2015 'A' Level
H2 Biology
Mark Scheme

PAPER 1 (MCQ)

Question Number	Key	Question Number	Key
1	C	21	A
2	D	22	C
3	D	23	A
4	A	24	C
5	C	25	D
6	A	26	C
7	B	27	B
8	B	28	A
9	B	29	B
10	C	30	C
11	D	31	C
12	A	32	D
13	D	33	B
14	A	34	C
15	A	35	B
16	D	36	A
17	D	37	C
18	D	38	A
19	A	39	B
20	B	40	B

PAPER 2 (CORE)

QUESTION 1

Fig.1.1 shows a diagram of a hepatocyte, which is a type of cell found in the liver.

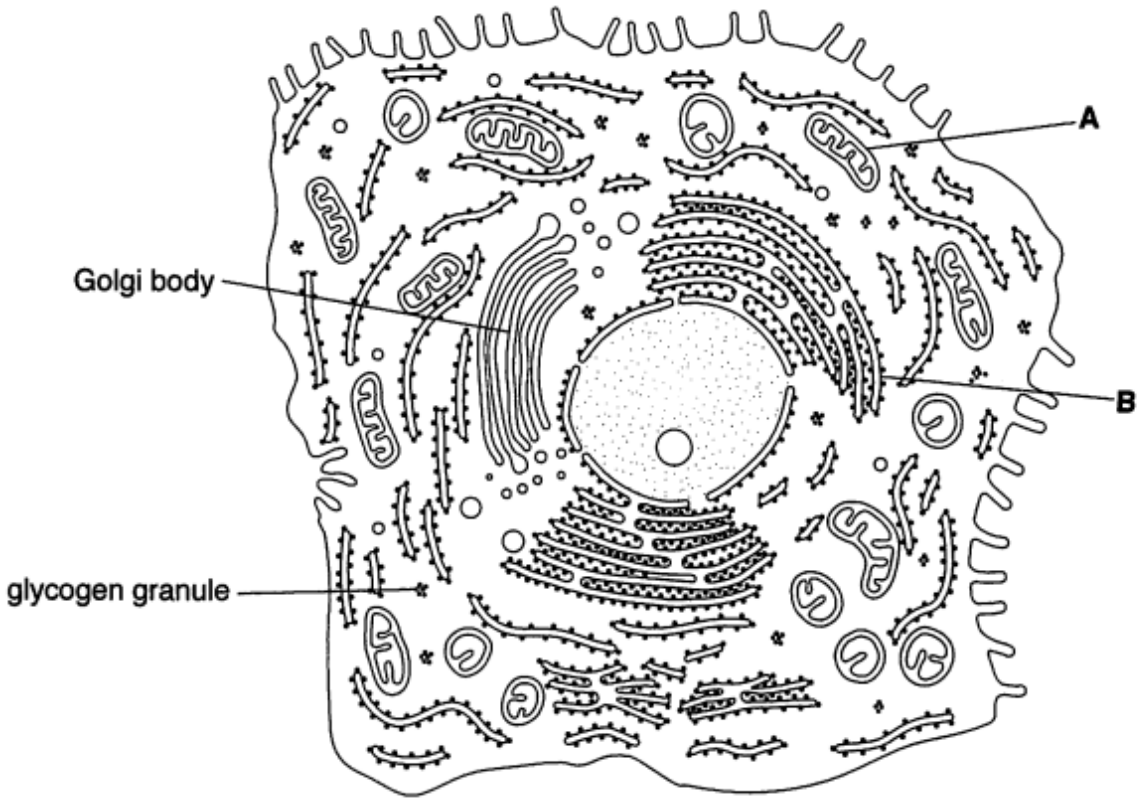


Fig. 1.1

(a) Identify the structures labelled **A** and **B**, as shown in Fig. 1.1, **[6]**

For each structure, state **two** features that can be seen in Fig. 1.1.

structure **A:** mitochondrion

feature 1 : double membrane

feature 2 : cristae

structure **B** : rough endoplasmic reticulum

feature 1 : cisternae / flattened membrane-bound sacs

feature 2 : bound ribosomes

Examiner's comment: The question stated that features of the identified structures should be visible in Fig.1.1. A few candidates mentioned features that could not be seen, e.g. the presence of DNA. A very small number of candidates confused the cristae of the mitochondria with the cisternae of the rough endoplasmic reticulum.

(b) Describe **two** functions of the Golgi body. **[2]**

1 **Further** post-translational modifications of proteins and lipids received from the ER (rER and sER) e.g. formation of glycoprotein, glycolipid ;
2 Sorting and packaging of proteins before being targeted to various cellular locations ;
3 Synthesis and transport of lipids ;
4 Formation of secretory vesicles (containing enzymes or proteins for release outside cell) or lysosomes ;
5 Involved in carbohydrates synthesis for the **formation of cell walls in plants** *(Ref: 9648/P1/2010)*

Examiner's comment: Candidates were able to describe a wide range of valid functions of the Golgi body.

(c) Suggest **two** advantages to eukaryotic cells of having membrane-bound organelles. **[2]**

1 Provides a boundary between the cytoplasm and specific contents within the membrane-bound organelles so that separate compartments can be formed within a cell ;
2 Increase surface area for reaction to occur ; E.g. Folding of inner mitochondrial membrane results in a greater surface area for attachment of more proteins and enzymes of the electron transport chain and ATP synthase for higher rates of ATP synthesis.

Examiner's comment: Many candidates considered compartmentalisation to be an advantage of having membrane-bound organelle. Since compartmentalisation is only another way of describing this feature, responses citing compartmentalisation required further qualification and relevant details.

(d) Explain the role of glycogen in animal cells. **[2]**

1 Stored in large quantities without having any great effect on the water potential of cells and can be prevented from diffusing out of cells ;
2 Stored in large quantities within the limited space in animal cells ;
3 Ease of breakdown allow glycogen to be a good energy source for respiration and to raise glucose levels quickly when blood sugar level drops in animals.

Examiner's comment: Candidates were largely aware of the role of glycogen in animal cells.

[Total: 12]

QUESTION 2

Fig.2.1 shows a diagram of DNA replication.

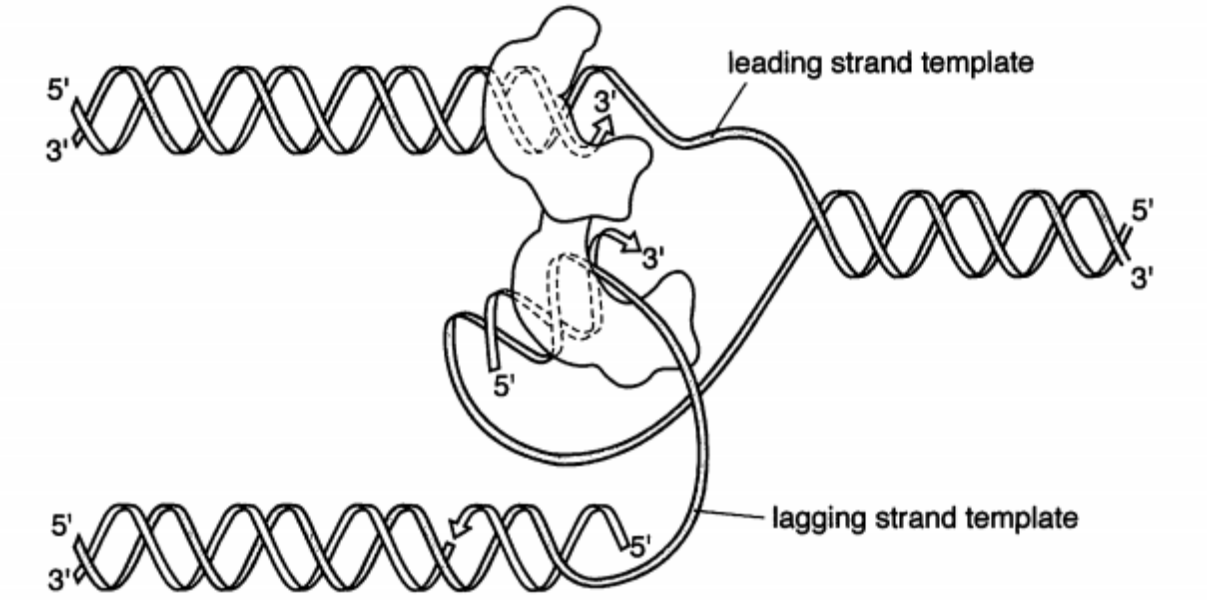


Fig. 2.1

(a) Describe how replication of the lagging strand template occurs. **[3]**

(Compulsory point)

1 After the DNA unzip at the origin of replication, RNA primers with bases complementary to bases on DNA template strand, were synthesised by DNA primase ;

(any two points ; point 2 to 7)

2 DNA polymerase III catalyses addition of free deoxyribonucleoside triphosphates to the free 3'OH end of each RNA primer ;

3 Complementary base pairing occur between newly added nucleotides and those on lagging strand template ;

4 DNA polymerase III catalyses formation of phosphodiester bonds between adjacent deoxyribonucleotides ;

5 DNA polymerase I hydrolyses the RNA primers ;

6 And fills the gaps with complementary deoxyribonucleoside triphosphates / deoxyribonucleotides ;

7 DNA ligase catalyses the formation of a phosphodiester bond between 2 Okazaki fragments.

Examiner's comment: Most candidates understood the significance of the antiparallel strands of DNA to replication of the lagging strand template and were able to provide full responses.

(b) State **two** ways in which DNA replication,

(i) differs from transcription, **[2]**

Any two:

	DNA Replication	Transcription
Template strands	**Both strands** of the **entire DNA double helix** are used as templates.	Only **specific regions** of **one strand** of the DNA double helix is used as a template.
Enzyme involved in polymerization reaction	**DNA polymerases** are used to add corresponding free deoxyribonucleoside triphosphates (complementary to those on the DNA parental strands) to the growing DNA chain	**RNA polymerase** is used to add corresponding free ribonucleoside triphosphates (complementary to those on the DNA template strand) to the growing RNA chain
Requirement for primers to initiate process	DNA polymerase III requires a (free 3'OH end on) **RNA primer** for the **initiation of replication**	RNA polymerase does **not** require a RNA primer for the initiation of replication
Nucleotides incorporated	**Thymine deoxyribonucleoside triphosphate** is used in addition to adenine, cytosine and guanine deoxyribonucleoside triphosphates.	**Uracil ribonucleoside triphosphate** is used in addition to adenine, cytosine and guanine ribonucleoside triphosphates.
End-product of process.	A **DNA** molecule is formed.	mRNA, tRNA and rRNA are formed.

Examiner's comment: Many candidates provided full and detailed answers that clearly stated how DNA replication

(ii) is similar to transcription, **[2]**

1. Both processes involve complementary base-pairing of nucleotides with a DNA template ;
2. Polymerases were used to catalyse formation of phosphodiester bonds between nucleotides on elongating strand ;
3. Both occur in nucleus.
4. Both involve the unwinding and unzipping of DNA molecule.

Examiner's comment: Most candidates were able to state two similarities between DNA replication and transcription.

(c) The symbols below represent the main components of RNA.

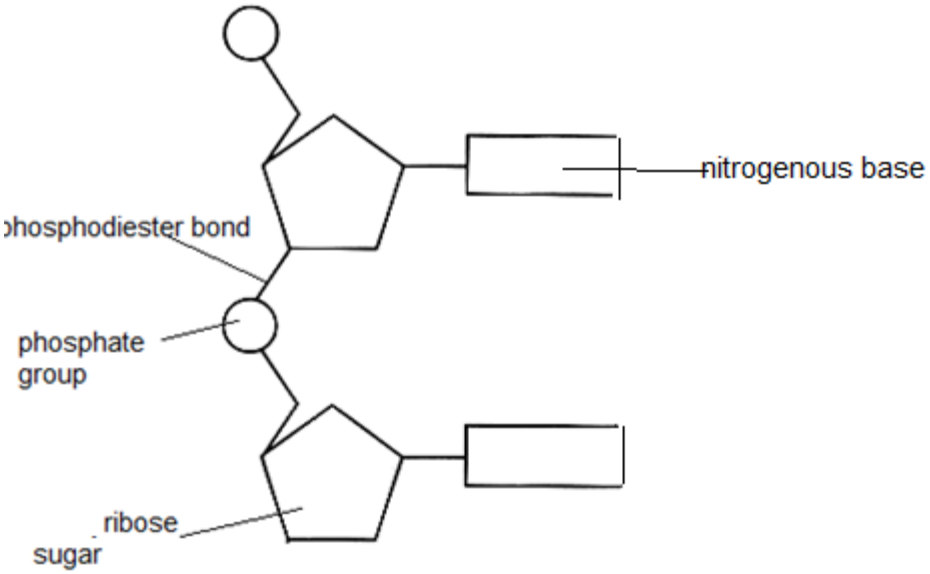
In the space below, draw a short section of mRNA that is made up of **two** nucleotides, using these symbols to represent the main components. Add lines to show the position of any bonds between the components.

Label and name the components and the covalent bond that links the nucleotides.

labels: phosphate group
phosphodiester bond (must have 2 label lines drawn)
ribose sugar
nitrogenous base

Correct drawing of 2 nucleotides: 1m
4 correct labels : 2m
2 correct labels : 1m

[3]



Examiner's comment: Candidates found this a challenging question. Although candidates were instructed to draw a section of mRNA made up of two nucleotides, many included only one phosphate group. Some drew mRNA as a double-stranded molecule or labelled bases as nucleotides.

[Total: 10]

QUESTION 3

3 Fig. 3.1 shows a diagram of the *lac* operon, which is found in some types of bacteria.

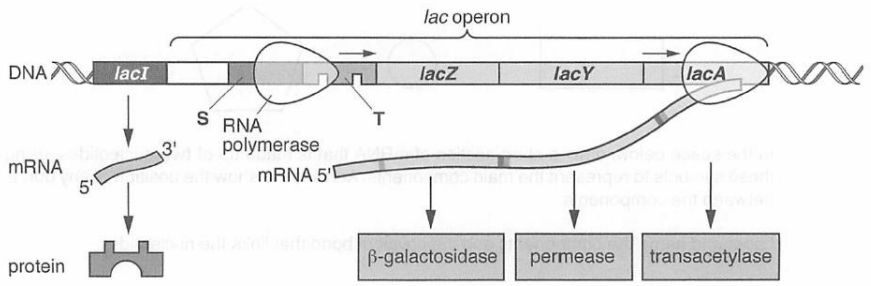


Fig. 3.1

(a)(i) Name the structures S and T, as shown in Fig. 3.1.

……[2]

S: promoter
T: operator

(ii) Identify a structural gene in Fig. 3.1 and explain what is meant by the term, structural gene.

……[1]

1 Structural gene is a region of DNA that codes for proteins that has an enzymatic function in lactose metabolism, e.g. ***lacZ/ lacY/ lacA***

(iii) Identify a regulatory gene in Fig. 3.1 and explain what is meant by the term, regulatory gene.

……[1]

1 Regulatory gene, e.g. **LacI gene**, encodes for a specific protein product, ***lac* repressor**, which regulates expression of the structural genes;

Examiner's comments:
Some candidates did not link the role of a regulatory gene to the production of a protein or polypeptide.

(b) Using Fig. 3.1, describe how the presence of lactose induces a bacterium to use lactose as a respiratory substrate.

……[3]

1 Lactose enters via the qualified leaky membrane and isomerizes to form allolactose, an <u>inducer</u> ;
2 allolactose binds to the <u>allosteric site</u> of the lac repressor ;
alters its 3D conformation (at the DNA-binding site) ;
3 lac repressor is <u>inactive</u> and is no longer able to <u>recognise and bind</u> to the <u>operator</u> (lacO) ;
4 RNA polymerase can access and transcribe the **structural genes**, acZ , lacY and lacA ; β-galactosidase, lac permease and lactose transacetylase are synthesised

[Total: 7]

QUESTION 4
QUESTION 4

Fig. 4.1 shows the development of a metastatic cancer in the colon over a period of ten or more years. Metastatic is a term used to describe cancer that is spreading from one organ to another. *APC*, *ras* and *p53* are tumour suppressor genes. *(Erratum notice by SEAB – The third sentence should read: APC and p53 are tumour suppressor genes. ras is a proto-oncogene.)*

(a) State two environmental causes of cancer. [2]

1 Chemical carcinogens e.g. tar in cigarette smoke causing lung cancer ;
2 Ionizing radiation e.g. UV light causing skin cancer ;
3 Both causes mutations that lead to cancer.

(b) With reference to Fig. 4.1, explain why cancer development is a multi-step process. [3]

1 accumulation of many mutations, 4 tumour suppressor genes mutation, 1 proto-oncogene mutation, chromosomal mutations and other events, **over time** before metastatic cancer resulted ;
2 mutant alleles of tumour suppressor genes, *APC* and *p53*, are usually recessive and would require two loss of function mutations of normal alleles resulting in loss of arrest of cell division, loss of ability for DNA repair and loss of apoptosis ;
3 mutant alleles of proto-oncogenes, *ras*, behave as dominant alleles and would only need one gain of function mutation of normal alleles resulting in stimulation of the cell cycle / cell keeps dividing ;
4 subsequent chromosomal mutations and other events such as angiogenesis where there is formation of new network of blood vessels to the cancer cells causes cells to lose cell adhesion, to break loose and enter the bloodstream to spread cancer cells to other tissues ;

Examiner's report:
(b) Candidates did not always make full use of the information provided in Fig. 4.1.

(c) Describe how dysregulation of the checkpoints of cell division may lead to cancer. [2]

1 Cell cycle checkpoints control / regulate the cell cycle progression / cell division ;
2 Dysregulation of
G1 checkpoint may result in DNA replication of damaged / defective DNA ;
G2 checkpoint may result in cell cycle progression despite DNA damage during DNA replication / cells escaping apoptosis even if there is irreparable damage ;
M checkpoint may result in cell cycle progression even if there is non-disjunction ;
3 With accumulation of mutations over time, eventually there is uncontrolled cell division as the rate of cell division is greater than that of cell death, leading to cancer ;

Examiner's report:
(c) Many candidates did not refer to the cell cycle in the context of dysregulation of the checkpoints of cell division and many did not link cancer to uncontrolled cell division.

(d) Outline the role of tumour suppressor genes in the development of cancer. [3]

1 Tumour suppressor genes are **genes** whose normal **protein products** inhibit cell division when there is DNA damage to allow more time for the cell to repair its DNA or causes apoptosis if DNA is beyond repair to help prevent **inappropriate cell cycle progression** ;
2 In cancer development, both alleles of a tumour suppressor gene undergo a loss of function mutation ;
3 Mutant alleles code for **abnormal / non-functional** tumour suppressor **protein** or whose gene products are under-expressed resulting in loss of arrest of cell division, loss of ability for DNA repair and loss of apoptosis ;

QUESTION 5

(a) Using the symbols for the alleles stated above, draw a genetic diagram to show the expected phenotypic ratios for the offspring of the test crosses if inheritance is Mendalian. **[4]**

F_1 phenotypes:	Black coat, Straight hair	x	White coat, Wavy hair
F_1 genotype:	BbHh		bbhh
F_1 gametes	(BH) (Bh) (bH) (bh)		(bh)

F_2 genotypes:
Punnett square:

	BH	Bh	bH	bh
bh	BbHh (Black coat, Straight hair)	Bbhh (Black coat, Wavy hair)	bbHh (White coat, Straight hair)	bbhh (White coat, Wavy hair)

F_2 / Progeny phenotypes:	Black coat, Straight hair	Black coat, Wavy hair	White coat, Straight hair	White coat, Wavy hair
F_2 / Progeny phenotypic ratio:	1	1	1	1

Mark scheme:
1 F_1 phenotype and genotypes
2 Parental gametes – (Gametes **must** be circled)
3 F_2 genotypes correspond to phenotypes
4 Expected F_2 phenotypic ratio

Examiner's comment: Some candidates tried to derive the outcomes shown in the table from a cross between two heterozygotes, rather than the described test cross. Others did not go on to show the expected phenotypic ratio.

(b) Explain why there is a greater number than expected of the parental phenotypes. **[3]**

1 The genes for coat colour and hair shape are located on the same chromosome; they are linked genes ; no independent assortment of genes ;
2 Crossing over ***occasionally*** breaks the linkage between the 2 genes on the same chromosome / ***probability*** of crossing over between the 2 genes is ***low***; forming recombinants;
3 Hence, there is a greater proportion of **gametes** with the parental combination of alleles than recombinants; when ***fertilisation occurs***, this would lead to a greater number of parental phenotypes **compared with** unlinked genes which would produce the parental phenotypes in relatively equal proportions.

Examiner's comment: Most candidates were aware of the involvement of linkage but many did not go on to explain how this results in a greater than expected number of the parental phenotypes.

(c) Describe how it is possible for progeny with black coats and wavy hair to be produced from these test crosses. **[3]**

3 Crossing-over during prophase I of meiosis occurred between the 2 genes in the **gamete-producing cells of the parents with BbHh genotype**;

4 Leading to the **exchange** of alleles of the same gene locus between non-sister chromatids and results in the formation of **new combination of alleles** (Bh) in the gametes;

5 **During fertilization**, when these gametes **fuse** with the gametes from the homozygous recessive parent, offspring with genotype Bbhh (black coat and wavy hair) were formed.

Examiner's comment: Most candidates were able to partially address this question, but fewer developed complete explanations for the occurrence of the non-parental phenotypes.

[Total:10]

QUESTION 6

Fig. 6.1 shows a diagram of insulin binding to an insulin receptor on the surface of a cell.

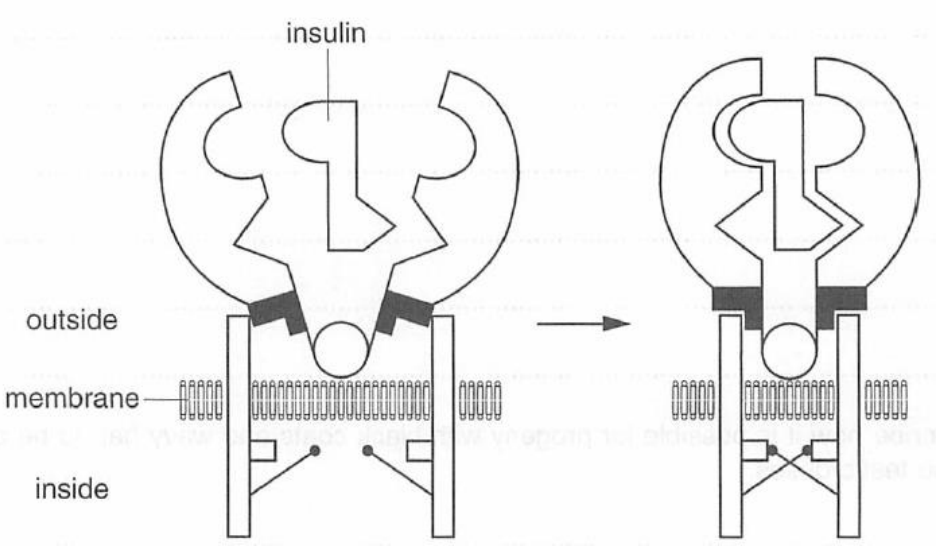


Fig. 6.1

(a) Receptors for some hormones are found within their target cells.

Explain why insulin receptors are found on the cell surface membranes of target cells and never within the cells. **[2]**

1 Its ligand, insulin, is a protein and is too large to diffuse through the plasma membrane
2 Hence it has to bind to receptors found on the cell surface membrane.

Examiner's comments:
The key consideration in locating the receptors is whether or not insulin can cross the cell surface membrane.

(b) Use Fig. 6.1 to explain how the presence of insulin is able to trigger a response inside the target cell. **[3]**

1 Insulin is complementary in shape to the ligand-binding site of the insulin receptor.
2 Binding of insulin to insulin receptor causes a change in 3D conformation of the receptor and activates the tyrosine kinase domains in the cytoplasmic tail.
3 Each receptor phosphorylates the tyrosine residues at the cytoplasmic tails of the other receptor via the addition of a phosphate from an ATP molecule in a process known as auto-crossphosphorylation.

Examiner's comments:
A number of candidates did not comment on the specificity of the binding between insulin and its receptor and the resulting conformational change, suggesting they had not made use of the information provided in Fig. 6.1.

Fig. 6.2 summarises the main effects of insulin that can occur in different target cells.

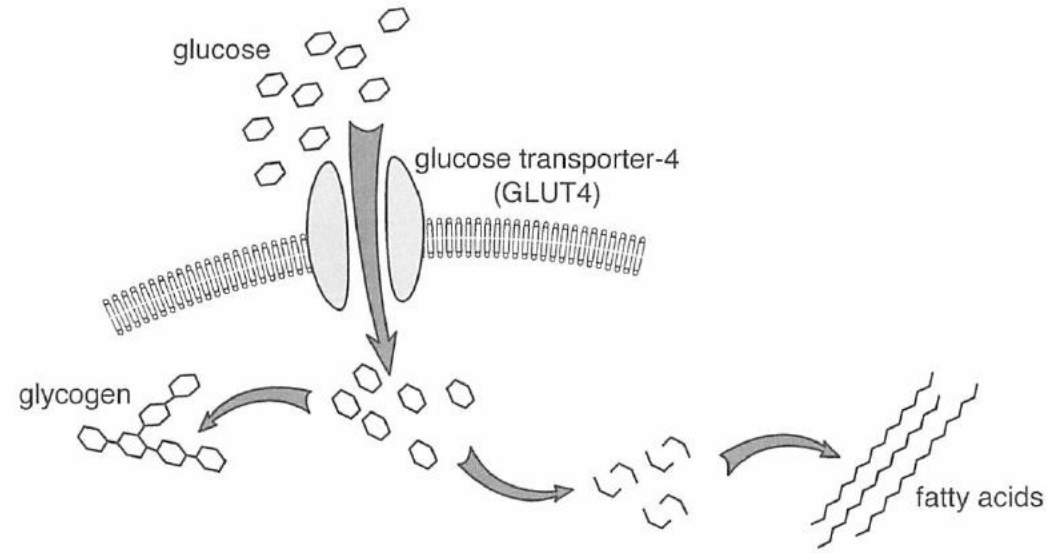


Fig. 6.2

(c) Using Fig. 6.2, describe the main effects of insulin on different target cells. **[6]**

1 Insulin increases the number of glucose transporter-4 on the cell surface membrane of target cells.
2 Insulin increases the uptake of glucose through glucose transporter-4 into target cells.
3 Insulin increases conversion of glucose to glycogen in liver for storage
4 Insulin the rate of glucose oxidation.
5 Insulin increases conversion of excess glucose to fat
6 Insulin results in the lowering of blood glucose level back to the normal level.

Examiner's comments:
Many candidates gave detailed answers prompted by consideration of Fig. 6.2.

[Total: 11]

QUESTION 7

(a) State what is meant by the term biological species. **[2]**

1 A species refers to a group of closely related organisms which are capable of interbreeding in nature to produce viable, fertile offspring.
2 Members of one species usually cannot produce fertile offspring with members of another species.

Examiner's comments:
A small number of candidates did not refer to the population of fertile offspring from breeding between members of the same species.

(b) Explain how new species arise. **[5]**

1 geographical isolation due to physical barrier
/ physiological isolation when female and male sex organs are not compatible
/ behavioural isolation when little or no sexual attraction between females and males of different species
/ temporal isolation when species breed at different seasons or times of the day.
2 leads to reproductive isolation;
leads to disruption to gene flow in the ancestral population;
/ no interbreeding between the organisms (name organisms)

3 processes of natural selection and genetic drift occur;

4 Genetic variations exist in a population due to mutation.
5 Different ecological niches/environments exert different selection pressure;
Individuals at a selective advantage will survive to reproduce, passing down their alleles to the offspring;
6 Over many generations, a change in allele frequencies will occur;
Accumulation of genetic differences between the two subpopulations over time will cause them to ultimately become unable to interbreed to produce viable, fertile offspring;
7 New species are formed via allopatric speciation or sympatric speciation

Examiner's comments:
Most candidates demonstrated thorough knowledge and understanding of speciation. Some accounts lack balance, especially where candidates concentrated on describing as many isolating mechanisms as possible without considering other relevant aspects. Some referred in general to passing on of genes from one generation to the next without considering the influence of the relative benefit of different alleles on this process.

Fig. 7.1 shows the phylogenetic tree of three species of closely related butterflyfish based on nucleotide sequences, with ages estimated from fossil and biogeographical data.

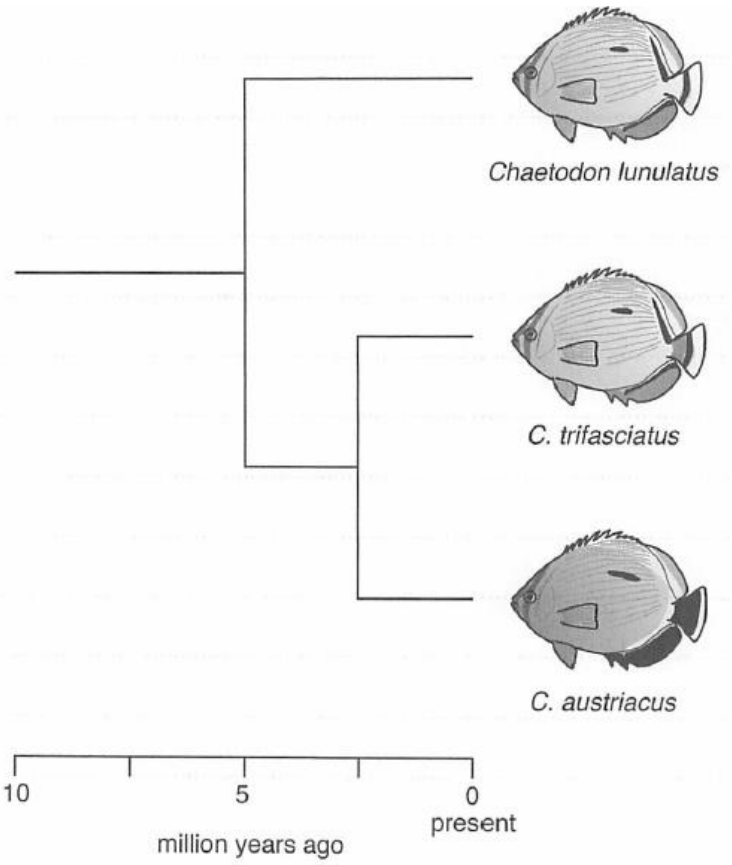


Fig. 7.1

(c) Describe the advantages of using nucleotide sequences in reconstructing phylogenetic relationships. **[3]**

1 Molecular data is quantifiable and open to statistical analysis;
Large quantities of data are required for statistical analysis; however there is little morphological data available

2 Molecular data is unambiguous and objective while morphological data may differ depending on the way in which it was classified.

3 Molecular data is not affected by convergent evolution while morphological data can be affected by convergent evolution.

4 Molecular data provides a clear model of evolution by comparing the nucleotide and amino acid sequence as the rate of molecular change in genes and proteins is regular like a molecular clock. Morphological evidence could be due to convergent evolution as similar morphology may not have been inherited from common ancestor.

Examiner's comments:

Most candidates understood the advantages of using nucleotide sequences in reconstructing phylogenies and many provided detailed responses. Some candidates did not consider time or the 'molecular clock'.

Fig. 7.2 shows the current distribution of these three species.

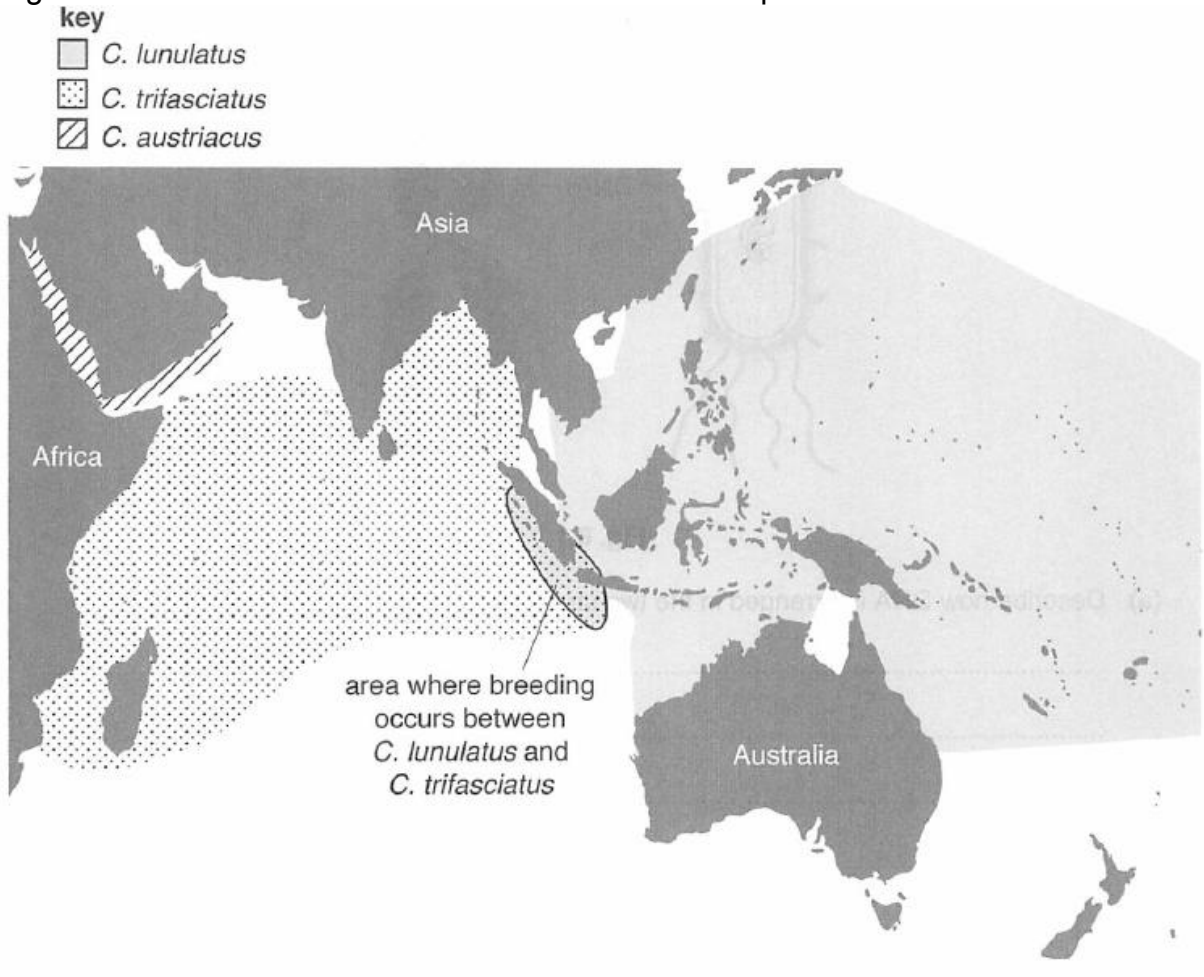


Fig. 7.2

(d) Suggest, with reference to Fig. 7.1 and Fig. 7.2, why breeding between *C. lunulatus* and *C. trifasciatus* is possible. **[3]**

1 The two species diverged from a common ancestor just 5 million years ago; hence they may share many homologous characteristics which make interbreeding possible.

2 From Fig. 7.1, the two species are highly similar in morphology (e.g. fin shape and colour), hence there may be an absence of behavioural and physiological barriers between them.

3 From Fig. 7.2, the two species overlap in their geographical distributions in the waters around Indonesia, hence there is no geographical barriers to prevent gene flow between the two species.

Examiner's comments:
Candidates who had read the question carefully and made full use of all the information supplied in both Fig. 7.1 and Fig. 7.2 were able to provide detailed responses.

[Total: 13]

QUESTION 8

(a) Describe how DNA is arranged in the two structures. [4]

Bacteria DNA

1 Circular DNA located in the nucleoid region
2 Highly folded and coiled, associated with DNA-binding proteins

Metaphase chromosome

3 DNA wound around histones to form nucleosomes; interactions between nucleosomes coil to form a chromatin fibre;
4 Chromatin fibre attach to non-histone protein scaffold to form looped domains; **further** packing to form the metaphase chromosome with two DNA molecules of sister chromatids held together at centromere;

(b) State two ways in which the organisation of genes found in these two structures differ and suggest one advantage of this to the bacterium. [3]

Two differences

1 For bacteria DNA, genes that code for products with related functions are grouped into an operon and are located adjacent to each other;
for eukaryotic DNA of the metaphase chromosome, genes exist as discrete regions along the chromosome with non-coding DNA between them;
2 There is only one copy genes in the bacterial chromosome, while there are two copies of genes identical to each other along the chromosome;

One advantage

3 Allows bacterium to adapt rapidly to changes in the environment as it can quickly switch on or off transcription of the group of genes involved in same metabolic pathway as the genes in each operon are under the control of the same promoter.

[Total:7]

QUESTION 9

(a) Describe the structure of a homologous pair of chromosomes at the start of meiosis. [6]

1 DNA replication occurs during S phase of interphase before the start of meiosis resulting in two DNA molecules in one homologous chromosome ;
2 Homologous chromosomes each exist as a duplicated chromosome which consists of 2 chromatids held together by the centromere ;
3 One DNA molecule in one chromatid ;
4 A homologous pair of chromosomes is also known as a bivalent which can be seen to be joined at several points called chiasmata along the length in prophase I of meiosis.
5 A homologous pair of chromosomes have the same length, same centromere position, same gene loci and same staining pattern in a karyogram.
6 Homologous chromosomes may have slightly different DNA sequences due to alleles at corresponding gene loci.

Examiner's report:
Good responses were provided by many candidates, referring to relevant features such as chromatids, centromeres, bivalents and gene loci. Some candidates discussed DNA organisation rather than the structure of a pair of homologous chromosomes.

(b) Outline the behaviour of chromosomes during meiosis. [8]

1 In prophase I of meiosis, chromatin shorten and thicken by coiling to form chromosome structure called duplicated chromosome (also known as double structure) and tighter packaging of their components;
2 Homologous chromosomes (homologs) pair up along their lengths, aligned gene by gene precisely for synapsis to occur ;
3 Kinetochore microtubules attach to the centromeres of the two homologs ;
The homologous pairs then move toward the metaphase plate pulled by kinetochore microtubules during metaphase I of meiosis;
4 In anaphase I of meiosis, homologous chromosomes separate and move towards the opposite poles, guided by the shortening of the kinetochore microtubules ;
5 Homologous chromosomes reach opposite poles of the cell in telophase I of meiosis followed by cytokinesis ;
6 Moving from prophase II to metaphase II, duplicated chromosomes arrange themselves at the equator of the new spindle ;
7 Centromeres of each duplicated chromosome divide ;
Non-genetically identical chromatids of each duplicated chromosome separate to become two individual chromosomes and move toward opposite poles during anaphase II of meiosis;
8 Chromosomes reach opposite poles of the cell and uncoil, lengthen to form chromatin in telophase II, followed by nuclear envelope re-form around the chromosomes and cytokinesis forming four daughter haploid cells that are genetically different from parent cells due to crossing over in prophase I.

(c) Explain the role of the nuclear envelope and centrioles during meiosis. [6]

1 Nuclear envelope starts to fragment and disappear during prophase I of meiosis to allow spindle fibres to attach to the duplicated chromosomes and the free movement of duplicated chromosomes to the metaphase plate ;
2 Nuclear envelope re-forms around the chromosomes at each pole in telophase I and II to prevent entanglement of the de-condensing chromosomes and prepares the cell for cytokinesis.
3 Centrioles move to opposite poles of cell to determine polarity of cells during prophase of meiosis ;
4 Centrioles **organizes the synthesis and assembly of spindle fibre** for example the kinetochore microtubules attached to the kinetochores on duplicated chromosomes ;
5 Shortening of Kinetochore microtubules ensure proper separation of homologous chromosomes during meiosis I and chromatids during meiosis II preventing chromosomal aberration due to non-disjunction events ;
6 The cell elongates as the polar microtubules and astral microtubules from the centrioles lengthen and comes into contact with the plasma membrane, respectively ;

QUESTION 10 (TNG)

10 **(a)** Describe the role of NAD and FAD in cellular respiration. [6]

(b) Outline the main stages of the Krebs cycle. [8]

(c) Explain how ATP is produced in anaerobic respiration. [6]

[Total: 20]

(a) Describe the role of NAD and FAD in cellular respiration. [6]

1 NAD and FAD act as coenzymes
2 used in glycolysis, link reaction and Krebs cycle (as hydrogen/electron acceptor)

3 Reduced NAD and reduced FAD act as electron donor during <u>oxidative phosphorylation</u> (Accept: NADH, $FADH_2$)
4 electrons are transferred from reduced NAD and reduced FAD to oxygen
5 via a series of electron carriers with increasing electronegativity and in order of decreasing energy levels
6 energy released during the transfer of electrons used to pump H^+ ions from the mitochondrial matrix into the intermembrane space / generate proton gradient
7 potential energy released as H^+ ions diffuses from intermembrane space to matrix through ATP synthase
8 synthesis of ATP from ADP and Pi;

(b) Outline the main stages of Krebs cycle. [8]

1 Acetyl CoA and oxaloacetate undergo a <u>condensation</u> reaction to form citrate
2 Citrate is <u>isomerised</u> to isocitrate
3 Isocitrate undergoes <u>oxidative decarboxylation</u> form α-ketoglutarate
4 α-ketoglutarate undergoes <u>oxidative decarboxylation</u> to form succinyl CoA
5 CO_2 is released and NADH is produced

6 ATP is formed by <u>substrate level phosphorylation</u>
7 Reduced NADH and $FADH_2$ are produced via <u>dehydrogenation</u> during the intermediate steps
8 <u>Oxaloacetate</u> is regenerated to receive more acetyl groups

(c) Explain how ATP is produced in anaerobic respiration. [6]

1 Under anaerobic conditions, oxygen is no longer available as the final electron acceptor
2 No electron transport down the ETC and Kreb's cycle occur as NADH and FADH2 remains reduced
3 Cells undergo fermentation in the cytosol
4 In animals, pyruvate to lactate and NAD^+ is regenerated
5 In plants/yeast, pyruvate is decarboxylated to ethanal with the release of CO_2. Ethanal is reduced by NADH to ethanol and NAD^+ is regenerated
6 NAD^+ is used in glycolysis to continuously generate ATP via substrate level phosphorylation
7 Net formation of 2 molecules of ATP per molecule of glucose oxidized during glycolysis

Examiner's comments:
Not all candidates recognised that the electron transport chain and Krebs cycle do not occur in anaerobic respiration. Many did not give complete accounts of alcoholic fermentation.

PAPER 3 (APPLICATIONS)

QUESTION 1

The polymerase chain reaction (PCR) is a three-stage process.

(a)(i) Describe what occurs in the first stage at 95˚C [2]

1 DNA denatures and the hydrogen bonds between complementary base pairs are broken;
2 The double-stranded DNA separates into single strands;

(a)(ii) the second stage. [2]

1 The mixture is then cooled to 55°C to enable the annealing of forward and reverse DNA primers to the single DNA strands
2 The primers bind to complementary sequences flanking the target sequence to be amplified at the 3' ends of single DNA strands

(b)
(i) Suggest and explain why Taq DNA polymerase is now obtained from genetically modified E.coli. **[2]**

1 Large quantities of Taq DNA polymerase can be cloned;
2 This meets the demand for its use in PCR as it is unlikely the naturally-occurring polymerase will be enough to meet the demand; also, harvesting large amounts of the Thermus aquaticus bacteria may disrupt their natural ecosystems;

(ii) With reference to your knowledge of PCR, explain why a half-life of 40minutes at 95˚C allows many cycles of PCR before the enzymes need to be replaced. **[3]**

1 A single cycle of PCR was short; first stage at 95˚C is about 1 minute;
2 the cycle repeats about 25-30 times, the total time the enzyme spends at 95˚C is about 30 minutes which can occur within a single half-life of the enzyme;
3 hence all the cycle would have been completed even before the enzyme activity drops before 50%;

Examiner's comments:
Candidates who focused on why Taq polymerase is thermally stable and speculated on how often it needs replacing were not addressing the question. This was also true for candidates who repeated information given in the stem concerning the meaning of the half-life of an enzyme.

(c) Explain how RFLP analysis of DNA samples from imported grain and non-GM grain can show whether or not the imported grain has been genetically modified.

[6]

1 The difference in the DNA nucleotide sequence of **GM and non-GM** grain would result in the differences in the restriction sites on homologous chromosomes;
2 This results in differences in restriction fragment lengths at the RFLP loci when the genomic DNA is digested with the same restriction enzyme, giving rise to unique RFLP patterns;

3 **Genomic DNA** samples from the **imported grain and non-GM** grain are digested by the same restriction enzyme; gel electrophoresis separate the different restriction fragments based on fragment length;
4 The fragments are then transferred to nitrocellulose membrane; a radioactive probe complementary to the RFLP loci will hybridise; X-ray autoradiography is then performed to visualise the pattern of bands, which is the DNA fingerprint for that DNA sample;

5 The DNA fingerprint of the imported grain should be **compared** with those of the non-GM grains; if it is not genetically modified, there should be many bands in common with the non-GM grains;
6 However, if the DNA fingerprint of the imported grain has many bands that are not found in the non-GM grain which shows their genomes differ significantly, the imported grain may have been genetically modified.

Examiner's comments:
A small minority did not mention that the samples would need to be cut by the same restriction enzyme and a very few candidates mismatched the use of UV light with autoradiography.

[Total:15]

QUESTION 2

The plant *Ajuga reptans*, variety 'Burgundy Glow' is grown in many gardens in Europe and North America. It can be grown by plant tissue culture in order to reduce the problems associated with more traditional techniques of increasing plant numbers.

(a) Outline **three** different problems associated with traditional techniques of increasing plant numbers.

……..[3]

1 new plants are not **disease-free**, leading to lower **yield** / **quality**

2 **genetic modifications are not** possible, cannot introduce **desirable traits** to plants

3 Take up **more space** and cannot be **grown intensively**

4 **Dependent on climate** changes so plants cannot be produced continuously/at any time of year, **lack of flexibility** in meeting consumer demand

5 Tough to **standardise** the conditions for growth and obtain many batches of identical plants to ensure product **uniformity**

[Any 3]

Experiments were carried out in order to determine which synthetic hormones (plant growth regulators) would result in the best yield of new plants through tissue culture.

The explants were taken from growing tips of the plant and after sterilisation were placed in Murashige-Skoog (M-S) medium with two different hormone treatments, X and Y, as shown in the Table 2.1.

After 14 weeks, the total number of new shoots on each explant was counted and these shoots were then classified into one of the three grades, 1, 2, and 3.

Grade 1		grade 3
Small shoots	----------→	well-developed shoots
No roots		many long roots

Table 2.1 shows the results.

Table 2.1

treatment	mean number of new shoots per explant ± standard deviation (*s*)	percentage of new shoots at each grade ± *s*		
		grade 1	grade 2	grade 3
X	46.7 ± 18.4	71.2 ± 10.6	25.6 ± 9.1	4.2 ± 3.1
Y	13.7 ± 12.6	29 ± 21.4	30.5 ± 12.6	39.7 ± 25.3

(b)(i) With reference to the mean number of new shoots per explant produced under treatment Y (13.7 +/- 12.6), explain what the standard deviation figures indicate about this result.

……[2]

1 Standard deviation is the deviation from the mean number of new shoots per explant and determines the range of the number of new shoots per explants observed, in Treatment Y, the mean number of shoots per explant ranges from 1.1 (13.7 – 12.6) to 26.3 (13.7 + 12.6)
2 An indication of the reproducibility of the data collected. Since standard deviation for Y is high, results are not very reproducible

Examiner's comments:
Many candidates simply quoted figures for standard deviation, rather than explaining and interpreting them.

(ii) Describe the patterns shown by the data in Table 2.1.

……[5]

1 Treatment X generates more shoots per explant compared to Treatment Y, mean number of shoots per explant is higher in treatment X, 46.7+/-18.4, than in treatment Y, 13.7+/-12.6
2 Treatment X generates less developed shoots. Majority of new shoots under Treatment X falls under grade 1 (with small shoots and no roots), with progressively decreasing percentages of grade 2 and 3 shoots.
3 ref. quoting of data for all 3 grades
4 Treatment Y generates more developed shoots. Majority of new shoots under Treatment Y falls under grade 3 (with well-developed shoots and many long roots), with relatively similar proportions for grades 1 and 2
5 ref. quoting of data for all 3 grades
6 There is greater variation in the number of shoots produced with treatment Y compared to treatment X, ref. quote standard deviations

Examiner's comments:

This question required clear statements about the patterns shown by the data in the table. Most candidates were able to identify some of the patterns, but a proportion quoted figures without attempting to describe any underlying patterns.

All of the new shoots in the three different grades were collected and planted out in soil in order to grow into plants that can be sold.

(c) With reference to the information provided, suggest why the explants that had undergone treatment Y subsequently grew into larger and better quality plants than those that had undergone treatment X.

……..[3]

1 There are more grade 2 and 3 shoots from Treatment Y compared to Treatment X
2 Ref. data for both treatments (e.g. 70.2% for grades 2 and 3 in total for treatment Y, 29.8% for grades 2 and 3 in total for treatment X)
3 Grade 2 and 3 shoots are better developed (more roots and shoots), resultant plantlets are able to photosynthesize more/at faster rate.
4 More roots means better/more efficient absorption of water and mineral salts from the soil, resulting in better growth.

Examiner's comments:
This question required candidates to link differences in the results between treatment X and treatment Y with possible reasons for the improved growth and quality of plants undergoing treatment Y. Valid suggestions provided an underlying rationale for why a particular difference could lead to a larger and better quality plant.
Many candidates repeated information from (b)(ii) or simply restated the data given.

(e) It was noticed that a small number of plants produced from both treatment groups displayed different phenotypes, other than size and quality, from the original *Ajuga reptans*, despite being grown under the same conditions.

Suggest what may have caused these different phenotypes.

……..[2]

1 Random mutations could have occurred in the cells e.g. substitution of a base, which leads to different mRNA sequence and amino acid sequence
2 Different R group interactions between amino acids lead to different tertiary/3D configuration of proteins, leading to different/new phenotypes in resultant plants after rounds of cell division and differentiation.

Examiner's comments:

Most candidates recognised that mutation was involved in the emergence of these new phenotypes and many were able to elaborate their response to provide further details of the mechanism.

QUESTION 3

(a) Outline the formation of double stranded cDNA. **[3]**

1 Reverse transcriptase is first added to the mRNA to synthesize complementary DNA (cDNA).
2 RNAse is added to hydrolyse the mRNA template
3 DNA polymerase is added to synthesis the second DNA strand complementary to the cDNA template, producing a double stranded cDNA.

Examiner's comments:
Candidates displayed sound knowledge of the formation of cDNA.

(b) In order to create a cDNA library, recombinant DNA has to be produced using plasmids and cDNA.

Fig 3.1 shows cDNA and cleaved plasmids used in two different methods, **A** and **B**, for the production of recombinant DNA.

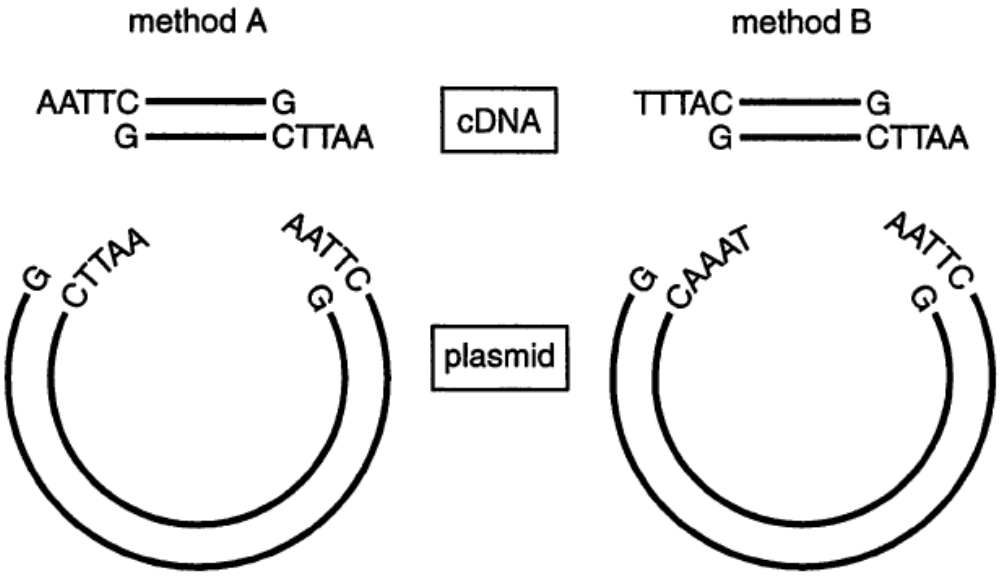


Fig. 3.1

With reference to Fig. 3.1, explain why method **B** will produce a better success rate of recombinant DNA formation, than method **A**. **[3]**

1 Method B produces restriction fragments with different types of sticky ends (TTTA and AATT) while method A produces restriction fragments with the same type of sticky ends on both ends (AATT).
2 In method B, when the cut cDNA and plasmids are mixed, the cDNA will be inserted only in one orientation into the plasmid while in method A, the cDNA can be inserted into the plasmid in two different orientations.
3 Hence, method B allows for a more specific recombinant DNA to be formed than method A.

Examiner's comments:

Candidates found this questions much more challenging. Many recognised that method B involved two different types of stick ends compared to a single type in method A. fewer went on to explain how this would affect the outcome of mixing the cDNA and plasmids.

(c) Using recombinant DNA technology, scientists introduced a gene coding for green fluorescent protein (extracted from a jellyfish) into the embryo of a zebrafish (Danio rerio). This caused the fish to fluoresce under both ultraviolet light and natural light.

By 2003, these genetically modified fish were being sold as pets in a number of countries, including the USA. In the USA, the fish had to be kept in heated aquariums.

The American Food and Drug Administration (FDA), which is responsible for regulating genetically modified organisms, stated:

> There is no evidence that these genetically engineered zebrafish pose any more threat to the environment than their unmodified counterparts which have long been widely sold in the United States. In the absence of a clear risk to the public health, the FDA finds no reason to regulate these particular fish.

(i) With reference to the information above, suggest why these genetically engineered zebrafish would **not** be a threat to the environment. **[2]**

4 The inserted gene does not confer any selective advantage or disadvantage to the zebrafish

5 Hence, even if the zebrafish is released to the environment, it will not upset the ecological balance of the ecosystem.

OR

6 The inserted gene codes for green fluorescent protein which is naturally found in jellyfish

7 Hence, there is no risk of releasing toxins into the environment even if the zebrafish is released into the environment.

Examiner's comments:
Many candidates were able to provide valid reasons to explain why the genetically engineered zebrafish would not be a threat to the environment.

Suggest why there is **unlikely** to be a health risk to people who keep these genetically engineered zebrafish. **[2]**

1 The zebrafish are kept as pets and not consumed as food.

2 Hence, there is no risk of ingesting secondary metabolites that are potentially toxic or allergenic to humans.

Examiner's comments:
Many candidates were able to make valid suggestions to explain why the genetically engineered zebrafish are unlikely to be a health risk to people.

[Total: 10]

QUESTION 4 (SPA PLANNING)

A business has been buying milk from the same supplier for a number of months. Recently, the business has found that the milk has been diluted with water.

How much water has been added can be determined by measuring the density of the milk.

The density of milk can be measured using a copper sulfate solution of standard concentration. When a small drop of milk is placed in copper sulfate solution in a measuring cylinder, a layer of copper proteinate forms around the milk and this prevents the milk and copper sulfate solution mixing. Since milk is denser than the standard copper sulfate solution, the drop of milk sinks to the bottom.

Fig. 4.1 shows the movement of the drop of milk through the copper sulfate solution.

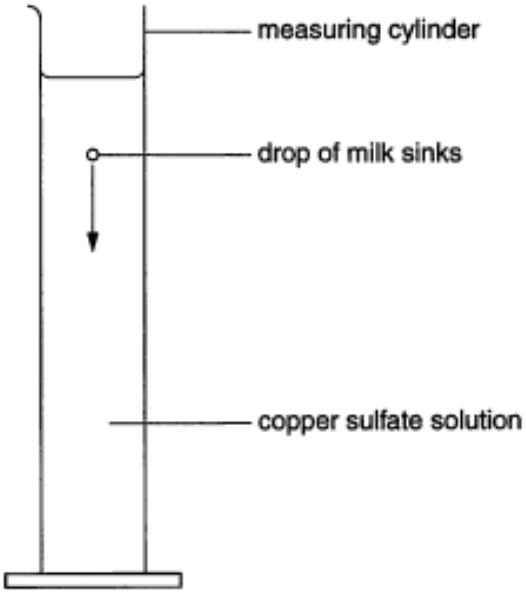


Fig. 4.1

The density of milk decreases as more water is added. The more dense the milk, the faster the drop will sink.

Using this information and your own knowledge, design an experiment to estimate the percentage of water added to the milk supplied to the business.

You must use:

- $10\,cm^3$ sample of the milk supplied to the business,
- $100\,cm^3$ undiluted milk,
- $100\,cm^3$ distilled water,
- $1\,dm^3$ $0.1\,mol\,dm^{-3}$ copper sulfate solution,
- $100\,cm^3$ measuring cylinder,
- $1\,cm^3$ syringe with needle attached,
- timer, e.g. stopwatch or stop clock.

You may select from the following apparatus and use appropriate additional apparatus:

- normal laboratory glassware e.g. test-tubes, beakers, measuring cylinders, graduated pipettes, glass rods, etc.,
- syringes.

Your plan should:

- have a clear and helpful structure such that the method you use is able to be repeated by anyone reading it,
- be illustrated by relevant diagrams, if necessary,
- identify the independent and dependent variables,
- describe the method with the scientific reasoning used to decide the method so that the results are as accurate and reliable as possible,
- show how you will record your results and the proposed layout of results tables and graphs,
- use the correct technical and scientific terms,
- include reference to safety measures to minimise any risks associated with the proposed experiment.

[Total: 12]

Theoretical considerations:

- Milk consists of water, proteins, sugars, and fats. When milk is diluted with water, these molecules become less concentrated due to the greater volume of water and thus overall density of the milk decreases.
- As % of water added to the milk increases, density of milk decreases. This will cause the drop of milk to take a longer time to sink in the copper sulfate solution.

- To measure the density of milk, time taken for diluted milk samples to sink from 70cm^3 to 20cm^3 mark on the 100cm^3 measuring cylinder can be recorded; average times calculated and a standard curve of the average time taken against % of water added to the milk can be plotted.
- The same experiment can be carried out with an equal volume of the milk supplied to the business; using the average time taken for the drop of supplied milk to sink, the corresponding % of water added can be read of the standard curve.
- A range of dilutions can be carried out on the undiluted sample of milk.

Variables:

Independent variable

- % water added to the milk
  10 different % (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100), using distilled water to dilute the undiluted milk.

Dependent variable:

- Time taken for drop of milk to sink from the 70cm^3 mark to the 20cm^3 mark in 100cm^3 of 0.1 mol/dm^3 copper sulfate solution, measured using stopwatch.

Variables to be kept constant

- Volume of milk dropped into copper sulfate solution (0.3cm^3), using syringe
- Concentration of copper sulfate solution used (0.1 $moldm^{-3}$)
- Volume of copper sulfate solution used (100cm^3), using measuring cylinder
- Starting point of release for drop of milk (80cm^3 mark), point to start timing (70cm^3 mark), point to stop timing (70cm^3 mark), using a stopwatch

Control:

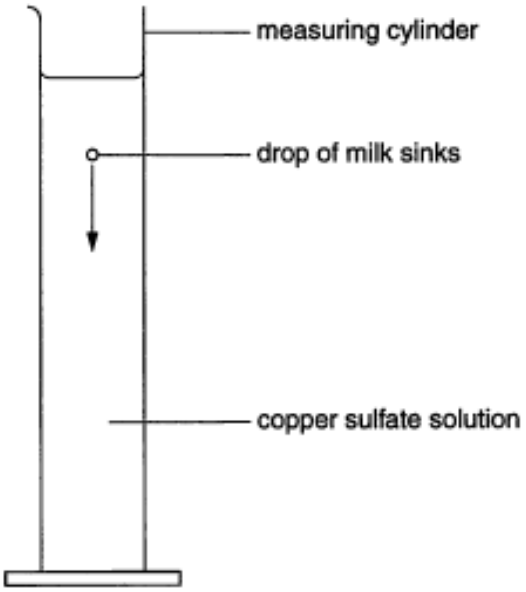
- Replace drop of milk with **equal** volume of distilled water. This is to prove that milk is denser than copper sulfate solution, and the presence of milk will result in the drop of sample to sink.

Procedure:

1. Make a range of dilutions using the stock sample of undiluted milk and distilled water, according to the dilution table below. Use 5cm^3 or 1cm^3 syringes to measure out the necessary volume.

Sample	% water added	Volume of distilled water added (cm^3)	Volume of undiluted milk added (cm^3)	Final volume (cm^3)
M	0	0.0	5.0	5
M1	10	0.5	4.5	
M2	20	1.0	4.0	
M3	30	1.5	3.5	
M4	40	2.0	3.0	
M5	50	2.5	2.5	
M6	60	3.0	2.0	
M7	70	3.5	1.5	
M8	80	4.0	1.0	
M9	90	4.5	0.5	

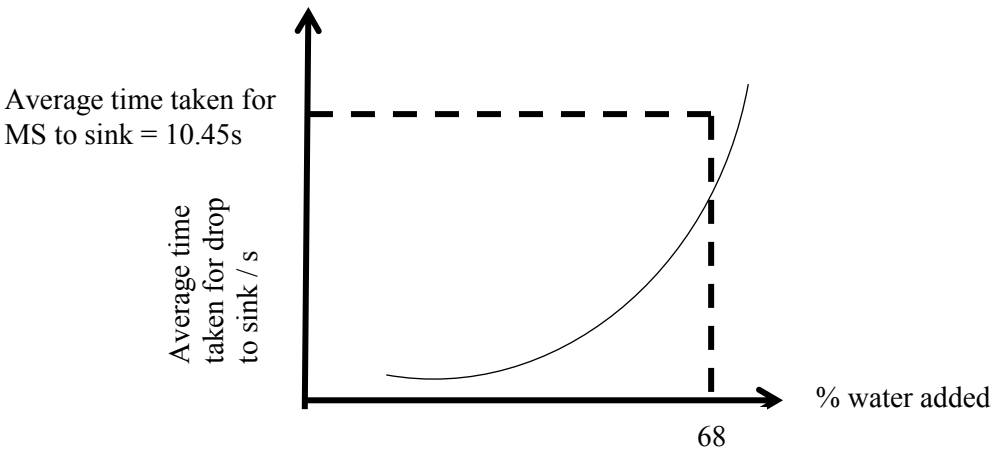
2. Run a pilot test using 0.3cm^3 of the milk samples M to M9, as well as the supplied milk (MS) in 100cm^3 of 0.1mol dm^{-3} copper sulfate solution to determine the appropriate range of dilutions to use such that all the drops will sink and none will float – to ensure that meaningful readings can be obtained.
3. Fill a 100cm^3 measuring cylinder with copper sulfate solution up to the 100cm^3 mark. Using rubber bands, make markings outside of the measuring cylinder at the 20, 70, and 80cm^3 marks as shown in the diagram.
4. Stir each sample of milk using glass rod. Using the 1cm^3 syringe with attached needle, take 0.3cm^3 of M9 and release at the 80cm^3 marking.
5. When the drop of milk sinks to the 70cm^3 marking, start the stopwatch.
6. Stop timing when the drop of milk reaches the 20cm^3 marking.



7. Record the time taken for the drop of milk to sink from 70cm^3 mark to the 20cm^3 mark.
8. Repeat steps 4 – 7 for M8 to M. Also, for MS.
9. Repeat steps 4 – 8 two more times for each milk sample to ensure reliability of results.
10. Calculate average time taken for the drop of milk to sink from the 70cm^3 mark to the 20cm^3 mark using the data.

Sample	% water added	Time taken for drop of milk to sink /s			Average time taken for drop of milk to sink /s
		T1	T2	T3	
MS	unknown				
M	0				
M1	10				
M2	20				
M3	30				
M4	40				
M5	50				
M6	60				
M7	70				
M8	80				
M9	90				

11. For M and M1 to M9, plot a graph of the average time taken for the drops to sink against the % of water added.
12. From the graph, read the value for the % of water added that corresponds to the average time taken for the MS (supplied milk sample) drop to sink.



13. Repeat entire experiment 2 more times using new copper sulfate solution and milk to ensure reproducibility of results.

Risk and safety precautions

14. Copper sulfate solution may cause skin irritation. Wear gloves when handling solutions and wash with water immediately if it comes into contact with skin.
15. The needle that is attached to the syringe is sharp and can cause injury. Handle the syringe with care and over the needle with cap when not in use.

QUESTION 5

(a) Describe the cause and symptoms of the genetic disease, cystic fibrosis. [7]
(b) Explain how a disease such as cystic fibrosis, can be treated by gene therapy, using non-viral delivery systems. [5]
(c) Describe four problems that may be associated with using a viral vector to introduce an allele into the cells of a person with a genetic disease. For each problem, explain the related ethical issues that should be considered. [8]

(a) Describe the cause and symptoms of the genetic disease, cystic fibrosis. [7]

1 Autosomal recessive mutation in CFTR gene (cystic fibrosis transmembrane conductance regulator gene) on chromosome 7, which codes for CFTR protein.

2 Deletion of 3 base pairs (reject: one codon) leading to the loss of the amino acid phenylalanine, resulting in CFTR protein missing phenylalanine at position 508. This changes the primary structure of the protein, therefore modifying the 3D structure.

3 CFTR is a cAMP-regulated chloride channel important in transporting Cl^- ions **out** of epithelial cells.

4 Non-functional CTFR channels are unable to transport Cl^- ions out of epithelial cells.

5 This causes accumulation of Na^+ ions in the cell to neutralise Cl^- ions, resulting in high ionic concentration (i.e. water potential in cell drops),

6 Water is drawn into the cell / cannot leave the cell.

7 Thick mucus forms on the epithelial surface and blocks passageways (e.g. airways of lungs, pancreatic duct, bile duct, etc.)

8 This lead to trapping of bacteria that leads to inflammation and infection of lungs and poor rate of gaseous exchange /Blockage in pancreas, inadequate secretion of pancreatic enzymes. Malnutrition due to inability to absorb essential nutrients /Blocked sperm ducts & fallopian tubes (infertility)

9 Overall life expectancy of individuals will be reduced considerably

(b) Explain how a disease such as cystic fibrosis, can be treated by gene therapy, using non-viral delivery systems. [5]

1 Normal dominant CFTR allele is packaged into liposomes *in vitro*.
2 These liposome vectors are incorporated into a nasal spray, and sprayed into the nose and mouth of CF patients.
3 Liposomes fuse with the cell membrane of tracheal / lung epithelial cells or by endocytosis to form endosome / endocytic vesicle within cell, releasing the normal CFTR allele into the cell cytoplasm.
4 The introduced CFTR allele is then transported to the nucleus for transcription into mRNA and the functional CFTR protein is produced.
5 The functional CFTR protein embeds itself into the cell membrane and begins to transport Cl^- ions out of cells, thereby thinning the mucus as water moves out of the cell and dilute the thick and sticky mucus, alleviating the symptoms of cystic fibrosis.
6 Alternative non-viral delivery system involves the usage of a gene gun to introduce naked DNA directly into target cells through bombardment with particles coated with DNA.

Examiner's comments:
Most candidates limited their responses to one non-viral system for delivery of gene therapy, typically focusing on liposomes to package the normal allele.

(c) Describe four problems that may be associated with using a viral vector to introduce an allele into the cells of a person with a genetic disease. For each problem, explain the related ethical issues that should be considered. [8]

[2 marks per problem-ethics pair]

Problem:

Immune response

- Introduced vectors may stimulate the immune system and render viral vectors useless, thus, gene therapy using common viruses will be difficult, since patients would have acquired antibodies during the previously infection.

Ethics:

Safety

- Question of safety and efficiency of treatment and therefore unknown long term consequences to the well-being of the recipients. Patients should be informed clearly that the possible health benefits of gene therapy are not guaranteed. Healthcare providers and personnel must be educated and trained so that they can disseminate information thoroughly to patients. Pathogenic viruses may pose a threat to public health if health personnel leave the laboratories and spread to human population.

Problem:

Insertional mutagenesis

- Insertional mutagenesis: most transgenes are inserted ectopically if retroviruses are used
- May cause disruption of other useful genes. Eg. insert into tumours suppressor genes / proto-oncogenes.

Ethics:

Safety

- Question of safety and efficiency of treatment and therefore unknown long term consequences to the well-being of the recipients. Patients should be informed clearly that the possible health benefits of gene therapy are not guaranteed. Healthcare providers and personnel must be educated and trained so that they can disseminate information thoroughly to patients. Pathogenic viruses may pose a threat to public health if health personnel leave the laboratories and spread to human population.

Problem:

Difficult to get DNA to integrate into target cell genome

- Difficult to get DNA to integrate into target cell genome if adenoviruses are used
- Introduced gene may not segregate equally to daughter cells if the cells are actively dividing.

OR

Inserted gene may not expressed

- Inserted genes may not be expressed because they may be integrated into a highly condensed heterochromatic region.

Ethics:

Safety

- Question of safety and efficiency of treatment and therefore unknown long term consequences to the well-being of the recipients. Patients should be informed clearly that the possible health benefits of gene therapy are not guaranteed. Healthcare providers and personnel must be educated and trained so that they can disseminate information thoroughly to patients. Pathogenic viruses may pose a threat to public health if health personnel leave the laboratories and spread to human population.

Problem:

- Viruses are possible vectors used to introduce in alleles coding for desirable traits in germline therapy for enhancement instead of disease treatment.

Ethics:

Eugenics social policies

- Potential for non-therapeutic enhancement possibilities.
- Type of circumstances that determines whether genomes should be altered/ what is normal or a disability or disorder, and who decides.
- Concerns about the widespread use of gene therapy making society less accepting of people who have mild disorders / genetic diseases or less able / lead to new definitions of "normal (e.g. intellect / height / strength etc.)

Or

Interfering with life

- The unborn child who would be affected by germline gene therapy cannot choose whether to have the treatment.

Examiner's comments:

In general, answers were well structured with each problem clearly described and related to ethical issues. Some candidates concentrated largely on emotive issues but others provided a more balanced treatment including objective assessment of medical problems. Superficial and vague references to ethical issues needed further qualification.