8. Phage genome is excised;

Lytic cycle

9. Host cell metabolic machinery hijacked to synthesise phage proteins;
10. And new copies of phage genome;
11. Proteins self-assemble to form new phage structures;
12. Genome is packaged inside capsid head;
13. Lysozyme synthesised and breaks down bacterial cell wall;
14. Water enters via osmosis;
15. Cell swells and releases phage particles via lysis;

Max. 6m

Feature	Release of bacteriophage	Movement of water
16. Integrity of cell surface membrane	CSM lysed	CSM remains intact;;
17. Direction of movement of substance (A)	May be low to high or vice versa	From less negative to more negative water potential;;
18. Direction of movement of substance (B)	Inside to outside of cell	Inside to outside or vice versa;;
19. Transport protein	Does not require membrane transport protein	Requires membrane transport protein (in facilitated diffusion);;
20. Number of ways of movement across membrane	One way – release via lysis	Two ways – facilitated diffusion and osmosis;;
21. AVP;;		

Max. 3m

		QWC: Accurately describes both reproductive cycles and gives at least 2 correct differences between the process, with use of correct scientific terminology;;	
5	**(a)**	Stem cells have different potency levels and can differentiate into all cell lineages in a living organism. Describe the role of **two** named stem cells of different potency levels and suggest ways to identify different cell types in an organism.	[15]
		1. Embryonic stem cell; 2. Pluripotent; 3. Able to differentiate into almost any cell type; 4. Forming 3 germ layers; 5. Endoderm, mesoderm, ectoderm; 6. To form any organ; 7. Except those that forms the placenta and umbilical cord; 8. Blood stem cell; 9. Multipotent; 10. Able to differentiate into all blood cell types; 11. Myeloid stem cell; 12. Develops in the bone marrow; To form 13. Granulocytes → 14. Monocytes can form macrophages for inflammatory response; 15. Erythrocytes for oxygen transportation; 16. Megakaryoctyes forms platelets for blood clotting; 17. Lymphoid stem cell; 18. Migrates to lymph nodes / spleen / thymus; 19. And further differentiates into lymphocytes; 20. B lymphocytes for humoral response / antibodies; 21. T lymphocytes for cell-mediated response; 22. Natural killer cells that destroy infected cells; Max 10m Ways to identify different cell types 23. Microscopy; 24. Using distinguishing cell features / morphology; 25. Or by cell staining; 26. Epigenetic analysis / DNA methylation analysis; 27. Using e.g DNA methylation patterns in genome; 28. Molecular profiling (e.g. RT PCR); 29. To identify using marker genes expressed / gene expression patterns; 30. Cell surface markers analysis (e.g. flow cytometry, immunohistochemistry); 31. To identify cell surface proteins as biomarkers; 32. AVP;; Max. 4m QWC: Accurately describes the roles of 2 stem cells and at least 2 ways on how to identify different cell types, with use of correct scientific terminology;;	

	(b)	Outline how antibody diversity is achieved and the ways viruses avoid detection from the hosts' immune system.	[10]
		1. Somatic recombination;; 2. DNA rearrangement of variable regions; 3. VDJ segments of heavy chain gene; 4. And VJ segments of light chain gene; 5. When expressed, variable regions form the antigen-binding site; 6. To recognise and bind different antigens of pathogens; 7. Somatic hypermutation;; 8. In the variable region; of heavy and light chains; 9. At a higher than normal rate of mutations; 10. Resulting in affinity maturation; 11. Class switching;; 12. DNA rearrangement at constant region; of heavy chain gene; 13. Results in antibodies with different effector functions; Viruses avoids detection from immune systems by 14. Antigen shift; 15. Rearrangement of genetic material from different viruses; 16. Antigenic drift; 17. Accumulation of mutations on genes coding for antigens; 18. Integrating its genome in that of a non-dividing host cell; 19. And not expressing / limited expression of its viral proteins; 20. Host cell gene silencing; 21. Such as preventing MHC class II expression; 22. AVP;; QWC: Accurately describes at least 2 processes generating antibody diversity and at least 2 ways to avoid detection, with use of correct scientific terminology;;	
			[Total: 25]