H

RAFFLES INSTITUTION
2024 Year 6 Preliminary Examination

Higher 2

CANDIDATE NAME

CIVICS GROUP | 2 | 4 | S | 0 | 3 | | INDEX NUMBER | | | | | | |

BIOLOGY

9744/03

Paper 3 Long Structured and Free-response Questions

11 Sept 2024

2 hours

Candidates answer on the Question Paper.

Additional Materials: Writing paper.

READ THESE INSTRUCTIONS FIRST

Write your index number, CT group & name in the spaces at the top of this page.
Write in dark blue or black pen.
You may use a HB pencil for any diagrams or graphs.
Do not use staples, paper clips, glue or correction fluid.

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

Section B
Answer any **one** question in the writing paper provided.

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.

At the end of the examination, **hand in your essay question SEPARATELY.**

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

For Examiner's Use	
Section A	
1	**/ 30**
2	**/ 11**
3	**/ 9**
Section B	
4 or 5	**/ 25**
Total	**/ 75**

This document consists of **19** printed pages.

Raffles Institution
Internal Examination

For Examiner's Use

Section A

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

1 The human papillomavirus (HPV) is sexually transmitted and can cause the development of cervical intraepithelial neoplasia (CIN). Patients with CIN are observed to have abnormal growth of cells that line the cervix. This can lead to the development of cervical cancer.

Fig. 1.1 shows the structure of the HPV.

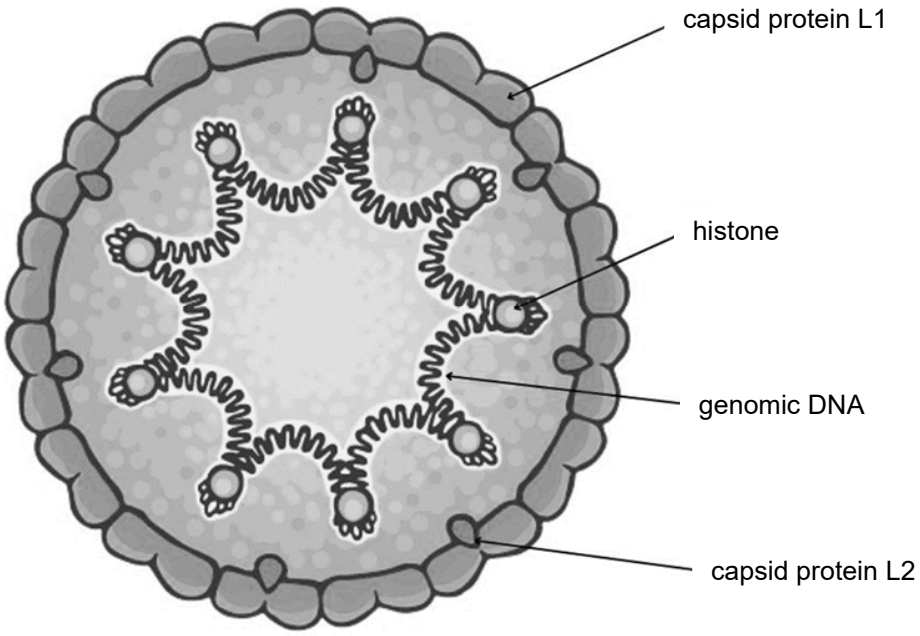

Fig. 1.1

(a) With reference to Fig. 1.1, compare the structure of HPV with a T4 bacteriophage.

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Similarities
1. The genome of both viruses are enclosed by a protein ***capsid*;**
OR
Both viruses have a ***capsid*** which is made up of several individual protein subunits (capsomeres);
2. Both viral genomes are made up of ***DNA***;

Differences

For Examiner's Use

3. The HPV has DNA associated with histone protein, while the T4 bacteriophage DNA does not associate with histone protein;
4. spherical capsid vs icosahedral capsid head
5. T4 bacteriophage has a sheath, collar, tail and tail fibres (name any 1) while the HPV has does not;

(A only if no mark point 4 or 5: The HPV has a spherical shape, while the T4 bacteriophage has a complex shape)

6. The HPV has 2 types of capsid proteins, L1 and L2, while the T4 bacteriophage has only 1 type.

A recent study assessed the impact of routine vaccination against HPV on the development of CIN. Fig. 1.2 shows the effect of HPV vaccination on the percentage of women found to have the most severe grade of CIN by cervical screening over the years.

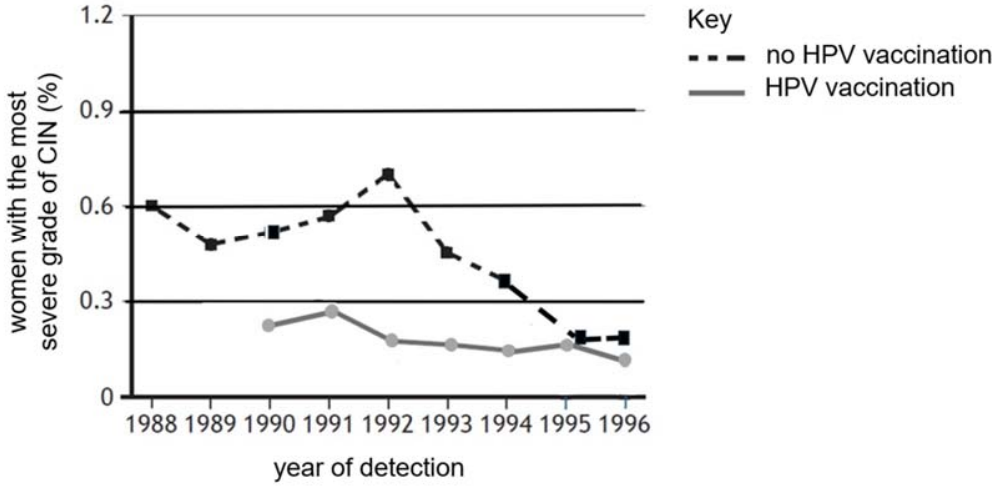

Fig. 1.2

(b) With reference to Fig. 1.2, explain how the data support the suggestion that vaccination against HPV could lead to herd immunity.

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1. Vaccination programme started in 1990 and resulted in lower (A: low) % of women with CIN amongst the vaccinated women, preventing transmission of HPV;
2. Due to % of vaccinated women reaching sufficiently high numbers/ number reaching critical threshold from 1992;
3. Resulting in a decrease in the percentage of non-vaccinated women with the most severe CIN from 0.7% to 0.2% from 1992 (R: 1990) to 1996 (A: 1995).

(c) Fig. 1.3 shows the number of cases of cervical cancer and death rate from cervical cancer in females of different ages in one year.

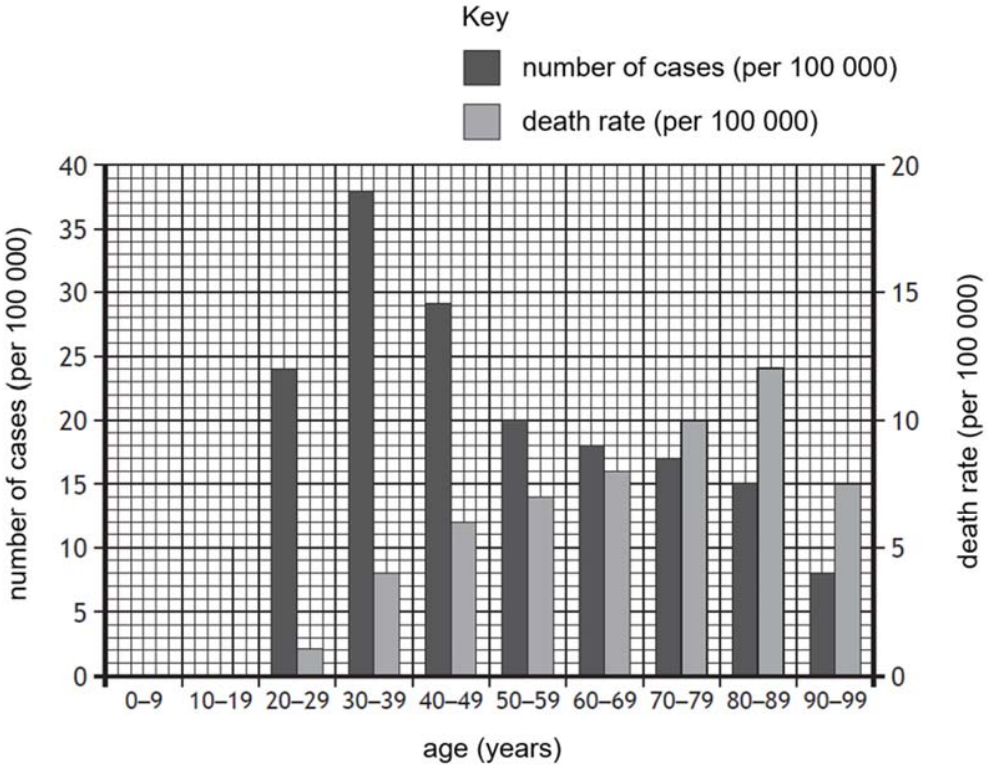

Fig. 1.3

With reference to Fig. 1.3,

(i) describe changes that occur in the number of cases of cervical cancer from the ages of 20–29 to 90–99.

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1. As age increases from 20-29 to 30-39 the number of cases of cervical cancer increases from 24 to 38 per 100 000/increases by 14 per 100 000;
2. and then from ages 30-39 to 90-99, the number of cases of cervical cancer decreases from 38 to 8 per 100 000/decreases by 30 per 100 000;
   (must quote values with units for both axes correctly to award marks)

For Examiner's Use

(ii) Suggest a reason for the decrease in the death rate from cervical cancer between ages 80–89 and 90–99.

……

……[1]

Many females with cervical cancer have died before this age;
OR
Many females with cervical cancer died of other causes;

(iii) Express as a simplest whole number ratio, the number of cases compared to death rate at ages 30–39. [1]

38:4 =19:2

_____: _____
number of cases : death rate

(iv) The human papillomavirus (HPV) is commonly associated with cases of cervical cancer. Many countries have a vaccination programme against HPV.

Use information from Fig. 1.3 to suggest why females are given the vaccine in their teenage years.

……[2]

1. There are no cases below 20/in teenage years/between 0-19/10-19
   OR
   From (age) 20-29/after teenage/in adult years there are cases (of cervical cancer);
2. Thus girls are given the vaccine before they are likely to be sexually active/ infected by the HPV;

(v) The HPV vaccination programme is being extended to boys' schools.

Explain the advantages of:

offering the HPV vaccine to boys,

……[2]

Advantages of vaccinating boys
1. protects boys from becoming infected so the virus is less likely to get passed on to girls during intercourse;
2. greater chance of developing herd immunity ;
3. help to protect girls who cannot be vaccinated;
4. protects boys from becoming infected and developing other types of HPV-related cancer;

and carrying out the vaccination programme in schools.

……[1]

Advantages of vaccination in schools

5. more boys / girls likely to be vaccinated because they are not reliant on a doctor's appointment being made / available;
6. seeing others being vaccinated might encourage others to get vaccinated;
7. inoculating school children is likely to be before they become sexually active so less likely to get infected;

(vi) Similar to HPV infections, infections caused by the bacterium, *Chlamydia trachomatis,* are also sexually transmitted.

Explain why the HPV vaccine will not protect girls from *C. trachomatis*.

……[3]

1. Since the immune response is specific to the antigen/pathogen/HPV;
2. and Chlamydia has a different antigen compared to HPV/the vaccine;
3. upon infection with Chlamydia, there will not be memory cells present specific to Chlamydia / memory cells specific to only HPV;
4. and so no plasma cells to produce ***antibodies**** against Chlamydia;
5. Therefore the Chlamydia will not be destroyed before it causes disease;

(d) Like HPV, the human immunodeficiency virus (HIV) may lay dormant in the body for years without causing symptoms.

HIV is an example of a virus that spreads from other animals to humans where it causes disease.

Chimpanzees can carry the simian immunodeficiency virus (SIV), which is similar to HIV. It is thought that chimpanzees who carry antibodies for SIV do not become ill if infected with HIV. This has been investigated by scientists who are developing potential vaccines for HIV.

Tests were carried out to see if antibodies against SIV present in chimpanzees bind to HIV antigens. Test strips which contained several different HIV antigens were prepared.

When samples are applied to the test strip, a line will appear in the control region. If the sample contains antibodies to the HIV antigens present on the strip, additional lines will also appear.

Samples of chimpanzee faeces were collected from a number of sites in Gabon in central Africa. The faecal samples were prepared and then applied to the test strips.

(i) Suggest why the investigators collected faecal samples rather than blood plasma samples from chimpanzees.

……...[1]

any one from:

1. easier to collect faeces without use of specialised medical tools;
2. storage of faeces does not require stringent storage conditions compared to blood plasma samples;
3. faecal samples can be collected remotely without harming the chimpanzees or investigators / no risk of investigators being attacked by chimpanzees;

4. faecal samples are non-invasive / less stressful for chimpanzees / limits human interaction / no risk of infection / no need to anesthetise chimpanzees;

The scientists collected 608 faecal samples from 224 individual chimpanzees.

(ii) Describe how an investigator can prepare the faecal samples before applying them to the test strips.

……[2]

1) make into a suspension / solution / liquify faeces and – accept procedure involving adding water (e.g: use of blender)
2) filter / centrifuge to remove solids;
3) apply supernatant to test strip;

(e) Samples from humans and chimpanzees were applied to the test strips.

1. Blood plasma samples from humans who are infected with HIV (HIV+)
2. Blood plasma samples from humans who are not infected with HIV (HIV−)
3. Faecal samples from chimpanzees who are infected with SIV (SIV+)
4. Faecal samples from chimpanzees who are not infected with SIV (SIV−)

The results of typical samples from the above four groups are shown in Fig. 1.4.

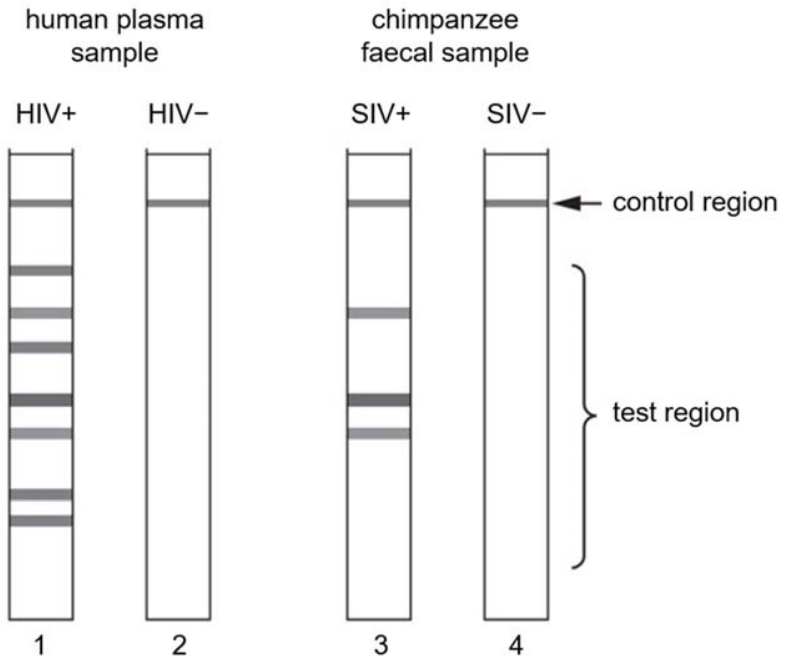

Fig. 1.4

With reference to Fig. 1.4, suggest conclusions that can be drawn about whether SIV antibodies bind to HIV antigens.

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1. SIV antibodies will bind to some HIV antigens;
2. 3 bands out of 7 in the HIV+ sample are recognised by SIV antibodies;
3. some SIV and HIV antibodies have similar antigen binding sites with complementary shape to the HIV antigens;
4. HIV– people and SIV– chimpanzees do not contain antibodies that bind to HIV antigens;
5. The test results are valid as there is a line in each of the control regions/ if the control works, it shows that the test kit works/is not damaged;

(f) HIV infection may lead to HIV/AIDS which, if left untreated, may cause death. The effects of SIV infection in chimpanzees are usually less severe.

A group of scientists investigated the effect of SIV infection on the life expectancy of a population of chimpanzees living in the wild.

- The population of 94 chimpanzees was observed for a nine-year period.
- The ages of all the chimpanzees in the population were estimated at the start of the nine-year period.
- The chimpanzees were observed each day.
- The numbers of dead and absent chimpanzees were recorded each day.
- A statistical test was also carried out.
- Faecal samples of all the chimpanzees in the population were tested for SIV antibodies.

The results of the investigation, including the *p* value of a statistical test to see if there are differences in the percentage survival between the two groups of chimpanzees, are summarised in Table 1.1.

Table 1.1

SIV status	original number of chimpanzees in population	number of chimpanzees who died	*p* value of a statistical test
SIV–	77	11	0.031
SIV+	17	7	

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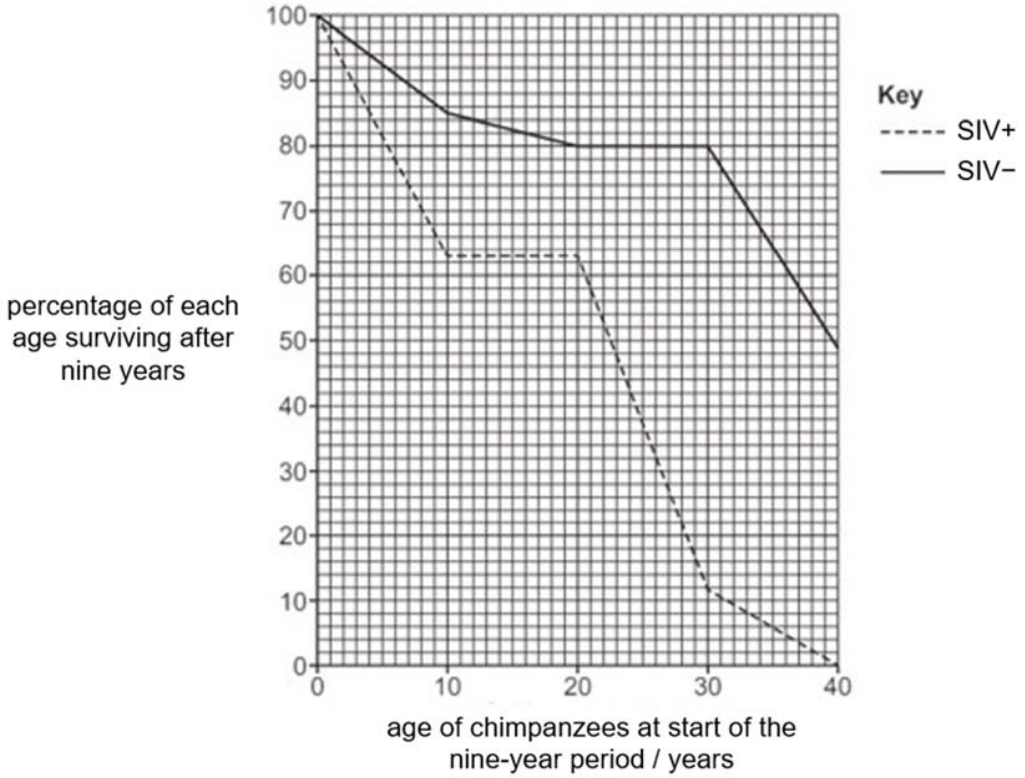

Fig. 1.5

Fig. 1.5 shows the percentage of chimpanzees of each age remaining in the population.

(i) Comment on the results shown in Table 1.1 and Fig. 1.5.

Table 1.1 .....

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Fig. 1.5 .....

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.....[4]

Table 1.1
1. At 5% level of significance, $p<0.05$, hence the difference in % survival between the 2 groups is ***significant**** and not due to chance;
2. SIV infection reduced the % survival: (quote data) 14.3% SIV– died compared to 41.2% SIV+;

Fig. 1.5

3. 100% of SIV+ and SIV– newborn chimpanzees survived past the age of 9;
4. (SIV+ *versus* SIV-) For every age group beyond newborns, % survival after 9 years is lower for SIV+ compared to SIV–;
5. Infected chimpanzees do not live past the age of 49 but SIV– have a 49% chance of living past 49;
6. (Young *versus* old) % survival after 9 years is lower for older chimpanzees for both SIV+ and SIV–;
7. Death occurs at all ages in SIV– chimpanzees due to causes other than SIV;

AVP: Earlier start age for the steep decline for the SIV+ compared to SIV-;

(ii) Clinical trials on HIV vaccination sometimes involve testing the vaccines on chimpanzees.

Discuss the suitability of using chimpanzees for such trials.

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Suitable
1) Chimpanzees are evolutionarily related to humans/ molecular / biochemical homology between humans and chimpanzees so adaptive immunity responses will be similar;
2) More public outcry if humans are used, so a suitable alternative has to be considered/ public find it more acceptable to use animal models rather than humans;

Not suitable
1) Chimpanzees cannot give consent/ suffer unnaturally just for humans' benefits
(R: mention of 'ethical concerns' without further elaboration)
2) *Idea of* chimpanzees' immune response is not entirely similar to human's, so wrong inference would be made;
3) SIV+ chimpanzees produce antibodies that can bind to HIV antigens and this may affect conclusions about effectiveness of vaccine in stimulating production of HIV antibodies;

[Total: 30]

2 Termites are a group of insects which consume a wide variety of plant material in the form of wood and leaf litter. They are able to breakdown cellulose into hexose and pentose oligomers due to the presence of symbiotic cellulolytic bacteria which produce a variety of cellulases.

(a) One step in the digestion of cellulose involves the cleaving of a cellulose chain to form cellobiose units. The digestion starts from the C4-hydroxyl group of cellulose that is not involved in bond formation. A cellobiose unit is a disaccharide with the formula $(C_6H_7(OH)_4O)_2O$.

The type of cellulase involved is called β–1,4 glucanase.

Show on Fig. 2.1 how the section of cellulose is cleaved to form cellobiose.

Use arrows to indicate the sites of hydrolysis. You do not need to include any monosaccharides that did not form cellobiose.

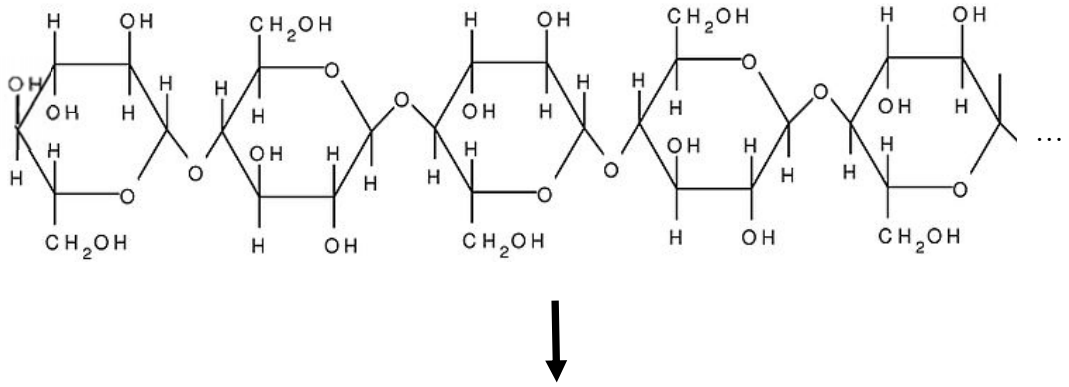
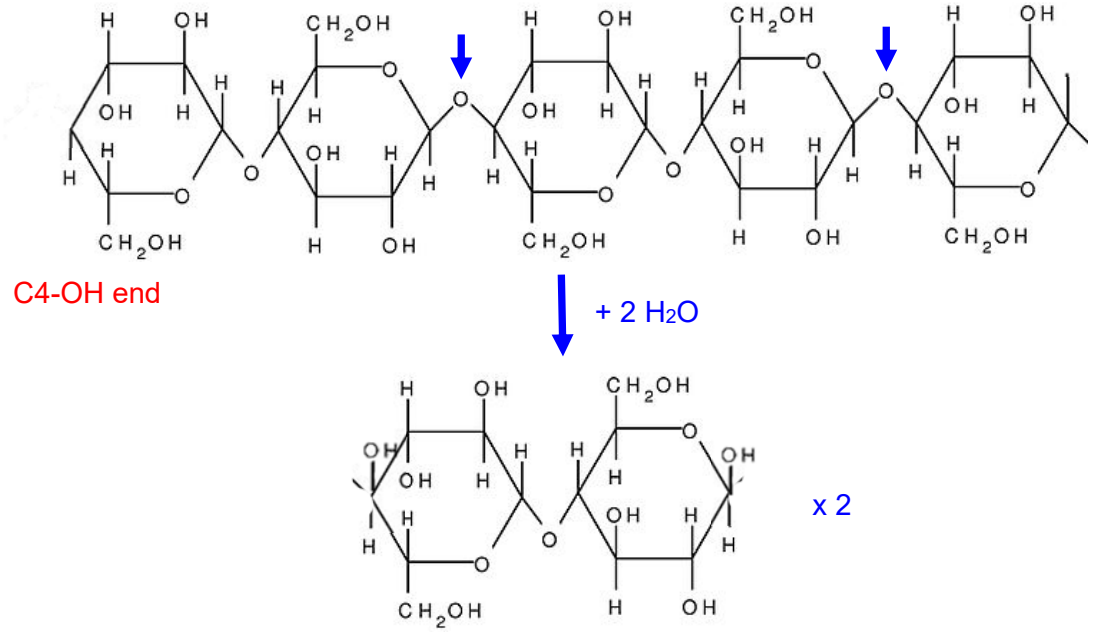


Fig. 2.1

[3]



1. Correct location indicated of the hydrolysis (2nd and 4th bond from the left)
2. Correct cellobiose disaccharides drawn (x 2 cellobiose)
3. Water molecule (x2) indicated

(b) To identify the region of the gut most heavily populated by cellulolytic bacteria, scientists investigated the amount of cellulose, cellulase activity and glucose content in various locations along the alimentary canal of termites - hepatopancreas (hepatop.), anterior hindgut (ant. hindgut) and posterior hindgut (post. hindgut), including the faeces. They also measured the same variables in the leaf litter consumed by the termites.

The results of the study are shown in Fig. 2.2. The mean amount of cellulose, cellulase activity and glucose content are shown as the middle line of the boxplots.

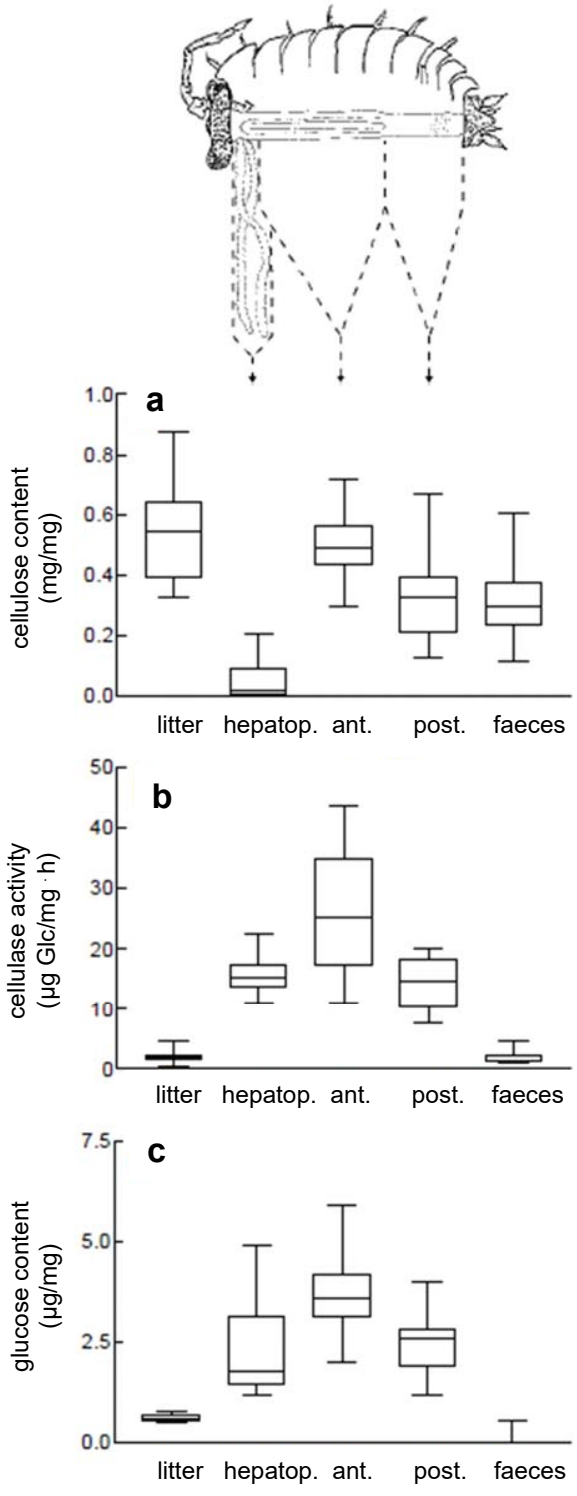

a
cellulose content (mg/mg)
1.0
0.8
0.6
0.4
0.2
0.0
litter
hepatop.
ant.
post.
faeces
b
cellulase activity (µg Glc/mg · h)
50
40
30
20
10
0
litter
hepatop.
ant.
post.
faeces
c
glucose content (µg/mg)
7.5
5.0
2.5
0.0
litter
hepatop.
ant.
post.
faeces

Fig. 2.2

With reference to Fig. 2.2,

(i) suggest where majority of the cellulolytic bacteria were found;

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1. ***Anterior hindgut****;
2. This is the region that had the highest mean cellulase activity 25 µg Glc/mg.h and/or highest mean glucose content 3.75 µg/mg;
3. Indicating the highest rate of (A: most) digestion of cellulose to glucose;

(ii) suggest why there was very little to no glucose found in the faeces of the termites;

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……..[1]

Most/All of the glucose was absorbed by the termite cells to be used for respiration;

(iii) explain why leaf litter was included in this study.

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……..[1]

The leaf litter acts as a ***control****;
to show that cellulose was indeed present in the leaf litter the termites consumed;
or
to show that the cellulase enzymes were present in the termite gut and not from consumption of the leaf litter;
or
to show the baseline amount of glucose present in the leaf litter consumed to get an accurate representation of the glucose produced via digestion of cellulose;

(c) Termites are also known to produce methane as a by-product in the process of breaking down cellulose. The global methane emission from this source is estimated to be 20 million tons each year.
Explain how termites can worsen the effects of climate change as global temperature increases.

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1. Increasing temperature increase metabolism/ reproduction rate in termites, resulting in larger termite population;
2. Increase temperature increase enzymatic activity in bacteria in termites, resulting in an faster methane production;
3. Increase in methane production causes increased ***greenhouse**** effect, trapping more heat in the atmosphere;
4. This causes a furtherincrease in temperature, resulting in a ***positive feedback**** loop;

[Total: 11]

3 The method for producing seedless watermelons was developed by Professor H. Kihara, a Japanese scientist at Kyoto University in 1939. His methods yielded commercially available seedless watermelons in 1951.

Production of viable triploid (3n = 33) watermelon seeds using colchicine, a chemical that is used to induce polyploidy in plants, is shown in Fig. 3.1. Colchicine works by interfering with mitosis, preventing the proper assembly of microtubules in the mitotic spindle.

Colchicine is first applied to diploid (2n = 22 chromosomes) watermelon seedlings to obtain tetraploid (4n = 44 chromosomes) plantlets. Tetraploid female plants are then crossed with normal diploid male plants to obtain triploid seeds.

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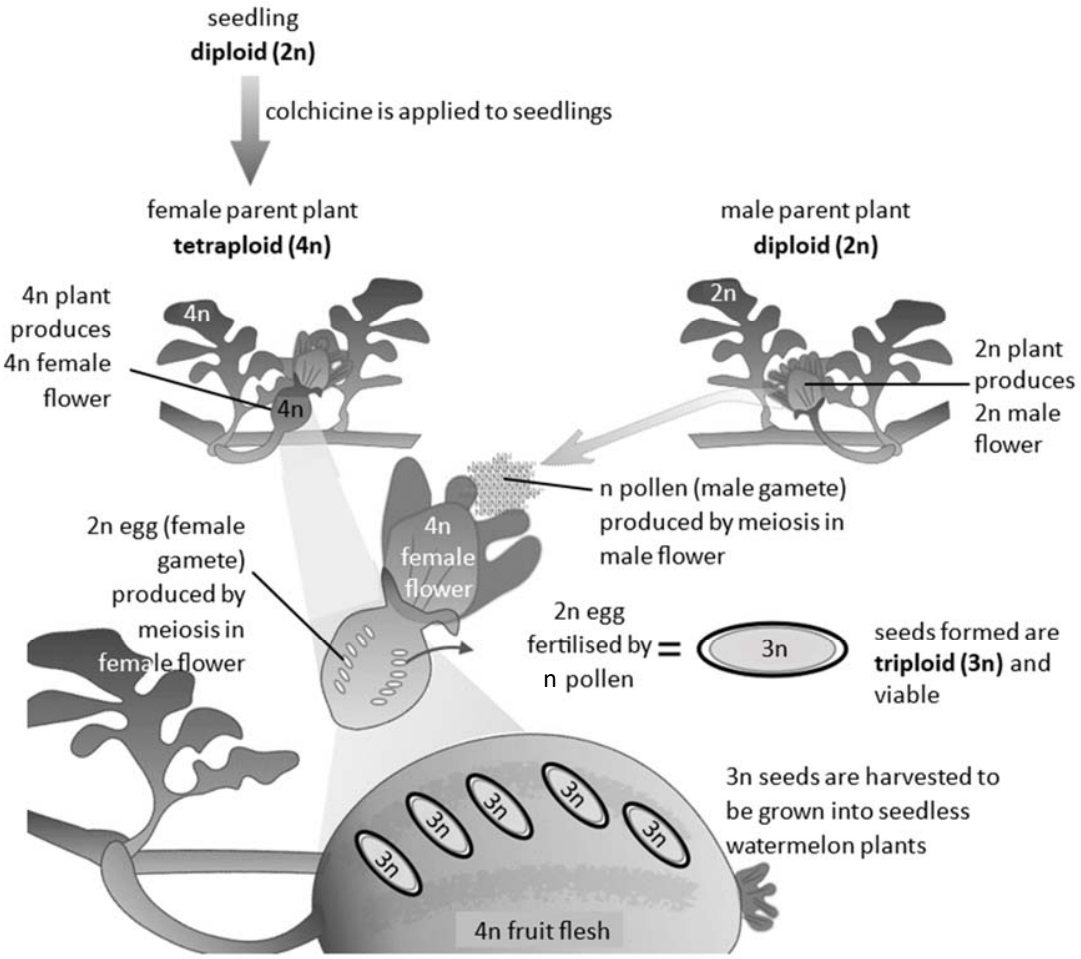

Fig. 3.1

Seedless watermelon fruits are stimulated to form when haploid (n = 11) pollen from normal diploid male plants pollinate female flowers from triploid plants.

(a) (i) Explain how application of colchicine to diploid (2n = 22 chromosomes) watermelon seedlings resulted in formation of tetraploid (4n = 44 chromosomes) plants as seen in Fig. 3.1.

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1. Diploid cell undergoes ***mitosis****;
2. During prophase, chromatin condenses to form 22 chromosomes each with 2 ***sister chromatids****;

3. As the mitotic spindle is not able to form, ***non-disjunction**** occurs,
4. Centromeres divide and the 2 sister chromatids of each chromosome now form daughter chromosomes.
5. Daughter chromosomes (A: sister chromatids) cannot be pulled to opposite poles, resulting in the doubling of the chromosomes in the cell/44 chromosomes/4 copies of each chromosome;

(any 3)

(ii) Using  to represent one chromosome in a haploid set of chromosomes, fill up the cell diagrams in Fig. 3.2 to show how application of colchicine to diploid (2n = 22 chromosomes) watermelon seedlings resulted in formation of tetraploid (4n = 44 chromosomes) plantlets. Do note that only one cell of each plant is shown.

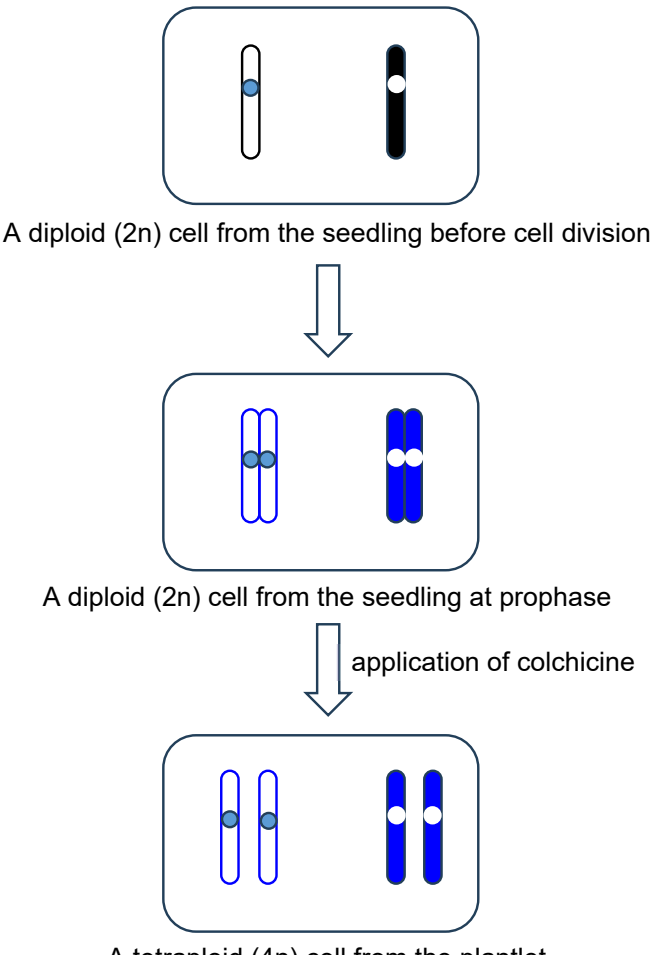

Fig. 3.2

[2]

(iii) Explain why gametes fail to form in the triploid (3n = 33) female flower.

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……..[1]

As there are 3 homologues of each chromosome, they are unable to pair up during synapsis, so meiosis cannot proceed.

(b) The identification of tetraploids can be based on stomatal density. Stomata density is not influenced by external factors such as temperature and water content of the plant tissue. Stomata counting is a suitable, easy and reliable method because their numbers in many plantlets can easily be estimated relatively quickly and easily.

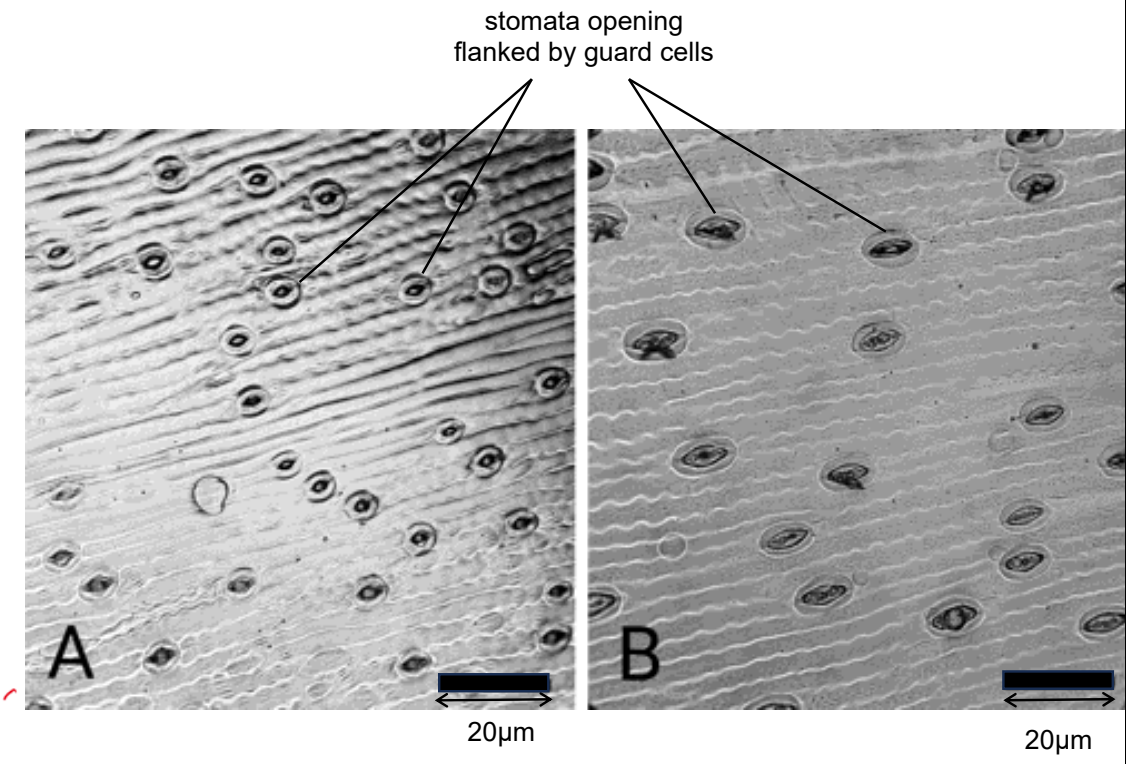


Fig. 3.3

The imprints of the diploid (2n) and tetraploid (4n) leaves shown in Fig. 3.3 were produced by applying nail varnish to the bottom side of the leaf. The dry nail varnish showing the difference in stomata density between the diploid (labelled B) and tetraploid (A) leaf was then peeled off and viewed under the microscope.

Stomata density is known to be lower in tetraploid (4n) leaf than diploid (2n) leaf.

Using the information in Fig. 3.3 and counting only whole stomata,

(i) calculate the stomata density in Fig. 3.3, A and B, and conclude which is the tetraploid (4n) leaf.

A has 30 (A: 31) stomata.
B has 13 stomata.

Area A = [(8.3/1.6) x 20] x [(8.0/1.6) x 20] = 103.75 x 100 = 10375 µm^2

Stomata density in A = 30 / 10375 = 0.00289 or 2.89 x 10^{-3} stomata / µm^2 [1]

Area B = [(8.3/1.6) x 20] x [(7.8/1.6) x 20] = 10115.625 μm^2
Stomata density in B = 13 / 10115.625 = 0.00129 or 1.29×10^{-3} stomata / μm^2 [1]

1. Formula for density (relevant working included) and correct units;
2. All measurements, stomata counts and calculations are correct;
3. Stating that B is tetraploid.

Tetraploid (4n) leaf: B [1]

[Total:9]

Section B

Answer **one** question in this section.

Write your answers on the separate answer paper provided.

Your answers should be illustrated by large, clearly labelled diagrams, where appropriate.

Your answers must be in continuous prose, where appropriate.

Your answers must be set out in sections **(a)** and **(b)**, as indicated in the question.

4 (a) Explain why the offspring produced by the same parents are different in appearance. [15]

A. Introduction

1. Differences in appearance in offspring is due to meiosis occurring during sexual reproduction giving rise to differences in phenotypes/ characteristics/ traits;
2. As different offspring have different genotypes/ genes and also their phenotypes influenced by different environmental factors;
3. Gene mutation gives rise to new alleles which code for new phenotype/ characteristic/ trait;
4. An accumulation of mutations in the same cell can lead to tumour formation/cancer development;
5. ***Crossing over**** of ***non-sister chromatids between homologous chromosomes**** occurs at ***prophase I**** at points called chiasmata;
6. Where equivalent portions of these chromatids break and rejoin, resulting in exchange of genetic material/alleles, hence new combination of alleles on the chromatid;
7. ***Independent assortment of homologous chromosomes**** at the equator/metaphase plate during ***metaphase I*;***
8. and separation of ***homologous chromosomes**** at ***anaphase I**** ultimately results in different combinations of parental chromosomes in the gametes;
9. Also, chromosomes with ***non-identical sister chromatids**** align randomly along the equator/metaphase plate during metaphase II and separate during anaphase II, giving rise to gametes with different allelic combinations;

B. Mendel's 1st law of segregation:

10. 1 gamete from each parent carrying 1 allele of each gene (ref to 1st law of segregation)

C. Fertilisation → greater variation in fusion of gametes

11. Random fusion of genetically different gametes results in even greater genotypic variation in zygote.

D. Chromosomal aberrations

12. Chromosomal aberrations can occur as changes in number of chromosomes or changes in the structure of chromosomes;
13. ***Non-disjunction**** in meiosis can result in aneuploidy or polypoidy;
14. Aneuploidy occurs when 1 pair of homologous chromosomes fail to segregate properly (A: Description of an example, e.g. Trisomy 21 leading to Down's syndrome)
15. Polyploidy occurs when all pairs of homologous chromosomes fail to segregate properly, resulting in offspring have extra sets of chromosomes (A: Description of polyploid plants);
16. Chromosomal translocation (A: deletion, inversion, duplication) where a chromosome breaks and a portion of it reattaches to a different chromosome;
17. Changes in gene expression as a result can lead to uncontrolled cell division and tumour formation/cancer development (A: description of changes in gene expression with elaboration);

E. Sex determination and X-inactivation

18. Offspring can inherit either the X chromosome or the Y chromosome from the male parent, resulting in offspring that could be phenotypically male or female;
19. 1 copy of the X chromosome is randomly inactivated (A: X inactivation) in females; eg. Tortoiseshell female cats;
20. Genetically identical females can look different since their phenotype is dependent only on the copy of X chromosome that is expressed;
21. Sex determination due to differences in chromosome number in ants, wasps/ Haplodiploidy system with elaboration – Male drones are haploid and females are diploid;

AVP other organisms, eg temperature dependent / birds – female heterogametic

F. Effect of environment on phenotype

Discontinuous variation

22. Sometimes, environmental factors also affect the discontinuous variation phenotypes;
23. e.g. any 1 below
    - fur that grows on shaved rabbit skin under ice is black (as tyrosinase enzyme is denatured at body temperature/ tyrosinase enzyme's optimum temperature is at low temperature)
    - diet of female bees determine whether it is worker bee (if feed on pollen) or queen bee (if feed on royal jelly);
    - low oxygen concentration result in carriers of sickle cell anaemia showing sickle cell anaemia;

- Diet contain phenylalanine results in brain damage in individuals with phenylkenoturia (PKU).

Genetic basis – Continuous variation

24. Other characteristics with intermediate phenotypes are controlled by multiple genes/ polygenic inheritance;
25. The effect of each gene on the phenotype is small and additive;
26. Environmental factors usually affect phenotype that show continuous variation;
27. Give a relevant example explaining how environment affects phenotype; e.g. more exposure to the sun results in tan skin colour/ nutrients of plants affecting height;
28. Even genetically identical twins can look different when growing up in different environmental factors;

Differences in gene expression/ epigenetic factors

29. Genetically identical offspring can look different due to differences in gene expression/ epigenetic factors;
30. State one example of mechanism of epigenetic effect at chromatin or genomic level, e.g. DNA methylation (caused by mother's diet) resulting in silencing of gene that determine coat colour in mice.

G. Genetic basis of variation

31. Complete dominance: Heterozygotes parents produce homozygous recessive offspring with a different phenotype (e.g. albinism, sickle-cell anaemia);
32. Basis: Dominant allele masks the phenotypic expression of the recessive allele in the heterozygote;
33. Co-dominance/ Incomplete dominance: Heterozygote offspring have a different intermediate phenotypes compared to homozygous parents (e.g. pink snapdragon flowers with red and white parents);
34. Gene interactions/Epistasis: where the expression of one gene is affected by the expression of another gene (e.g. white x white gives purple flowers)

QWC: 1 mark for sections A + 3 others.

(b) In normal cellular respiration, glucose is used in aerobic respiration in the presence of oxygen to produce energy. However, in cancer cells, it is observed that even in the presence of oxygen, there is an increased glucose uptake and lactic acid production.".

Despite being a less efficient process in energy production compared to aerobic respiration, cancer cells are still able to produce the same amount of energy in the same amount of time via this method.

Describe this method of energy production in cancer cells and suggest how it may be beneficial to cancer cells. [10]

Method of energy production:

1. Energy production in cancer cells takes place in the cytosol of the cell via ***glycolysis**** and lactic acid ***fermentation****;
2. Increase in the number of glucose transporters will increase glucose uptake;

3. Phosphorylation of glucose involves the initial investment of 2 ATP molecules which phosphorylate the two ends of glucose molecule forming ***fructose-1,6-bisphosphate****;
4. ***Phosphofructokinase**** is the enzyme involved in the second phosphorylation step;
5. The phosphorylated fructose-1,6-bisphosphate sugar splits/ undergoes lysis ultimately gives rise to two molecules of ***glyceraldehyde-3- phosphate (G3P)****;
6. Glyceraldehyde-3-phosphate undergoes ***oxidation by dehydrogenation**** to form 1,3-bisphosphoglycerate;
7. ***NAD**** is reduced to ***NADH**** in the process;
8. 1 ATP is produced by ***substrate level phosphorylation**** of 1,3-bisphosphoglycerate to glycerate-3-phosphate;
9. 1 ATP is produced by ***substrate level phosphorylation**** of glycerate-3-phosphate to ***pyruvate****;
10. Net of 2 ATP molecules produced for each glucose molecule oxidised;
11. During fermentation, pyruvate is reduced by ***lactate dehydrogenase**** to lactate with the regeneration of NAD;
12. NAD regenerated ensures steady supply of NAD is used for glycolysis to continue.

AVP: Glyceraldehyde-3-phosphate undergoes ***phosphorylation**** to form 1,3-bisphosphoglycerate;

How it may be beneficial to cancer cells:

13. Allow for ATP production even when oxygen supply is limited;
14. Allow for ATP production even when mitochondria is dysfunctional;
15. Increased glucose uptake allows cancer cells to "hoard" glucose and deprive neighbouring normal cells of glucose;
16. Allow for increased ATP production for increased cell proliferation;

QWC: 1 mark for describing at least one point from both parts of the question.

[Total: 25]

5 (a) Molecular techniques are used to detect genetic diseases, where some of the steps involve the concept of "complementarity".

With reference to the mutation that causes sickle cell anaemia, outline the molecular techniques used to determine the genotype of an unaffected individual and explain the role of "complementarity" in specific steps of the molecular techniques used. [15]

Basis of sickle cell anaemia

1. A single nucleotide base substitution in the ***β globin**** gene;

   R: "sickle cell anaemia gene"

2. Changes DNA triplet from CTT to CAT (A: CTC to CAC) / mRNA codon from GAA to GUA (A: GAG to GUG) (need to quote specific mutation either in DNA or mRNA);

   R: Thymine substituted for Adenine

Concept of complementary shape

3. Restriction enzyme's ***active site**** is ***complementary in shape**** and charge to specific DNA sequence;

4. Mutation results in the loss of a restriction site (R: splice site) (by *Mst*II enzyme) in Hb^S / mutant allele;

   A: creation of a new restriction site in mutant allele

5. When same restriction enzyme/ *Mst*II enzyme cuts both normal and recessive alleles, normal allele/ Hb^A allele gives 2 smaller fragments, while mutant allele/ Hb^S allele gives 1 large fragment;

Steps in molecular technique

6. Obtain genomic DNA samples from cheek swab / blood sample / hair follicle / any cell (A: any method or cells) from unaffected individual;

7. and amplify the target DNA using ***polymerase chain reaction****;

Concept of complementary base pairing / complementary shape

(In first step of PCR cycle, temperature is raised to 95 °C for denaturation of DNA: no marks)

8. In second step of PCR cycle, temperature lowered to ***64 °C**** to allow DNA ***primers**** to ***anneal****;

9. DNA ***primers**** bind specifically, via ***complementary base pairing****;

   R: RNA primers

10. to ***3'**** ends of each strand of the target DNA sequence; (need idea of both strands and target region)

11. Primers bind to flanking regions of target DNA sequence / ***β-globin**** gene;

12. ***Adenine**** base pair with ***Thymine****, and ***Guanine**** base pair with ***Cytosine****; (mark only once)

13. In third step of PCR cycle, temperature raised to ***72 °C**** to allow DNA ***extension/elongation****;

14. The ***active site**** of ***Taq polymerase**** is ***complementary in shape**** and charge to the ***3'-OH*** end of the primer / elongating strand and incoming free dNTPs;

15. Free ***dNTPs**** bind to template strand via ***complementary base pairing****;

Steps in molecular technique

16. DNA is separated according to size in ***gel electrophoresis****;

17. Double stranded DNA is denatured / made single-stranded and by alkaline solution / NaOH and transferred to a nitrocellulose membrane;

Concept of complementary base pairing

18. Carry out ***Southern blotting/nucleic acid hybridisation**** by incubating nitrocellulose membrane with ***single stranded radioactive probe**** ;

19. DNA probe will hybridise with DNA fragment/ target DNA sequence through ***complementary base pairing****;

Steps in molecular technique

20. Using ***autoradiography/*** placing ***X-ray film**** over the **nitrocellulose membrane**, the bands can be visualised.
(ref to Radioactivity of bound probes exposes film to form an image corresponding to bands that have base-paired to probe.)

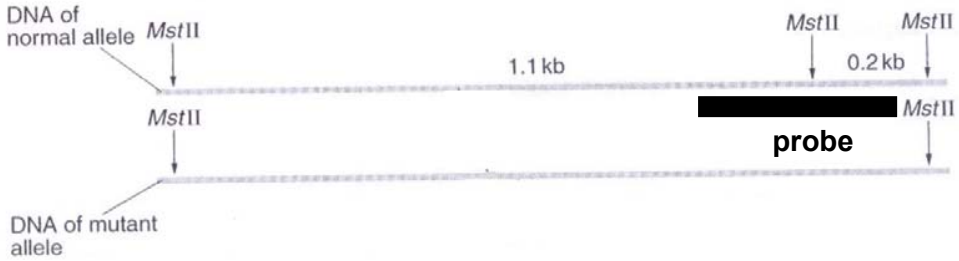
OR

Stain gel with ***ethidium bromide**** and visualise bands under ***UV**** (after gel electrophoresis).

Analysis

(Points 21 to 23 are relevant and awrded marks only if students included Southern blotting method);

21. State position / fragment that probe hydridises to;
E.g.



22. Normal allele gives rise to an intermediate and short fragment upon restriction digestion (with *Mst*II) and these fragments correspond to the 2 bands further away from the well;

23. Mutant allele gives rise to a single long fragment upon restriction digestion (with *Mst*II) and this corresponds to the 1 band closest to the well;

A: normal allele gives 1 band closest to the well and mutant allele gives 2 bands further way from the well
Pts 22, 23 bands need to match the choice of their probe position. Pts 22 and 23 cannot be credited from pts 21 is not stated.

24. If homozygous dominant, two (thicker) bands, whereas if heterozygous/ carrier, three bands.
ECF based on student answers for pts 22/23.
Can award based on PCR & gel electrophoresis.

QWC: 1 mark for mentioning complementary shapes + complementary base pairing + analysis

(b) Discuss how the fluidity of membranes is important to allow for different types of transport across membranes. [10]

1. The cell membrane is said to be fluid as it comprises of ***phospholipids**** and ***proteins**** which are free to move laterally within a layer;

2. Weak ***hydrophobic interactions**** between phospholipids and proteins can be easily broken and formed;

3. Description of a factor affecting membrane fluidity:

   - higher proportion of unsaturated fatty acid tails of hydrocarbon chains can lead to greater fluidity (ORA);
   - presence of cholesterol can regulate the fluidity of the membrane;
   - higher temperature will lead to greater fluidity of the membrane due to increased kinetic energy (ORA);

Transient pores can form

4. Fluidity allows for formation of transient pores;
5. Which facilitate simple ***diffusion**** of small, non-polar molecules (e.g. oxygen) across the membrane;
6. Water moves across the membrane via simple diffusion even though it is polar, as it is sufficiently small;

Transmembrane proteins can undergo conformational changes

7. Fluidity allows for conformational changes in the transmembrane region of ***carrier proteins****
8. This allows for ***facilitated diffusion**** where solute binding causes a conformational change of carrier protein that results in the solute being transported across the membrane down the concentration gradient;
9. This also allows for ***active transport**** where the conformational change can be caused by ***ATP**** binding to transport a solute against concentration gradient;

Membrane is able to move and change shape

10. Fluidity of the membrane allows for change in shape of the cell membrane that can be caused by rearrangement of the cytoskeleton;
11. Description of ***exocytosis****: Vesicles are able to fuse with cell surface membrane to release contents out of cell
12. Description of insertion of membrane proteins: transport proteins can be delivered to cell surface membrane when vesicles carrying transport proteins fuse with cell surface membrane;
13. Ref to named example – allow for regulation of transport e.g. increase in glucose transporter (GLUT) protein increase more glucose intake;
14. Hydrophilic molecules that normally are repelled by hydrophobic core can diffuse down concentration gradient via ***channel protein**** due to presence of a ***hydrophilic pore/channel*;***
15. Description of ***phagocytosis****: allows for formation of ***pseudopodia**** to engulf particle to be taken in;
16. Description of ***endocytosis****; allows for ***invagination**** of membrane for molecules to be taken in.
17. Endo / Phago: pinching off the cell surface membrane to form vesicle

Exo: pinching off from RER / SER Golgi apparatus toward cell surface membrane (any 1 process ref to pinching off)

QWC: 2 out of 3 for pts 4, 7 and 10

[Total: 25]

End of Paper