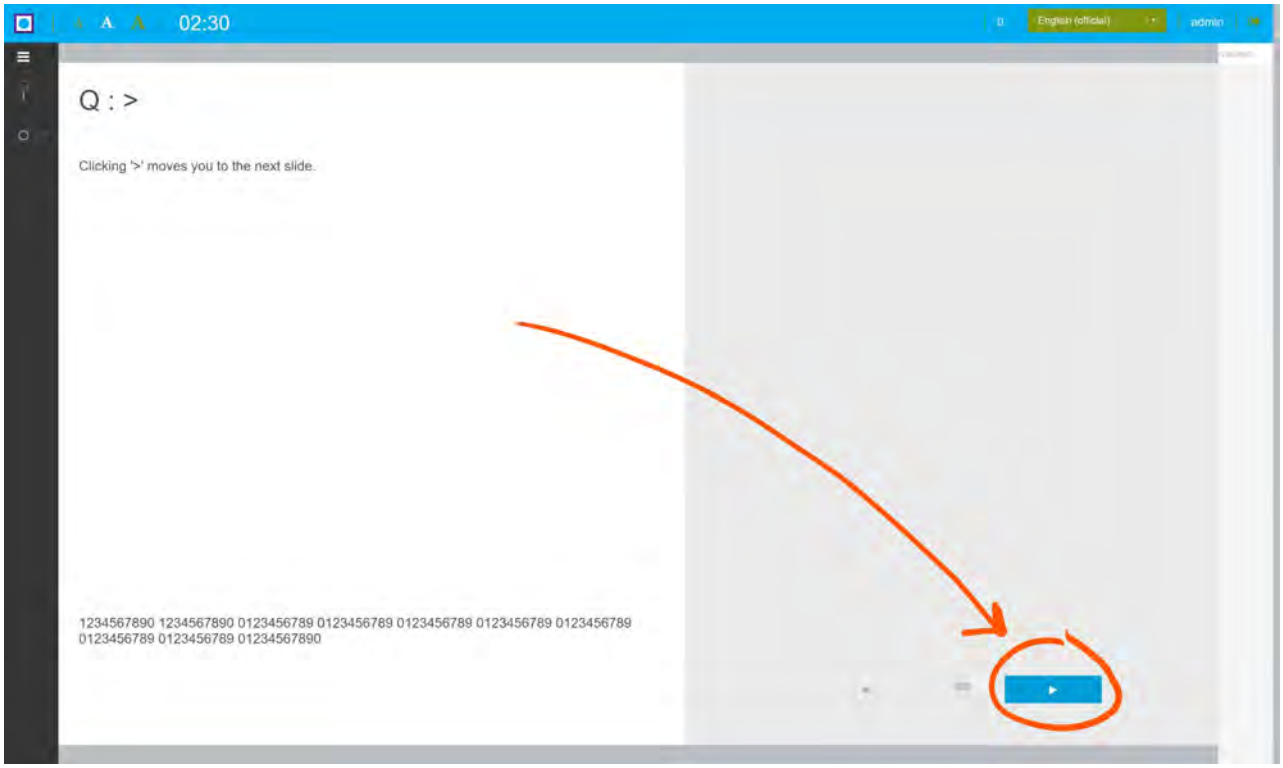
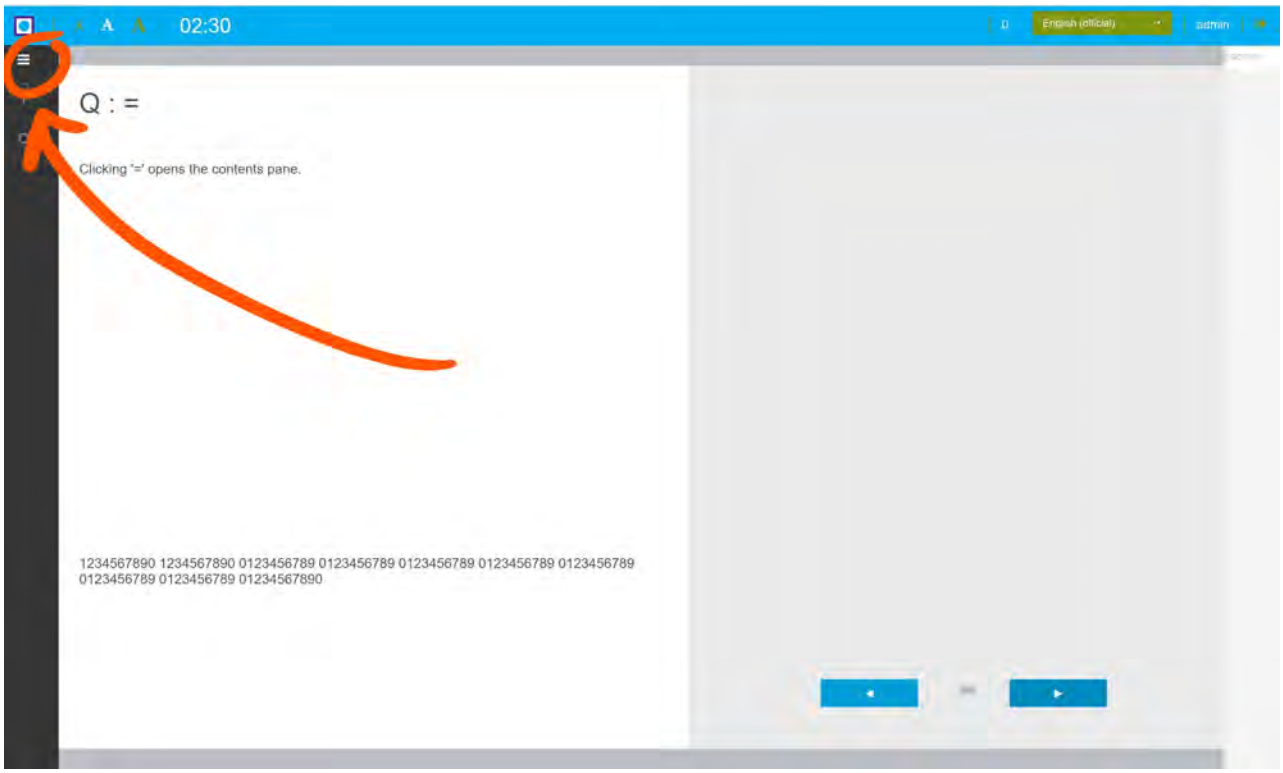
INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

NAVIGATING SLIDES

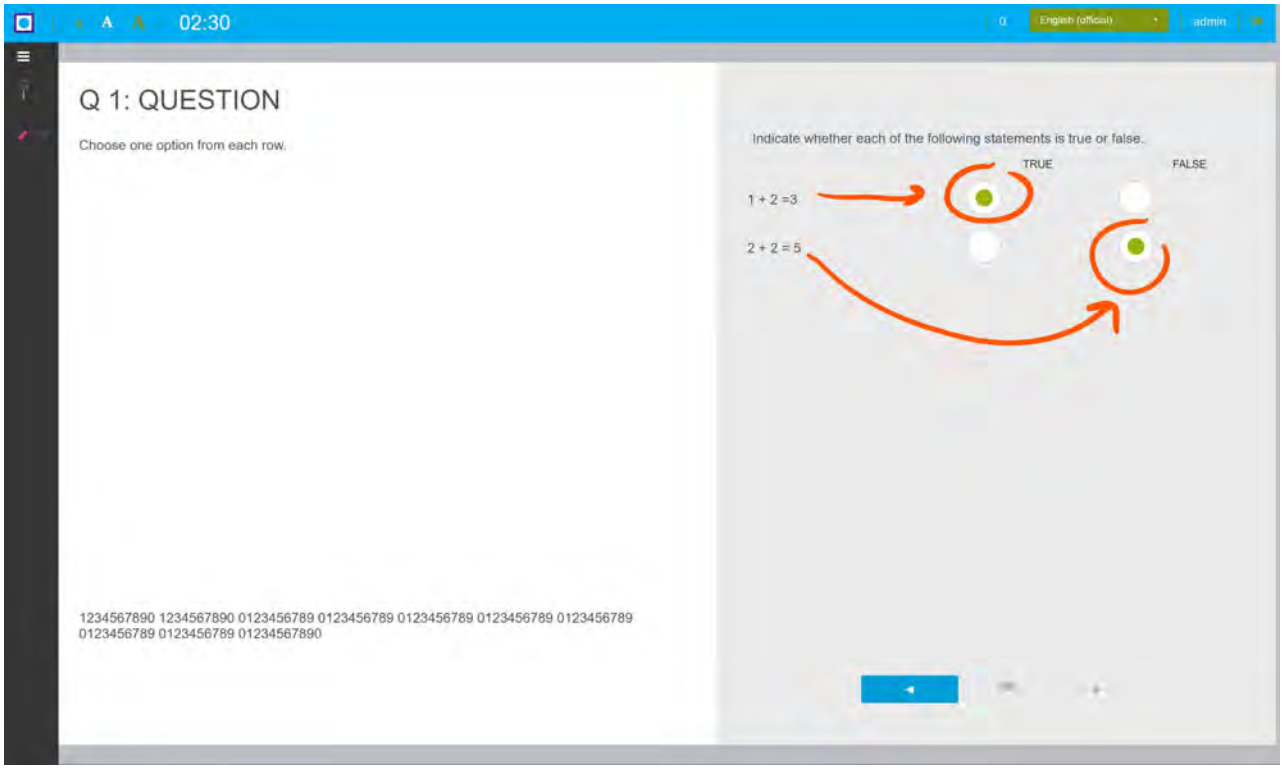
View all the introductory slides before moving to the first question. ***They contain essential and useful information.*** including instructions and useful scientific definitions.
Click '>' to move to the next page.



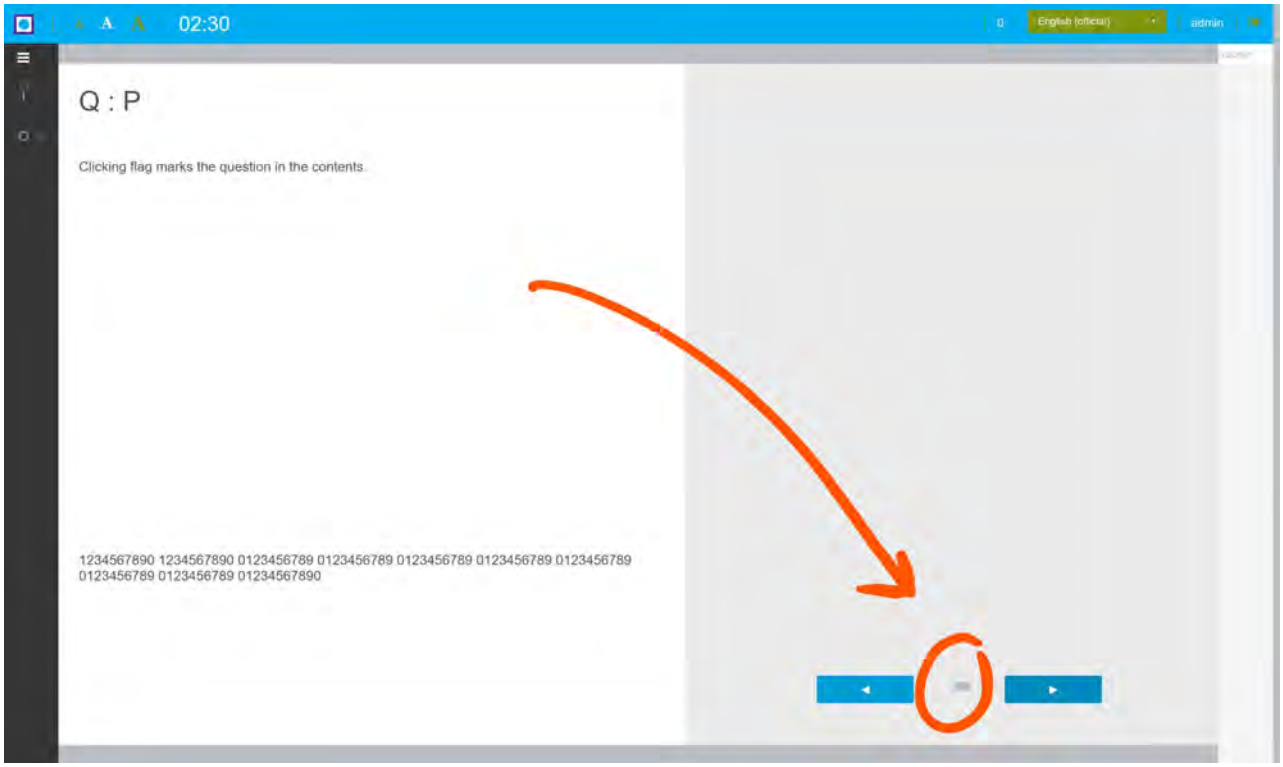
Click the burger to view the contents of the exam. Click it again to close the contents.
Then click '>'



For all questions, select one option per row. All your answers can be changed at any time during the exam, and will be saved automatically.



The flag icon can be used to mark questions in the contents pane.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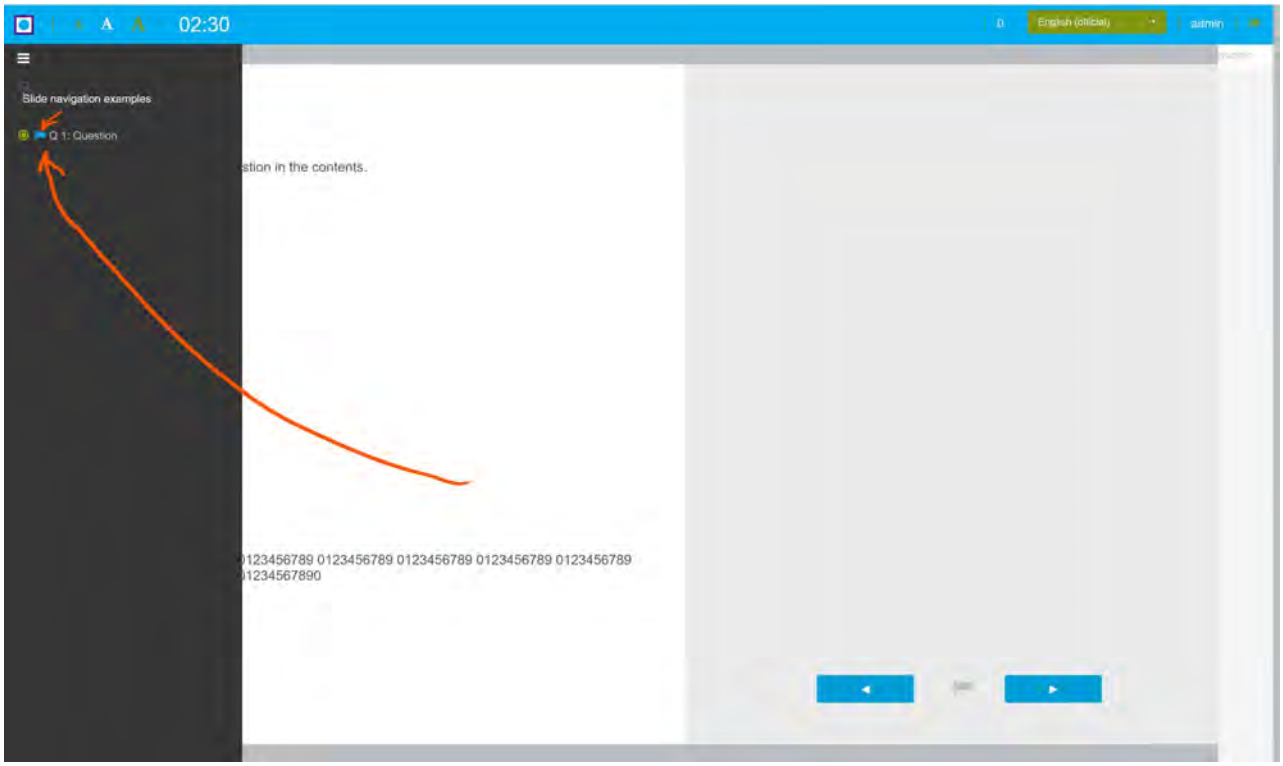
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INSTRUCTIONS FOR THE THEORY EXAMINATIONS

PAPER 1: 8.30AM - 11.30AM

Instructions

Each paper comprises 46 questions, which will be completed on a computer.

You MUST answer ALL parts of ALL questions. For multiple-true/false tasks, answer each statement with either 'true' or 'false'. Between none and all of the statements may be true. For calculations, choose the number nearest to the correct answer. You should make your best guess if you are unsure; you will not be penalised for incorrect guesses, but may gain marks.

Each correct answer will score 1 mark. Each incorrect or missing answer will score 0 marks.

You SHOULD attempt the questions IN ORDER, and come back later to any that you cannot answer. You can flag these by clicking the flag icon, and see your progress by opening the contents pane on the left-hand side. You may find that ideas explored in earlier questions help you answer later questions.

Some figures can be enlarged by clicking on them.

You can change the language you view the papers in by choosing an option from the top right corner.

You will need to use the information given to you in each question creatively, but you will never require advanced technical or specialised knowledge.

You MUST have the following equipment for this exam.

- Approved calculator
- Pen/pencil
- You will be provided with scrap paper. You MUST NOT bring any paper into, or out of, the exam room. A copy of this document will be available on the first page of each exam.

Regulations

You MUST NOT communicate with ANY other candidate at ANY time, whilst you are in the examination room.

You MUST NOT open ANY other windows on your computer.

You MUST NOT access ANY information that could unfairly help you whilst the examination is in progress.

If you require the assistance of a guide you should raise your hand, and remain facing forward until given further instructions.

You MUST NOT attempt to leave your computer station without the assistance of a guide.

If you experience technical problems, you MUST inform a guide IMMEDIATELY.

Good luck!

USEFUL SCIENTIFIC DEFINITIONS

USEFUL SCIENTIFIC DEFINITIONS

These terms have featured in IBO exams for many years, but you may not be familiar with their precise definitions.

WT	In all cases, *WT* refers to *wild-type*. Wild-type organisms have not been genetically manipulated, or otherwise chosen for a specific genetic property.
Knockout	*Knockout* refers to an organism which has had a specific gene, which is stated in the question, mutated such that no functional product is produced from it.
Haplotypes	A haplotype is a combination of alleles that occur on the same DNA molecule. For example, if genes A, B, C, D, and E are located on the same chromosome, and each gene has two alleles, this genomic region can have many different haplotypes (AbCdE, abcDE, ABCde etc.). If these genes are strongly genetically linked, some haplotypes will occur in the population more often than expected by chance, i.e. specific alleles of one gene will usually co-occur with specific alleles of the linked genes. Mutations within such a linked region create new haplotypes, descended from the old. Meiotic crossing over within the region breaks existing haplotypes and randomly recombines alleles thus eliminating the association between alleles over time.
mmHg	Millimeters of mercury. Biologists usually use mmHg as the unit for pressure. mmHg are directly proportional to Pascals and cmH_2O, but give rounder numbers in most biological situations.
Partial pressure (PGas)	Partial pressure measures the pressure that a gas would exert on its surroundings if only that gas was present. Partial pressures are noted as Pgas (e.g. Po_2 = partial pressure of oxygen). For example, the total pressure of atmospheric air, at sea-level, is 760 mmHg, and oxygen makes up 21 % of all the molecules in atmospheric air. Therefore the partial pressure of oxygen in atmospheric air is $Po_2 = 0.21 \times 760 = 160$ mmHg. The partial pressure of a gas in solution, is the partial pressure that the gas would have in air which is in equilibrium with the solution. For example, the partial pressure of oxygen in a glass of water exposed to atmospheric air for a long time will also be 160 mmHg. Hence, partial pressures are used by biologists to predict the rate and direction of gas transfer and equilibrium conditions. Partial pressures are NOT directly proportional to the concentration of the gas in a solution. Concentration depends on partial pressure, solubility, temperature etc.
Expression	Many DNA genes are transcribed to produce RNA, which is translated to produce a polypeptide. This folds, and may be modified, to give a functional protein. Unless stated otherwise, the expression level of a gene describes how much functional product it is generating through the combined action of these processes. Therefore, if expression is increased, more functional protein is being produced. This does not necessarily mean there is increased amounts of protein (it may be degraded quickly). The functional product may also need further steps to become activated.
Arrows	In scientific diagrams, arrows are taken to mean *leads to*, *activates*, *becomes,* or simply a label.
Flat-headed arrows	In scientific diagrams, flat-headed arrows are taken to mean *inhibits*, *blocks*, or *reduces*.

BLUEPRINTS OF LIFE

SEQUENCING

Frederick Sanger (1918-2013) invented protein, RNA and DNA sequencing, and Sir Shankar Balasubramanian (1966-present) invented high-throughput DNA sequencing. The National Health Service is sequencing an unprecedented 100 000 genomes from rare-disease patients, but different sequencing technologies have different merits for this purpose, as described below.

Technology	Maximum length of sequence fragments which can be read	Error rate	Total number of bases sequenced per sample per day
Sanger sequencing	900 bp	1 in 1000 bp	900 bp (1 fragment)
Illumina machines	200 bp	1 in 100 bp	$3x10^{11}$ bp (>$1.5x10^{9}$ fragments)
PacificBiosciences machines	5000 bp	1 in 10 bp	$4x10^{8}$ bp (>80 000 fragments)

	True	False
Illumina technology is best for finding new Single Nucleotide Variations (mutations to a single base) in the patient genomes.	X	
PacificBiosciences technology is best for assessing transcriptional changes by RNA sequencing.		X
PacificBiosciences technology is best for finding rearrangements of chunks of DNA in the patient genomes.	X	
Sanger sequencing is best for validating sequencing results before using patients' genetic information to guide clinical interventions.	X	

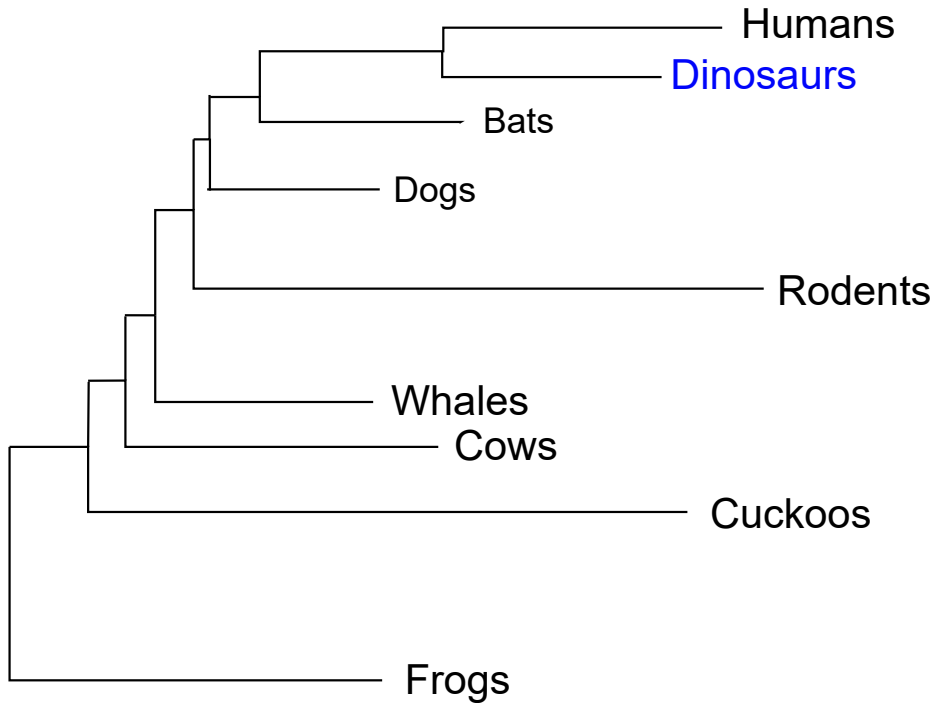
Explanation:

The question indicates how bio-technologies have evolved and diversified. The huge number of whole genome sequences that need to be generated for the 100000 genomes project places constraints on the machines which can be used. Figures are rounded to aid comparison, and cost is not included, but would not affect the conclusions.

- Illumina technology reads a sufficient number of bases to cover the entire genome several times over, so is appropriate for reading all the genomes, and each base is called with confidence.
- False. To assess transcriptional changes, total RNA must be sequenced many times over, to ensure all transcripts are captured, and that the frequency of each transcript fairly represents its abundance. PacBio doesn't read enough bases to achieve this. Futhermore it is too error prone to map small exons to the genome robustly.
- The only way to spot rearrangements is to sequence through the break-point at the border of the moved chunk. To stand a good chance of doing this, the largest possible continuous sequences need to be generated.
- By this point, a variant will be well characterised, so only a short region of known sequence needs to be analysed. Sanger sequencing is the quickest, cheapest and most targeted way to do this. Moreover, it is the only method with sufficient accuracy to base life changing decisions on. Therefore, ethical guidelines state sanger sequencing MUST be used to validate results before patients are informed of their genetic status.

ANCIENT DNA

Mark Thomas (1964-present) read the first DNA sequences from woolly mammoths *(Mammuthus primigenius)*. However, ancient DNA analysis remains difficult, due to degradation, contamination, and polymerase inhibitors found in the samples. Scientists attempted to extract DNA from a dinosaur fossil and analyse the sequence of one specific gene. The following phylogeny was produced after comparing the sequence against other species.



	True	False
When working with ancient samples, it is easier to recover DNA sequences from a mitochondrial gene than a nuclear gene of the same length.	X	
It is better to sequence very long DNA fragments from ancient samples, than very short fragments.		X
Adding a DNA fragment of a known sequence could show whether or not samples contain polymerase inhibitors.	X	
This dinosaur fossil extract is likely to contain contaminating DNA.	X	

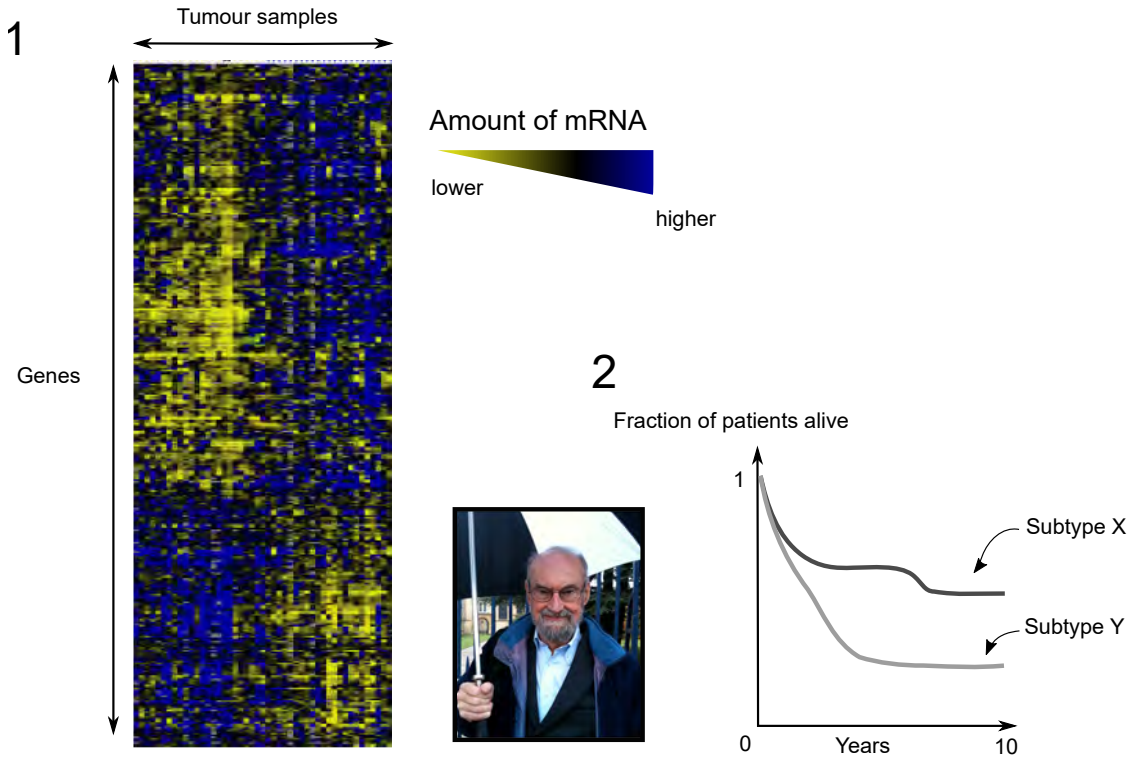
Explanation:
This question was inspired by Vasili Pankratov of the Belarus Olympiad.

Students are tested on their ability to design an experiment to give the best data with difficult samples, and to judge the quality of the resulting data.

- There are many copies of each mtDNA gene, and only two of each nuclear gene. Therefore, if a single specific gene is to be used to generate a tree, it will be easier to find a mitochondrial copy than genomic copy in heavily degraded samples.
- Very long fragments are contamination. Degradation over time means only small fragments of ancient DNA survive.
- If the artificial fragment is not recovered during sequencing, even though it was present as a positive control, then we know something inhibited the sequencing reactions.
- False, The tree is clearly ridiculous. It is more than likely a scientist contaminated the sample with their own DNA.

CANCER MICROARRAY

Sir Edwin Southern (1938-present) invented microarrays to analyse the expression of hundreds of genes simultaneously. A microarray has probes printed onto it which detect a specific complementary mRNA. These probe for mRNAs expressed from across the genome. Diffuse Large B Cell lymphomas (DLBCL) from tens of patients were analysed by microarray (1). Additionally, patient survival rates for two clinical subtypes of DLBCLs were measured (2).



	True	False
The data rule out patient survival rate being determined by differences in gene expression between DLBCL subtypes.		X
Gene expression data suggest there are two major subtypes of DLBCL distinguishable at the molecular level.	X	
Measuring the expression of a single gene is sufficient to distinguish subtypes of DLBCLs.		X
Each subtype of DLBCL has an equal number of relatively up-regulated versus down-regulated genes compared to other subtypes.		X

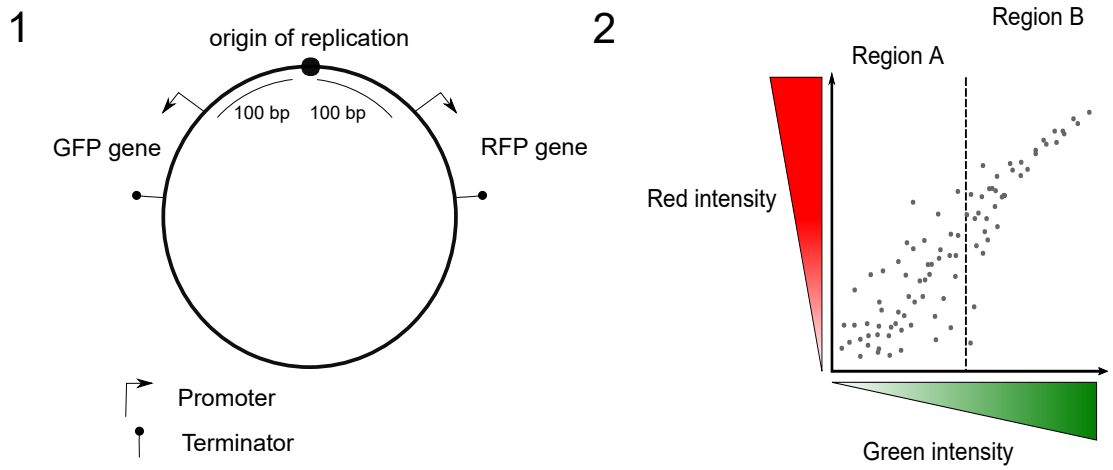
Explanation:

This question assesses how global changes in the transcriptome manifest in a clinical phenotype.

- According to the central dogma, RNA levels determine protein activity, determine cell function. Hence the difference in survival between tumour subtypes originate in gene expression changes. The micro-array data shows that there is a large variability in expression between tumours, validating this conclusion.
- It can be seen that the samples cluster into two broad columns, with good definition between them, and any further division into subtypes is less profound.
- There is no one gene that is solidly blue in one subtype, and solidly yellow in another. A group of (at least six) diagnostic genes must be analysed.
- If one subtype is taken as the reference, the other is seen to have ~2/3 of genes more highly expressed.

GENE EXPRESSION NOISE

A plasmid was inserted into bacteria (1). Red fluorescent protein (RFP) or green fluorescent protein (GFP) are produced from the genes in this plasmid whenever transcription factors bind to their promoters. The red and green gene promoter sequences are identical. Plasmid DNA replication starts at the origin of replication, and replication forks move outwards in both directions at the same speed. Replication occurs whenever replication factors bind to the origin of replication. The red and green fluorescence intensity of individual bacteria was measured (2).



	True	False
Replicating plasmids have unequal numbers of red fluorescent protein and green fluorescent protein genes.		X
On average, cells in Region A contain more replication factors than those in Region B.		X
On average, cells in Region A contain more transcription factors than those in Region B.		X
Increasing the activity of transcription factors is expected to increase the number of cells containing equal amounts of green fluorescent protein and red fluorescent protein.	X	

Explanation:

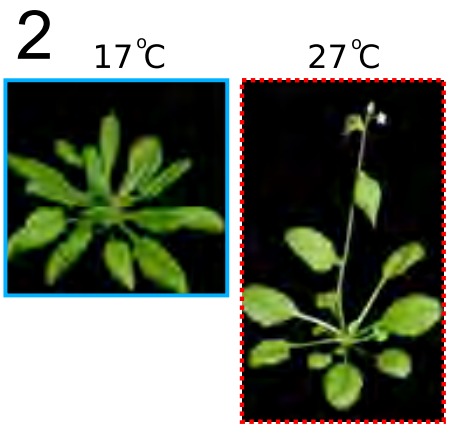
This question assess understanding of the process of gene expression, and encourages candidates to consider how the balance of expression between alleles changes in different situations.

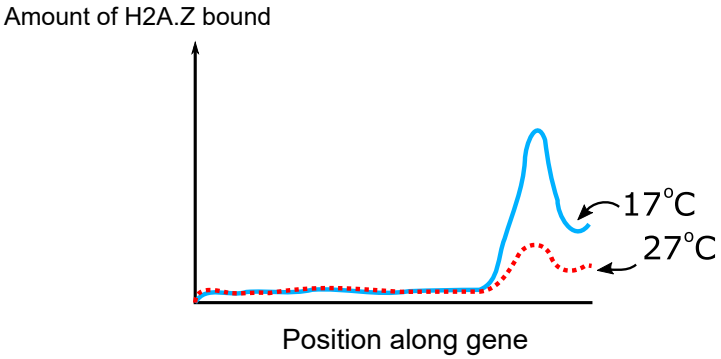
- The promoters are an equal distance from Ori, so replication factors will reach them at the same time.
- Cells with more replication factors will contain more plasmid copies, and therefore more copies of the GFP/RFP genes, and therefore highler fluresecnce
- More transcription factors leads to more RFP/GFP mRNA, which leads to more protein, which leads to more fluresence.
- More copies of each protein leads to high amounts of red and green protein, and so yellow cells. At low levels of transcription factor, both promoters cannot be saturated simultaneously, so cells tend to be more one colour, than the other. Hence there is a bigger deviation from the diagonal in region a.

EPIGENETICS OF FLOWERING

Dame Jean Thomas (1942-present) discovered that eukaryotic DNA is tightly wrapped around histone proteins. Sir Adrian Bird (1947-present) helped explain the epigenetic marks of DNA. For example, the classical histone H2A, can be replaced by a variant, H2A.Z.
H2A.Z occupancy on a pro-flowering gene (1) in *Arabidopsis* plants (2) of the same age, at different temperatures, was measured.

1





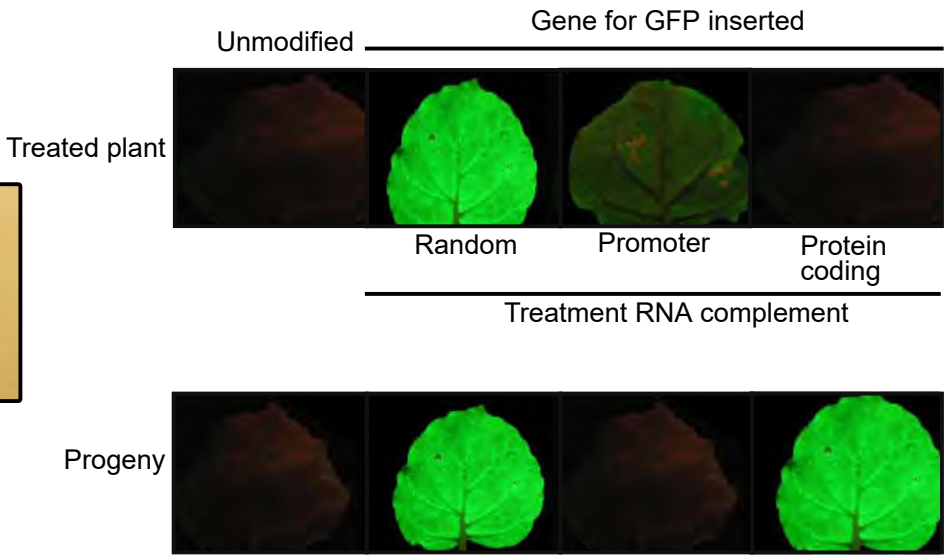
	True	False
Increasing temperature increases pro-flowering gene expression.	X	
H2A.Z enhances gene expression.		X
Temperature moves the locations to which H2A.Z binds in the genome.		X
Plants that flower early may have increased H2A.Z occupancy in this gene before flowering.		X

Explanation:
This question considers how the environment can influence epigenetics and so gene expression.

- The plant at higher temperature has a flowering stem.
- At high temperature, the plant flowers, showing the pro-flowering gene is more expressed, and there is less H2A.Z at the pro-flowering gene.
- The amount of H2A.Z bound is reduced at high temperature, but there is no evidence of it binding different places.
- Early flowering plants would have more pro-flowering gene expression, and therefore less of the transcriptional repressor H2A.Z bound at these genes.

GENE SILENCING

Sir David Baulcombe (1952-present) discovered RNA-directed gene silencing.
The gene for green fluorescent protein (GFP) was inserted into plants. The plants were treated with RNAs, which silence GFP, and were then allowed to set seed (the treatment RNAs are not present in the next generation). The treatment RNA sequence was complementary to either the promoter or the protein coding sequences of GFP, or random (non-complementary). The parent plants, their progeny, and controls were imaged under a UV light.



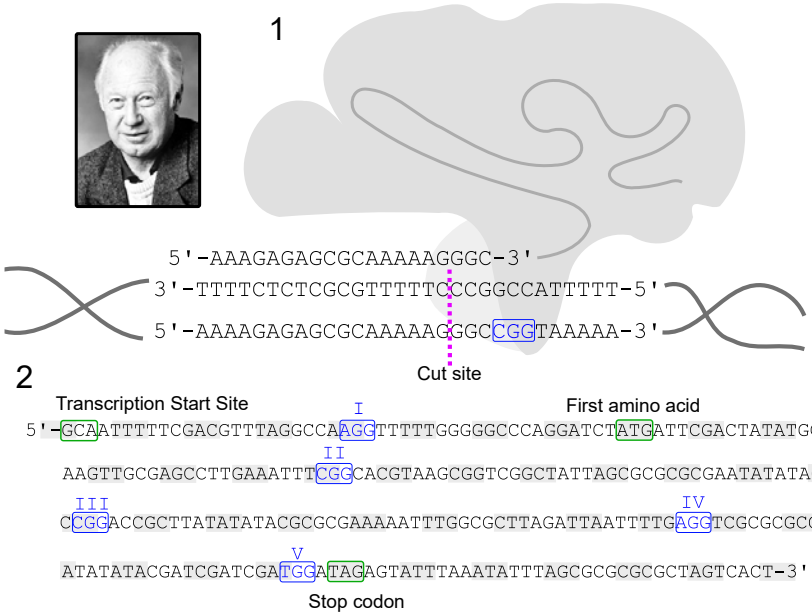
	True	False
Leaves expressing GFP appear darker under UV light.		X
RNA-directed gene silencing is possible without using treatment RNAs designed to target mRNA.	X	
Changes in the expression level of genes can be inherited from one generation to the next without mutating the DNA sequence.	X	
Silencing against the protein coding sequence is heritable.		X

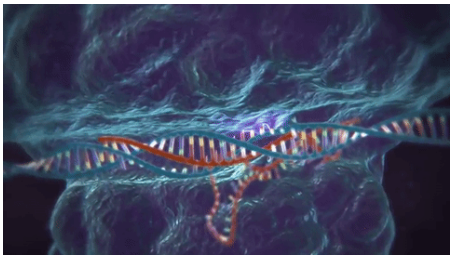
Explanation:
This question builds on the previous two to consider post-transcriptional regulation of gene expression and trans-generational epigenetic effects.

- The unmodified leaves are dark. The GFP transfected, without silencing, are bright green.
- The promoter is not transcribed, but siRNA directed against it can still block expression.
- GFP remains silenced in the progeny, even though the siRNAs are no longer present, when directed against the promoter. (The siRNA-protein coding treatment acts as a control to show siRNA does not mutate the DNA sequence it complements).
- This is not inherited, indicating it is less likely to be epigenetic.

GENE ENGINEERING

Michael Smith (1932-2000) invented site-directed mutagenesis. Recently scientists invented CRISPR-Cas9 technology to carry out mutagenesis more easily.
Cas9 protein from *Streptococcus pyogenes* is guided by a 20bp guideRNA which base-pairs with target DNA. This Cas9 enzyme can only make double strand cuts in DNA three bases upstream from a 5'-NGG-3' site (1). Double strand cuts are attacked by DNA degrading enzymes, and then re-joined. Gene X, which you want to stop from functioning with this enzyme, is shown (2).
An alternative way to knockout genes is to use a pair of modified Cas9's which can only make single strand cuts, and guideRNA's which target adjacent sites.





	1	2	3	4	5
You have five guideRNA sequences to choose from: 1 = 5'-ATTTTTCGACGTTTAGGCCA-3' 2 = 5'-AUUUUUCGACGUUUAGGCCA-3' 3 = 5'-AUAAAACGUGCAAAUCCGGU-3' 4 = 5'-UUUGCACGUUUAGGCCAAGG-3' 5 = 5'-UUUUUGGGGGCCCAGGAUCU-3'		X			

Explanation:

-

	I	II	III	IV	V
NGG site		X			

Explanation:

- Random nucleotide loss causes frameshift. More likely to disrupt protein if early in aa sequence. Non-coding region more likely to tolerate changes. II is the first site in the coding region.

	True	False
Using the pair of modified Cas9s and guideRNAs increases off-target damage to other genes.		X
The *Streptococcus pyogenes* genome has more NGG sites than expected by chance.		X
If the gene has no GG sequences, Cas9s from other species should be investigated for alternatives.	X	

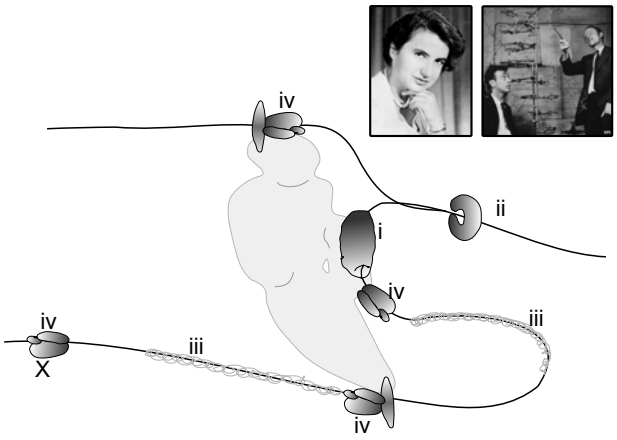
Explanation:

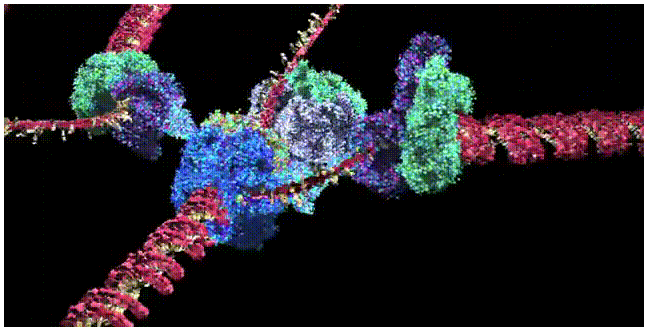
This question assesses whether students can analyses a DNA sequence to manipulate it like a molecular biologist. Additionally they need to think about different consequences of mutations, and how the technology can be refined.

- Whilst the Cas9s will bind more off-target sites which happen to be complementary to one sgRNA if more different sgRNAs are present, each only makes ss cuts. ss cuts are easliy repaired without mutagenesis, and it is extremely unlikely a pair will bind and produce a ds cut away from the target site.
- To prevent Staph from attacking its own genome, it must have fewer, and modify those present.
- It is possible (and actually the case) that different versions of the protein have different sequence preferences.

REPLICATION FORK

X-ray crystallography, invented at the Royal Institution, was used by Sir Francis Crick (1916-2004) and Rosalind Franklin (1920-1958) to discover the structure of DNA, and predict the mechanism of its replication. Building on their work, this is the current predicted structure of an *E. coli* DNA replication fork, which moves along DNA at 1000 bp/s.
i = Tube shaped clamp, which pulls on one DNA strand.
ii = Topoisomerase, which makes temporary cuts in one phosphodiester backbone.
iii = Single-stranded DNA binding protein.
iv = Different polymerases.





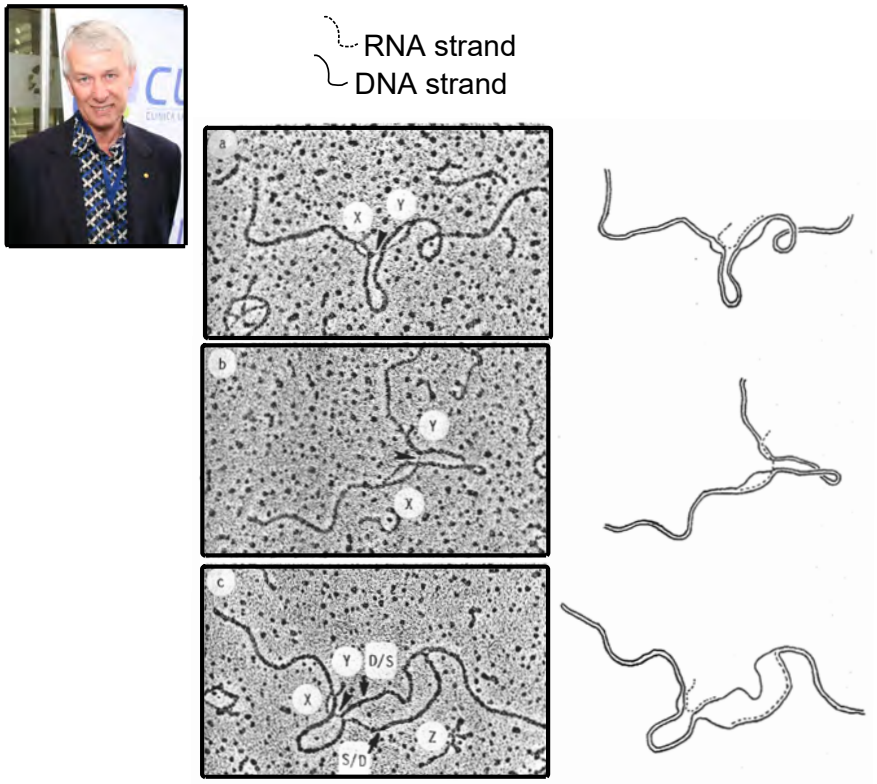
	True	False
Antibiotics which poison topoisomerase cause DNA ahead of the fork to become over twisted.	X	
An activity of polymerase complex X is to replace ribonucleotide uracil, with deoxyribonucleotide thymidine.	X	
Enzyme i is preferentially loaded at G/C rich (A/T poor) sequences.		X
Protein iii assists complementary base pairing.		X

Explanation:
This question requires students to know that DNA is a double helix, and that it is replicated semi-conservatively. This is alluded to in the stem. They are then assessed on their ability to grasp the dynamic geometry of the replication fork.

- As i tugs along one DNA strand, forcing the other to seperate from it, it forces the DNA infront to spin. (Imagine pulling apart two bits of string wound around each other) Given the extreme speed the fork moves, this rapidly causes the DNA infront to overwind, snap, or the fork to jam. Hence topoisomerase is needed to release the tension. Several antibiotics poison this topoisomerase.
- This polymerase acts on the lagging strand after the replication fork has passed. Therefore, it is sealing Okozaki fragments.
- This needs to clamp around one DNA strand. Thereofer, DNA strands must be seperated before it is loaded. A/T pairs have fewer H bonds, therefore origin of replication sites have an A/T rich region which melts easily, allowing it to be loaded.
- Binds single strand regions, which are primed by primase, but must loop round to reach a DNA polymerase which travels in the same direction as the replication fork.

SPLICING

Sir Richard Roberts (1943-present) discovered the fundamental structure of genes using adenovirus genomes. Under an electron microscope, Roberts identified adenoviral DNA and RNA strands. Eukaryotic mRNA was later found to be missing parts (introns) of the genes, because the RNA sequence was rearranged by splicing.



	True	False
Each adenovirus RNA is transcribed from the same DNA strand for the whole RNA.	X	
Adenovirus mRNA remains bound in a DNA-RNA hybrid duplex for a period of time.	X	
Transcription requires helicase activity (separates the two DNA strands).	X	
mRNA splicing of these Adenovirus genes uses a similar mechanism to typical Eukaryotic splicing.		X

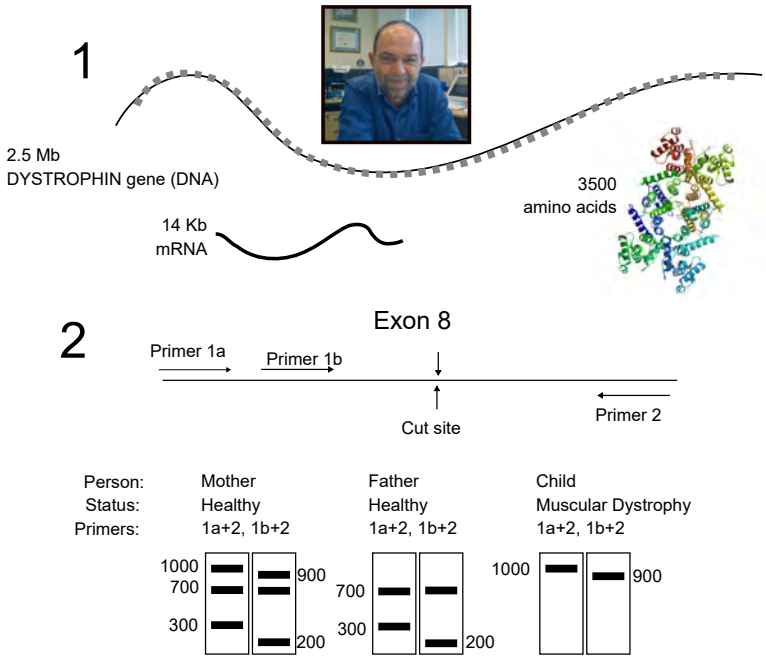
Explanation:

This question asks simple questions about these nobel prize winning images.

- The coding strand doesn't swap in any of these images
- Paired regions can be seen
- One DNA strands floats free of the hybrid duplex
- RNA is not cut, but transcription complex directly skips across loops of DNA

DYSTROPHIN

Muscular Dystrophy is caused by alterations to *DYSTROPHIN* (1). This is a very large gene, and its length affects the biology and diagnosis of Muscular Dystrophy.
RNA polymerase moves along DNA at 30 bp per second.
DNA polymerase has an error rate of 10^{-8} mistakes per base. Repair systems later correct 99% of mistakes.
Sir Alec Jeffreys (1950-present) invented DNA fingerprinting (RFLPs) whereby *DYSTROPHIN* exons may be amplified by PCR, treated with DNA-cutting enzymes (endonucleases), and separated on an agarose gel, according to length in bp (2).



	1 second	1 minute	1 hour	10 hours	1 day
Select the nearest time to the correct answer.					X

Explanation:
Must understand the process of gene transcription.

- Must transcribe the full 2500000 bases of DNA. 2500000/30 = 83333 seconds. 83333/60/60 = 23 hours.

	50	500	5000	50000	5000000
Choose the nearest number to the correct answer.			X		

Explanation:

- 2 500 000 * 10^{-8} * 0.01 = 0.00025 mistakes per division. 1/0.00025 = 4000 divisions

	100 bp	200 bp	300 bp	700 bp	1000 bp
Primer 1b binding site.	X				
Endonuclease cut site			X		
Primer 2 binding site.					X

Explanation:
1a------100bp-------1b---------200bp---------cut----------700bp-------2
The only way to get the observed banding pattern.

-
-
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	True	False
DYSTROPHIN is on the X chromosome.	X	
Muscular Dystrophy is dominant.		X
DYSTROPHIN protein could be made in bacteria with a 20 kb plasmid.	X	
Many muscular dystrophy patients have new (*de novo*) causal mutations.	X	

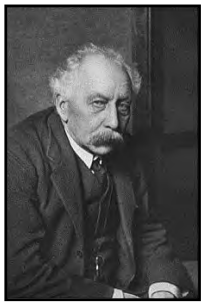
Explanation:
Students have to perform calculations to assess their understanding of molecular biology. The question then assesses whether they can use these results to infer features of the disease.

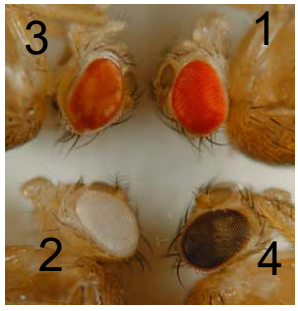
- Child only has one allele
- Mother has same allele as child, but is healthy
- mature mRNA is only 14 kbp
- Is a very long gene, with new alleles appearing often, and many ways to cause loss of function. Therefore the majority of patients have an allele that has never been seen before. Also very deleterious, so common alleles quickly lost.

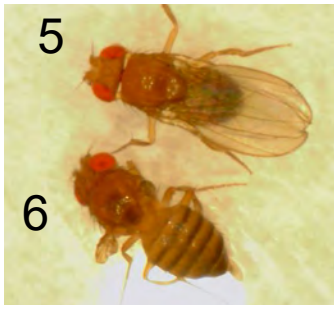
FLY EYES

William Bateson (1861-1926) founded the discipline of modern genetics. Reginald Punnett (1875-1967) created a technique to predict the frequency of phenotypes with Mendelian inheritance, but Edith Saunders (1865-1945) noticed some combinations of traits were not inherited in a Mendelian fashion. To investigate this phenomenon, the genetics of fly *(Drosophila)* traits can be analysed. A fictional *Drosophila* species was crossed according to the following sceme:
WT flies have red-eyes (1) produced by mixing brown and cinnabar pigments.
Mutating the *white* gene gives white eyes (2).
Knocking out the *cinnabar* gene gives cinnabar eyes (3).
Knocking out the *brown* gene gives brown eyes (4).
WT flies have functional wings (5), whilst mutations in the *vestigial* gene lead to vestigial wings (6).
White is carried on the X-chromosome. The genes *brown-cinnabar-vestigial* are carried on the same chromosome, in that order.
Males with white-eyes and normal wings are crossed to females with white-eyes and vestigial wings. All offspring (F1) are red-eyed females, or white-eyed males. No F1 flies had vestigial wings.
The F1 females are mated to a male that has white-eyes and vestigial wings. The female F2 flies from this cross are counted.

F2 (female) phenotype	Number of F2 females
White eyed, normal wings	500
Red eyed, vestigial wings	500
Red eyed, normal wings	50
White eyed, vestigial wings	50
Brown eyed, vestigial wings	5
Cinnabar eyed, normal wings	5





	True	False
The gene *vestigial* is on the X chromosome.		X
The gene *brown* is essential for making the brown pigment.		X
Mutations to *white* disrupt the products of both *cinnabar* and *brown*.	X	

Explanation:
This question was inspired by Kevin Moffat of Warwick University

This question assesses understanding of mendelean genetics, recombination and epistasis, which are alluded to in the stem.

- The parent females had vestigial wings, therefore all the male F1s would also have vestigial wings if this gene was X-linked.
- Mutations in Brown give brown eyes. Therefore, the brown pigment is unaffected.
- Both pigments must be lost to give white eyes.

	0	1	5	9	10
Brown to *cinnabar.*		X			
Brown to *vestigial.*					X
Cinnabar to *vestigial.*				X	

Explanation:
bbccVV male x wv female

gives bc+/++v females and wV/+v males

bcV/BCV female x bbccvv males

Gives
b-----c-----V = 500
b-----c------v

B-----C-----v = 500
B-----C------v

B-----C--x--V = 50
b------c------v

b-----c---x--v = 50
b-----c------v

b---x---C----v = 5
b-------c-----v

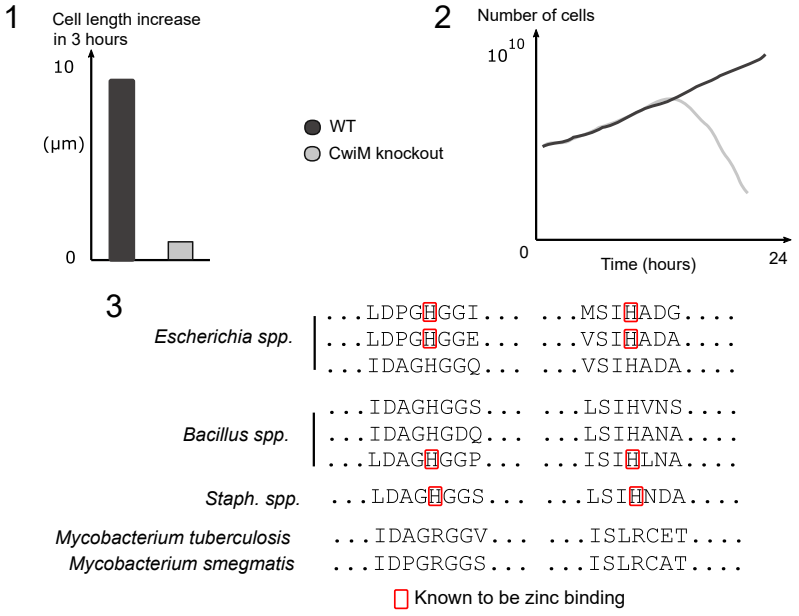
B---x---c----V = 5
b--------c----v

- c and b
  10/1110 = 0.9 cM
- B to v
  110/1110 = 9.9cM
- C to v
  100/1110 = 9 cM

BUILDING AND GROWING

PROTEIN EVOLUTION

Experiments found that CwiM is a regulator of *Mycobacteria tuberculosis* cell wall formation. The length (1) and number (2) of WT and CwiM knockout cells were assessed. Amino-acid sequence similarity analysis suggests that CwiM is homologous to zinc-dependent enzymes (3).



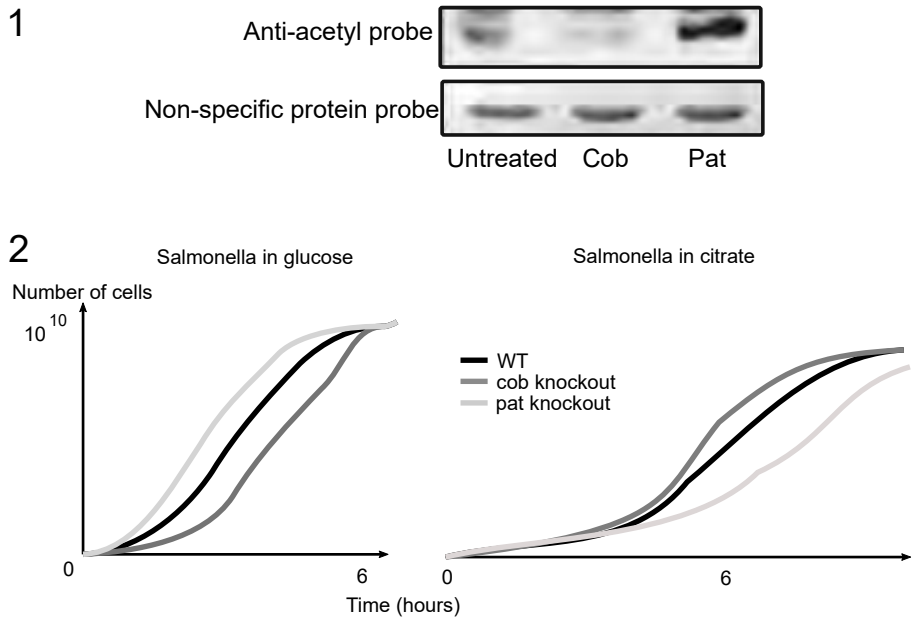
	True	False
CwiM is likely to be zinc-dependent.		X
CwiM increases cell survival rate.	X	
CwiM very likely plays a role in cell elongation in *M. smegmatis*.	X	
Cell elongation is required for survival.		X

Explanation:
This question assesses ability to understand how the role of genes is studied, and sequence alignments / structural data.

- It does not have the conserved Zn binding Histidine.
- The number of cells declines steeply in knockouts.
- The species are in the same genus, and have a very similar amino-acid sequence.
- Elongation is stunted well before cell survival is impacted.

ACETYLATION

Sir Edwin Southern (1938-present) invented a method to visualise specific biomolecules, by separating them according to size, transferring them to a membrane and using specific probes for them. Protein modifications, including acetylation can be visualised in a similar way (Western blotting). WT *Salmonella* had their proteins extracted, and acetylation of a metabolic enzyme was investigated after treatment with purified enzymes pat or cob (1). The role of pat and cob in *Salmonella* growth was assessed in knockout cells (2).



	True	False
Cob adds acetyl groups to proteins.		X
More pat treated protein than cob treated protein was used in this analysis.		X
Peak growth rate is equal whether *Salmonella* are feeding on glucose or citrate.		X
Growth on citrate is initially slow because it takes time for cells to reduce pat activity.		X

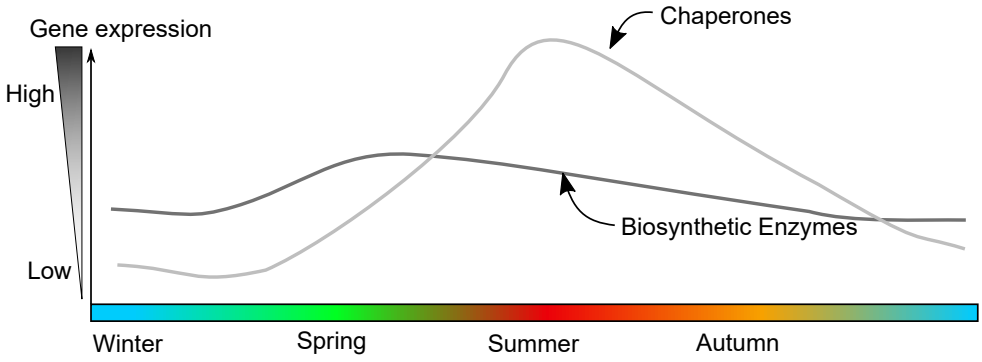
Explanation:
This question was inspired by Adam Heath of the University of Cambridge.

Topics of the previous question are extended to include post-translational modifications to gene products.

- Anti-acetyl staining is less intense after cob treatment.
- Total protein staining shows the same number of cells were loaded.
- The slope is less steep when growing on citrate.
- Pat knockouts also have a slow initial growth rate in citrate.

CORK

Cork oak *(Quercus suber)* produces suberin, a highly resistant material, from fatty acids, and uses it to reinforce the cell walls of cork. The expression of genes specific to cork tissue was measured across the year, and the genes were grouped into biosynthetic enzymes, or chaperone proteins. Sir Hugh Pellham (1954-present) discovered chaperones, which assist other proteins to fold properly.



	True	False
Cork tissue grows fastest in late spring.	X	
The enzymes produce hydrophilic molecules.		X
Increased expression of chaperones may allow cork to continue growing, despite higher temperatures.	X	

Explanation:
This question was inspired by Dimitar Epihov of The University of Sheffield.

This question builds on the themes of metabolism and post-translational modifications to understand how they affect development, and are influenced by the environment.

- Is true from field observations. Demonstrated by higher biosynthetic enzyme expression in late spring.
- Cork is strong and impermeable.
- Heat damages cells by causing proteins to denature. Chaperones can assist their folding, to prevent this. These chaperones are 'heat-shock proteins'. Their expression is highest in the hottest months.

QUININE BIOSYNTHESIS

The UK has undertaken to cure 1 billion cases of neglected tropical diseases, and develop new treatments, from 2017-2022. Quinine-like drugs are useful treatments for several diseases, but their synthesis by *Cinchona* trees requires many steps. Enzymes I and II were seen to catalyse a range of substrates into a range of stereoisomers, reversibly.

	True	False
Overexpressing enzyme I may increase quinine synthesis.		X
Modifying enzyme II may change the ratio of quinine:quinidine in plants.	X	
Nitrogen uptake in the roots may increase if enzyme II is overexpressed.	X	
Trees containing the largest amount of quinine, also contain the largest amount of quinidinone.	X	

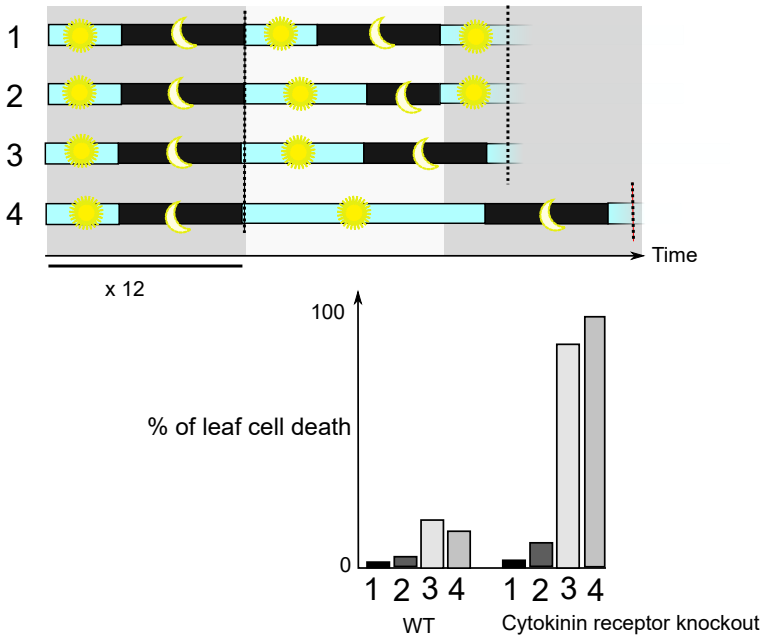
Explanation:

This question delves deeper into the regulation of metabolism at a systems level.

- Increases flux through Cinchoninone arm.
- Can alter which product it favours.
- Increased flux through these pathways requires incereased amino acid catabolism, and therefore increased anabolism, and therefore increased N uptake.
- Reaction is reversible, therefore at equilibrium, there is a stable ratio between the two.

DAY-NIGHT CYCLES

Cytokinin is a plant hormone involved in stress signalling, which may affect responses to day-night cycles. *Arabidopsis* plants were grown under 8 hours light / 16 hours dark for 12 days, then four different light / dark regimes for one cycle. Patches of cell death were measured in leaves after this.



	True	False
Increased light exposure is the major cause of cell death in cytokinin receptor knockouts in this experiment.		X
A condition of 12 h light and 12 h dark will lead to a high percentage of cells dying in cytokinin receptor knockout plants.		X
Cytokinin increases leaf stress.		X
Cytokinin helps adjust expression of circadian clock related genes.	X	

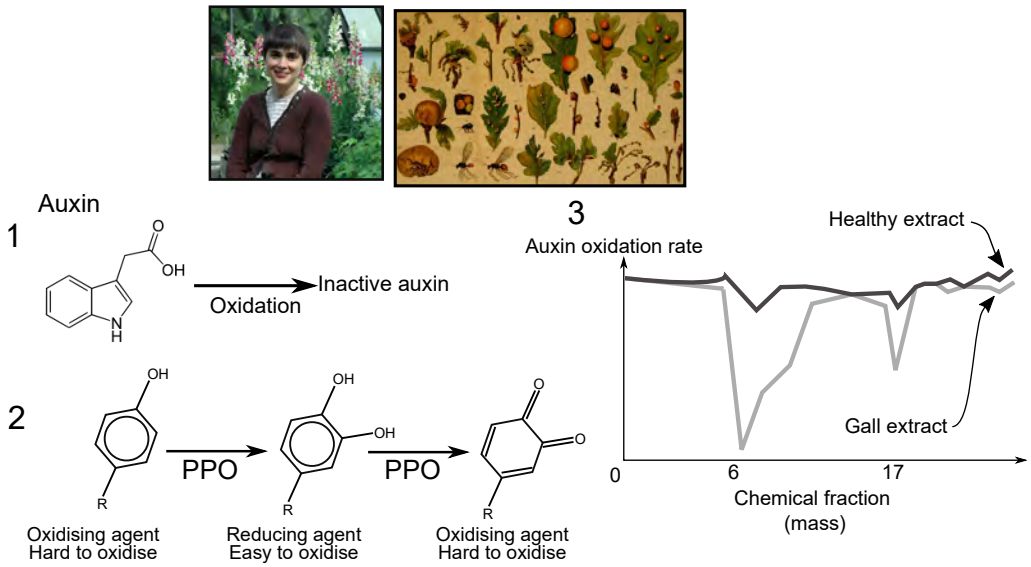
Explanation:
This question was inspired by Quyen Nguyen Van of the Vietnam Olympiad.

This question ties together the themes of metabolism, studying gene function, and environmental influence.

- Compare 2 to 3. Increased light exposure does not increase cell death to anywhere near the same extent as changing the overall day+night length.
- See above
- When the receptor is lost, death/stress increases.
- Accounts for the very severe inability of receptor knockout plants to cope with changed day+night lengths.

OAK GALLS

Dame Ottoline Leyser (1965-present) showed that auxin is a plant hormone with roles in environment-development interactions. For example, English oak *(Quercus robur)* is attacked by gall wasp larvae *(Cynipidae)*, which cause tumorous galls to grow.
Auxin can cause tumour growth, but auxin is degraded by oxidation (1). Plants use the enzyme PPO to control oxidation of phenolic chemicals (2), but auxin is *not* a phenolic compound.
Different chemical fractions (based on mass) were taken from galls and mixed with auxin and an oxidising agent. Auxin oxidation rate was then measured. Only fractions 6 and 17 contained mostly phenolic compounds (3).



	True	False
Auxin activity is high in galls.	X	
Compounds in fractions 6 and 17 are oxidised more easily than auxin.	X	
Phenolic compounds are responsible for oxidising auxin in galls.		X
Secretion of auxin-like compounds by these gall wasp larvae is necessary to drive gall formation.		X
An upregulation of PPO in galls, compared to healthy tissue, could explain these results.	X	

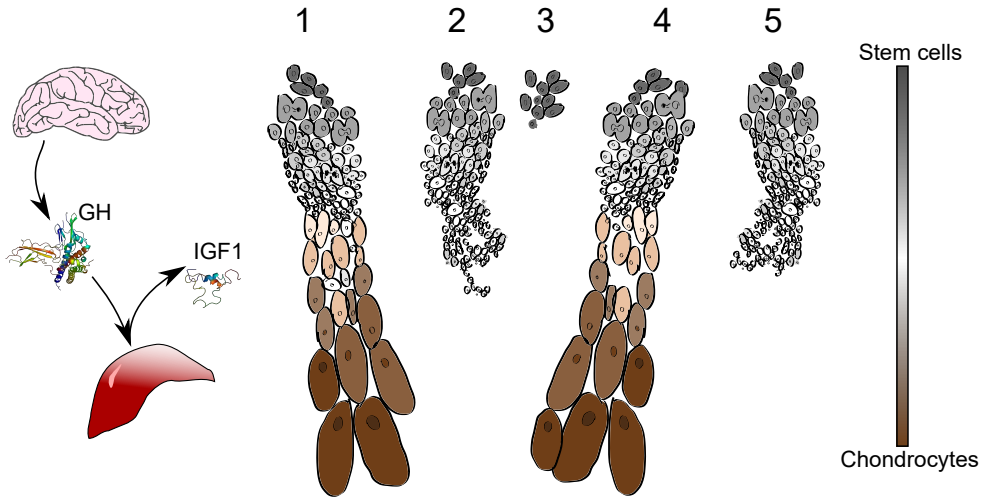
Explanation:
This is a complex question designed to thoroughly asses understanding of fundamental biochemistry, also with a developmental and metabolic theme.

Reference: Presence of auxin protectors in Eriophyes induced Zizyphus stem galls, 1980, Experientia, P. Tandon and H. C. Arya

- Would explain overgrowth, and auxin oxidation is slower in gall extracts.
- Explains why auxin oxidation is slower in the extracts - oxidising agents draw electrons of these compounds, before degrading auxin.
- The phenolic compounds dominate the fractions that reduce auxin oxidation.
- There is no need, since they act to reduce auxin oxidation instead.
- An upregulation explains how gall wasps increase the ability of phenolic compounds to protect auxin from oxidation. An increase in PPO gives more reducing agent.

GROWTH PLATES

Growth hormone (GH) is secreted by the brain, and can stimulate the liver to secrete insulin-like growth factor 1 (IGF1).
Bones, formed from chondrocytes, elongate from terminal growth plates as animals grow.
(1) A healthy, active growth plate. All the growth plates injected with GH activated.
(2) GH was injected into rat growth plates in combination with IGF1 inhibitors.
(3) All GH was inhibited in a rat.
(4) GH injected into a rat growth plate with a liver specific IGF1 knockout.
(5) GH was added onto stem cells in a dish.



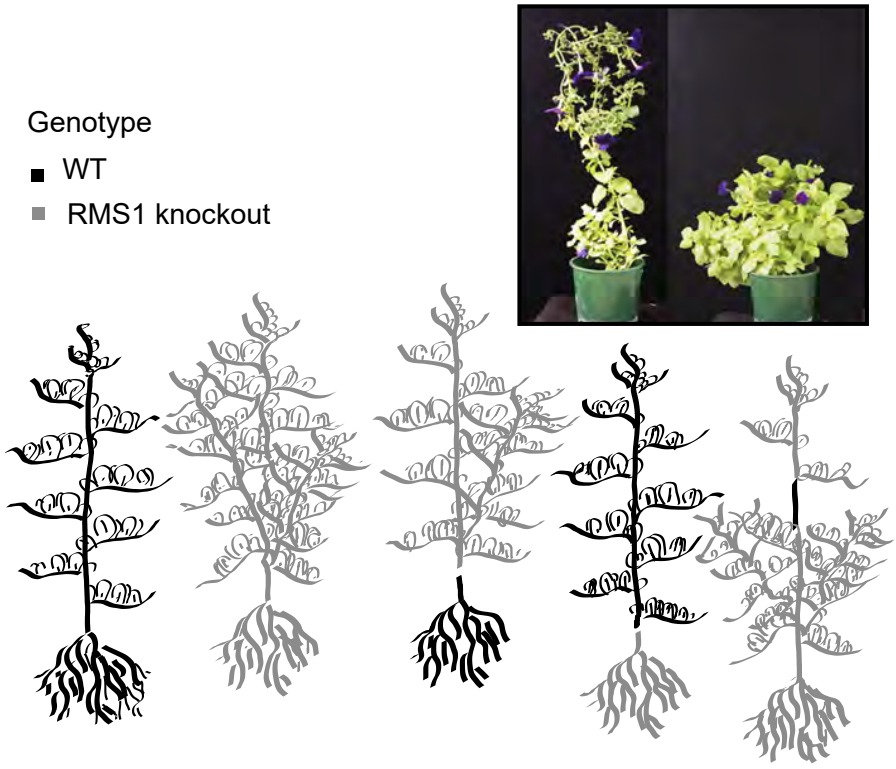
	True	False
Mutations in *GH* can lead to dwarfism.	X	
IGF1 is necessary for chondrocytes to enlarge.	X	
Injecting IGF1 would lead to bone elongation, even if GH is absent.		X
IGF1 is produced by chondrocytes, as well as the liver.		X

Explanation:
This question requires further consideration of growth, epistasis and non-genetic ways to study hormone function.

- GH knockouts show no stem cell proliferation.
- Without IGF1, stem cells proliferate, but do not enlarge and mature.
- GH stimulates stem-cell proliferation. Without prior proliferation, there will be insufficient numbers of chondrocytes for IGF1 to enlarge, so bones will not elongate.
- Growth plates appear normal even if liver IGF1 is blocked, therefore there is a non-liver source. However, this cannot be chondrocytes, since if GH is added to chondrocytes in a dish, they proliferate to resemble growth plates treated with an IGF1 blockade. IGF1 is actually released from cells surrounding the growth plate upon GH stimulation.

SHOOT BRANCHING

The *RMS1* gene encodes a signalling protein which is altered in modern crops. Its activity can be studied by grafting together seedlings from different plants, and monitoring their growth.



	True	False
RMS1 activity increases shoot branching.		X
The signal produced by *RMS1* travels towards the shoot tip rather than towards the roots.	X	
RMS1 activity in the root is sufficient to give WT plant architecture.		X
RMS1 activity in the root is necessary to give WT plant architecture.		X

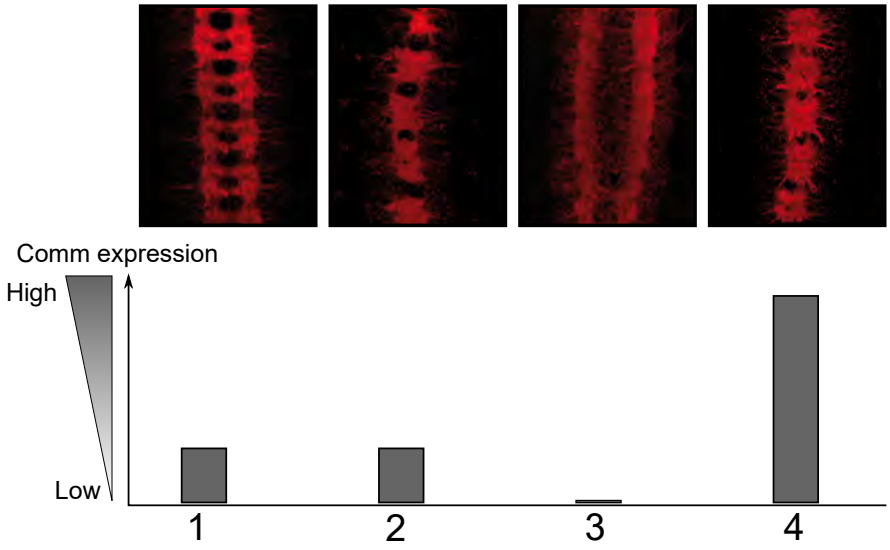
Explanation:
This question was inspired by Sam Deans of the University of Cambridge.

This is a more simple question, and considers ways to analyse plant physiology in a similar vein to the previous question.

- Knockouts show more branching.
- RMS1 knockout branches appear normal above a WT section of stem, but not below it.
- The plants' architecture is somewhat rescued, but still shows a failure to establish apical dominance fully.
- WT stem alone is sufficient to restore full apical dominance.

SLIT COMM ROBO

In *Drosophila* embryos, developing neurons (red) grow along two columns parallel to the midline.
Some traverse the midline and some do not, creating a ladder. Midline cells secrete a protein, called Slit, which is detected by the receptor, Robo. Comm, a transcription factor, controls Robo expression. To investigate how this guides neurons, WT (1), *Robo* knockout (2), *Comm* knockout (3), and *Comm* overexpression (4) flies were dissected.



	True	False
Neurons cross the midline more often in *Comm* knockout flies, than WT flies.		X
Neurons are repelled when they detect Slit.	X	
Comm acts to increase Robo expression.		X
Neurons increase Comm expression once they cross the midline.		X

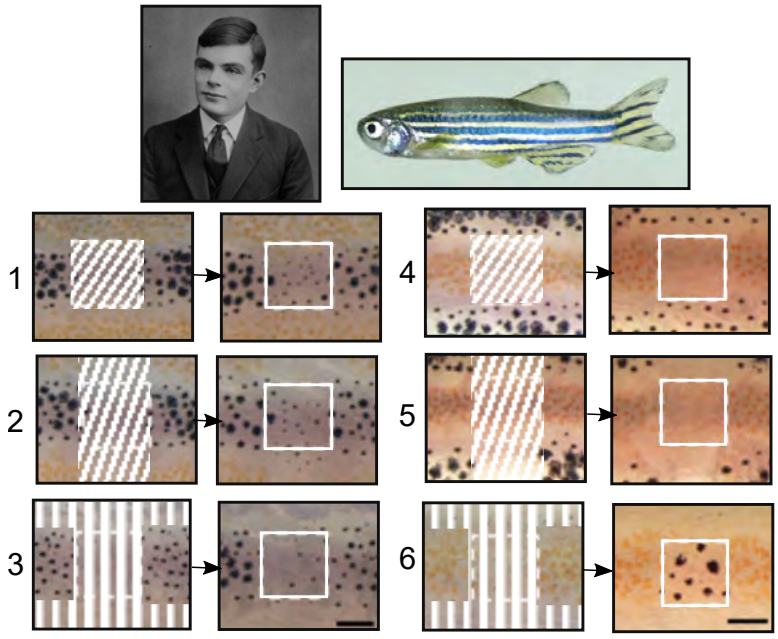
Explanation:
This question was inspired by Kevin Moffat of Warwick University

This is quite similar to the previous question, in a very different context, with added complexity.

- 3 shows more neurons running in parallel tracks either side of the midline, and not crossing it, than 1.
- Robo knockouts (2), cross the midline more frequently. This indicates that the robo receptor usually guides neurones away from the slit expressing cells of the midline.
- Com knockouts have the opposite phenotype to Robo knockouts, and Comm overexpression gives a similar phenotype to Robo knockouts. This indicates Comm is likely to repress Robo.
- A ladder can be produced if neurons cross the midline only infrequently. Therefore, after crossing, they should be more sensitised to Slit for a time, by increasing Robo expression. This requires Comm expression to be reduced upon crossing the midline.
  Neuron crosses midline > com expression decreased > robo upregulated > sensitised to slit > stronger repulsion from midline > does not cross again.

TURING PATTERNS

Alan Turing (1912-1954) famously invented the fields of computer science and artificial intelligence. Turing also published equations explaining spontaneous pattern formation in biology.
Dark zebrafish *(Danio rerio)* stripes are formed by collections of dark cells (melanophores), whilst brown stripes are produced by collections of lighter cells (xanthophores). To investigate whether zebrafish stripes are a Turing pattern, pigmented cells were surgically removed (dashed regions), and the appearance of melanophores from precursor cells was measured a fortnight later. The scale bar in the bottom right corner is 1 mm.



	True	False
Melanophore formation is stimulated by other melanophores immediately next to the precursor cells.		X
Precursor cells from different stripes are predetermined to become either melanophores or xanthophores.		X
Melanophore formation is stimulated by other melanophores >0.5 mm from the precursor cells.		X
Melanophore formation is stimulated by xanthophores >0.5 mm from the precursor cells.	X	

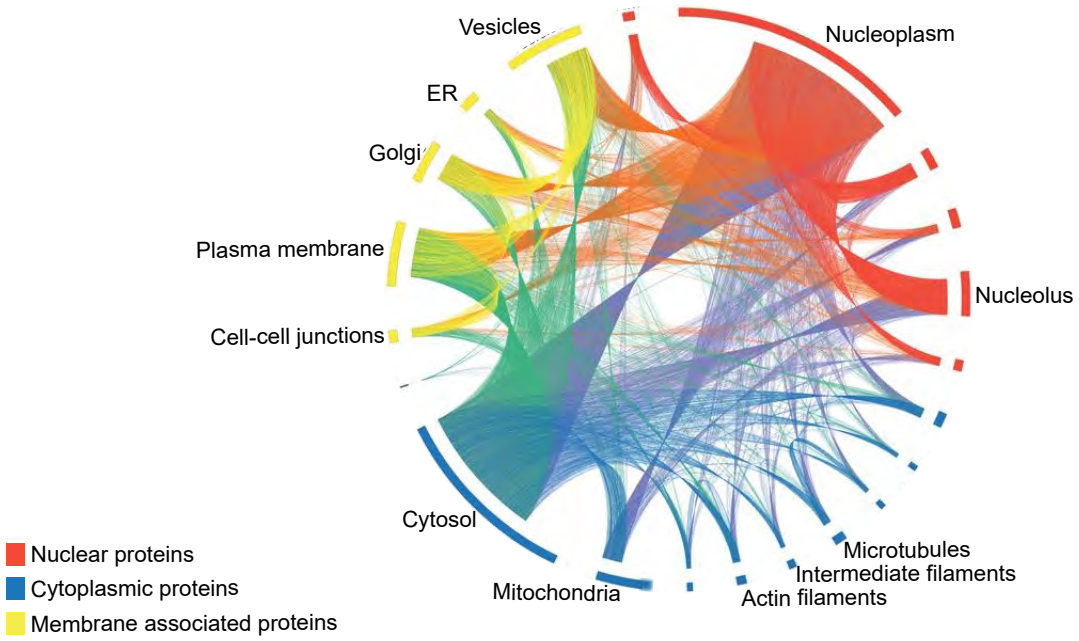
Explanation:
Turing patterns are explained by complex equations, but this question considers simple manipulations to get at the principals. Candidates ability to make observations and understand their meaning is assessed.

- Comparing 1,2&3 to 6, melanophores of the same stripe to not lead to a noticeable increase imediately next to them. And new ones do not encroach inwards from the edges.
- This is shown not to be the case in (6)
- Compare 4&5 to 6; melanophores are repressed by adjacent melanophore stripes.
- Compare 1&3: melanophores appear more frequently when there are nearby xanthophores. And a large number of large ones appear when inhibition by adjacent melanophores is blocked.

RESPONDING TO THE WORLD

CELL PROTEIN ATLAS

The location of proteins in a cell affects their function by determining which molecules they interact with. A collaboration of scientists in Sweden and Cambridge used microscopy to map the location of >12 000 human proteins in numerous cell types. In the diagram below, solid bars around the circumference represent all the different types of protein within each organelle, and lines are drawn between the same type of protein in different organelles.



	True	False
Most proteins are found only in a single organelle.		X
Most mitochondrial proteins are found only in the mitochondria.	X	
Most proteins that are found in multiple organelles are spread homogeneously through the cell.		X

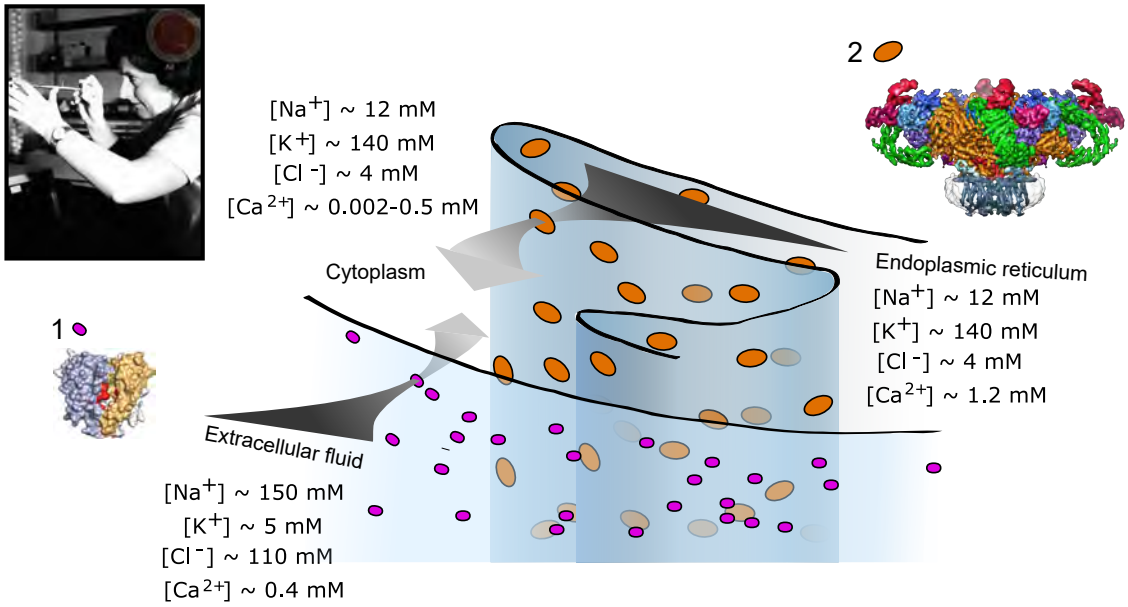
Explanation:

This question assesses the ability to understand 'big data'.

- Most of the lengths of the bars are connected to others.
- Most of the length of the mitochondrial bar is unconnected.
- Most links are between neighbouring organelles or sub-compartments.

CALCIUM SIGNALLING

Jean Hanson (1919-1973) discovered how muscles contract. Contraction is triggered by a build up of calcium in the cytoplasm, which is actively removed during relaxation. Calcium diffuses through plasma membrane channels (1), or endoplasmic reticulum membrane channels (2).
(1, purple) Plasma membrane channels, opened by depolarisation, pore width = 1 atom
(2, orange) ER channels, open by binding calcium on cytoplasmic face, pore width = 100s of atoms
The concentrations of the major biological ions in different compartments are shown.



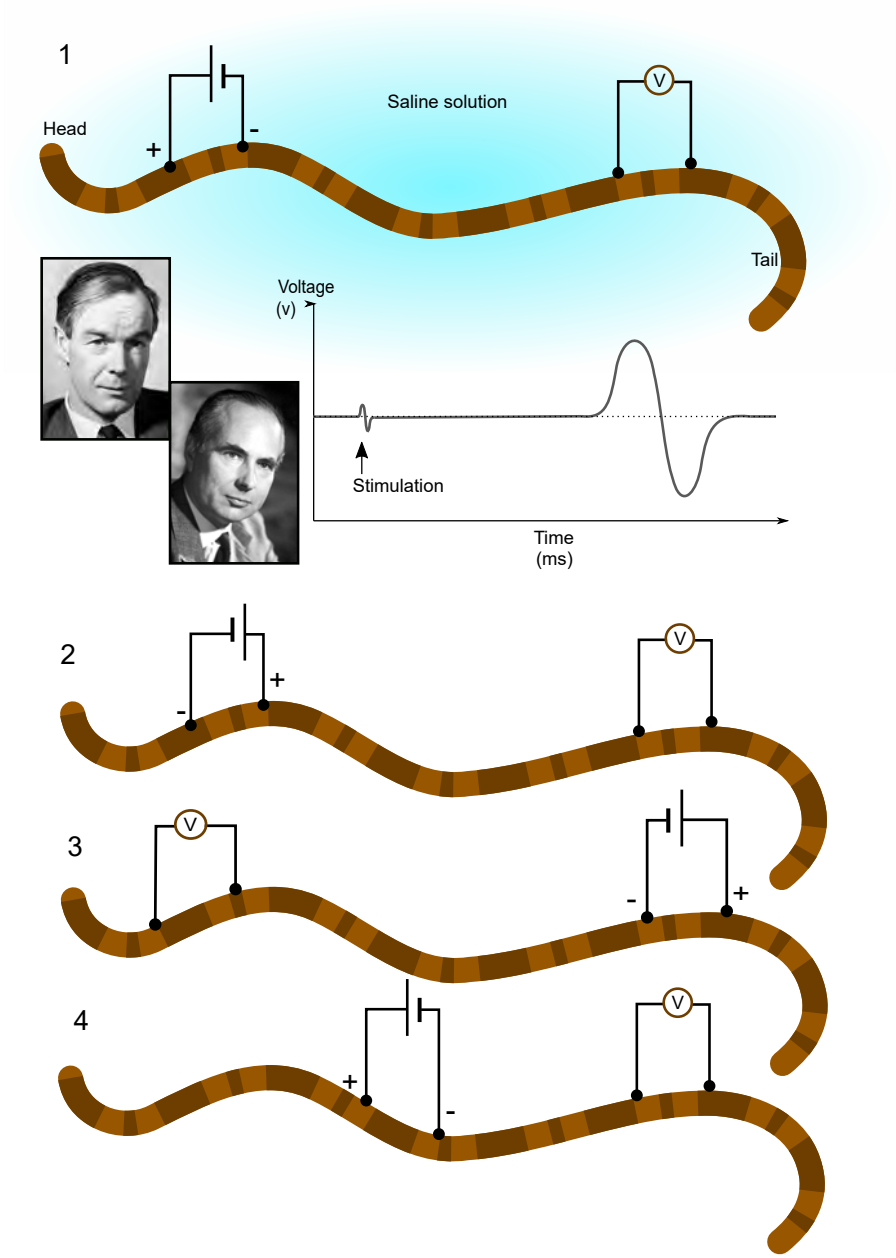
	True	Fasle
Peak calcium flow into the cytoplasm is greater from the intracellular than extracellular stores.	X	
Cytosolic calcium has to be moved against an electrical and chemical gradient to be returned to the endoplasmic reticulum.		X
Muscle performance is improved by artificially expressing channel (2) on the plasma membrane.		X
Peak calcium flow is greater in high frequency muscle than to low frequency muscle.	X	

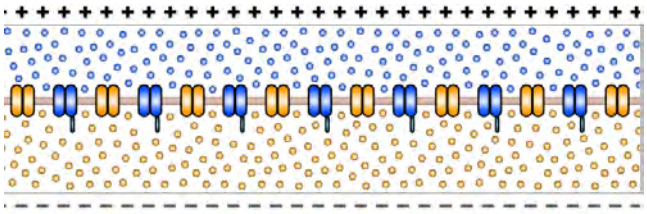
Explanation:
This question is about cellular physiology. Candidates need to consider multiple aspects of geometry and electrochemical gradients to understand how contraction is triggered.

- Intracellular channes have a much larger pore, to allow higher fluz; the ER membrane has a much larger surface area than the PM; and the Ca2+ concentration gradient is much larger between the ER and cytoplasm.
- The other ions are in equilibrium across the ER membrane. They are in disequilibrium across the PM. Therefore, Ca2+ must be pumped up and electrochemical gradient out of the cell, but just up a chemical gradient into the ER.
- The large diameter ER Ca2+ channels are also permeable to Na+ and K+, therefore they would severely disrupt muscle AP transduction, inhibiting performance.
- Must go in to cause contraction, and back out to cause relaxation, quicker.

ACTION POTENTIAL PROPAGATION

Sir Alan Hodgkin (1914-1998) and Sir Andrew Huxley (1917-2012) explained how action potentials occur and spread.
(i) Resting neurons have a negative voltage (membrane potential) across their membrane (more negative inside than outside).
(ii) If the potential becomes less negative, ion channels open, making the voltage become positive.
(iii) Channels shut in a time dependent fashion.
(iv) Channels cannot re-open until the membrane returns to the resting negative voltage.
(v) As one section of membrane becomes positive, electrical currents make adjacent sections less negative.
Artificial action potentials can be triggered and measured in a giant neuron which runs the length of worms. A stimulating pair of electrodes are placed close to each other, against the worm, and a voltmeter placed further along is used to record the passing action potential. The trace from setup (1) is shown. Other setups (2, 3 & 4) were also tested.





	True	False
If the voltmeter electrodes are swapped in setup (1), action potentials can still be measured.	X	
Action potentials can still be measured in setup (2).		X
Action potentials can still be measured in setup (3).	X	
Measured action potentials will have a larger magnitude (reach a higher voltage) in setup (4) than setup (1).		X

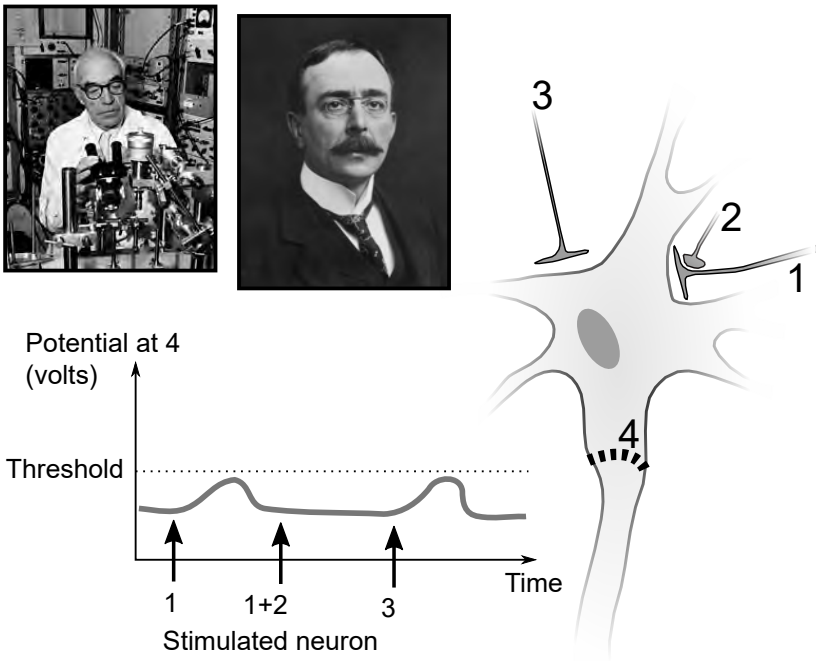
Explanation:
This question was inspired by Becky Peel of the University of Cambridge.

Electrochemical physiology is further explored, and a method to investigate it is analysed.

- The trace would simply be flipped upside down, since it is arbitary which electrode is measured relative to which.
- The + electrode forces a patch of PM to be stuck in a polarised state (+ on the outside), which prevents action potentials passing this point. (making this patch of membrane more hyperpolarised will not trigger an AP here).
- There is no inherant directionality to an axon, and the described mechanism does not dictate one.
- The described mechanism generates all or nothing APs.

SYNAPTIC INFORMATION PROCESSING

Sir John Eccles (1903-1997) and Sir Charles Sherrington (1857-1952) discovered the different roles of synapses, which dictate how neurons respond to stimulation from each other.
Neurons 1, 2 and 3 were artificially fired, and their ability to initiate an action potential at point 4 was assessed.



	True	False
Simultaneous stimulation from neurons (1) and (3) can fire (4).	X	
Neuron (2) stimulates neuron (1).		X
The potential of (4) becomes more negative when (2) alone fires.		X
If (3) was fired twice in quick succession, (4) would fire.	X	

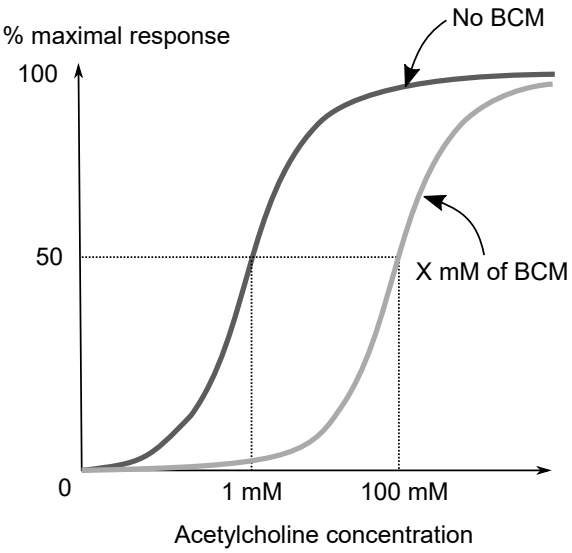
Explanation:
This question was inspired by Mats Carlberg of Sweden.

The exploration of electrophysiology culminates in exploring how the brain processes information.

- The combined depolarisations of both would exceed threshold.
- Neuron 2 blocks signals travelling down neuron 1.
- Neuron 2 does not innervate neuron 4.
- Testing the idea of frequency as well as networking. Two fires from 3 would lift 4 above the threshold.

RECEPTOR INFORMATION PROCESSING

Whilst Lewis Wolpert (1929-present) hypothesised graded responses to signals control animal development, Sir John Gurdon (1933-present) was the first scientist to relate the number of activated receptors to different cell responses.
The neurotransmitter acetylcholine can produce a graded response, but if the inhibitor BCM is above the critical concentration (>X mM), a cell's response to acetylcholine is significantly below maximal at all acetycholine concentrations.
Each acetylcholine receptor has one binding site for the neurotransmitter acetylcholine.



	True	False
BCM is a competitive inhibitor of the acetylcholine receptor (binds in the same way as acetylcholine).		X
Increasing the number of acetylcholine receptors increases cells' sensitivity to acetylcholine.	X	
Individual receptors produce 50 % of their maximal signal when treated with 1 mM acetylcholine.		X

Explanation:
This question was inspired by Kieran Toms of the University of Cambridge.

Synapses are explored in a more mathematical and biochemical way to explore another way in which cells process information.

- Above a critical level, no amount of Ach can give a maximal response, indicating a non-competitive mechanism.
- As BCM inactivates AchRs, sensitivity decreases, therefore, increasing the expression of AchR would increase sensitivity.
- Each receptor has one Ach binding site, so is therefore on, or off. (As explained above, these kinetics are explained by the presence of a 100 fold excess of AchRs gradually being inactivated by increasing BCM).

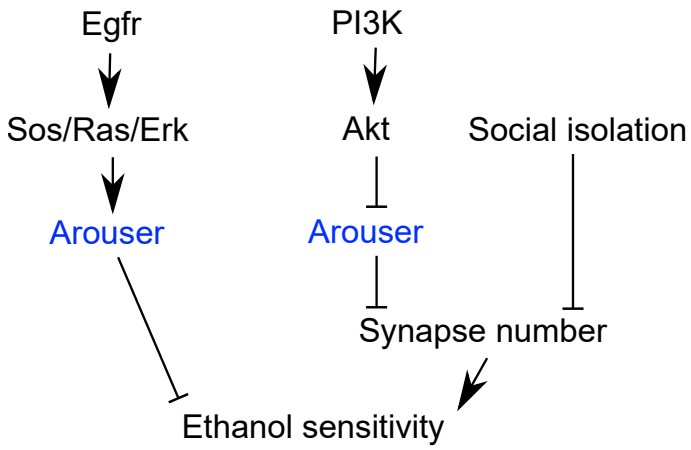
	1 %	25 %	50 %	75 %	100%
Choose the nearest proportion to the correct answer.	X				

Explanation:

- At X mM BCM, there are just enough AchRs to give a maximal response. At this level 100x more Ach is needed to give a 50 % response (affinity is only 1/100 of what it was). This is consitent with all but 1% of AchRs being inactivated by X mM of BCM.

DRUNK FLIES

The most important signalling pathways of development and cancer were discovered by epistasis analyses in fruit-flies *(Drosophila melanogaster)*. Such screens also showed the protein encoded by the Arouser gene mediates signals along the Epidermal growth factor receptor (Egfr) pathway in neurons. *Arouser* knockout flies are unusually sensitive to ethanol (get drunk easily).



	True	False
Wild type Arouser facilitates alcohol tolerance.	X	
Blocking Egfr activity increases alcohol sensitivity.	X	
Over-expression of *Akt* in *arouser* knockout flies increases alcohol sensitivity.		X
Socially isolating *arouser* knockouts makes them more like WT flies.	X	

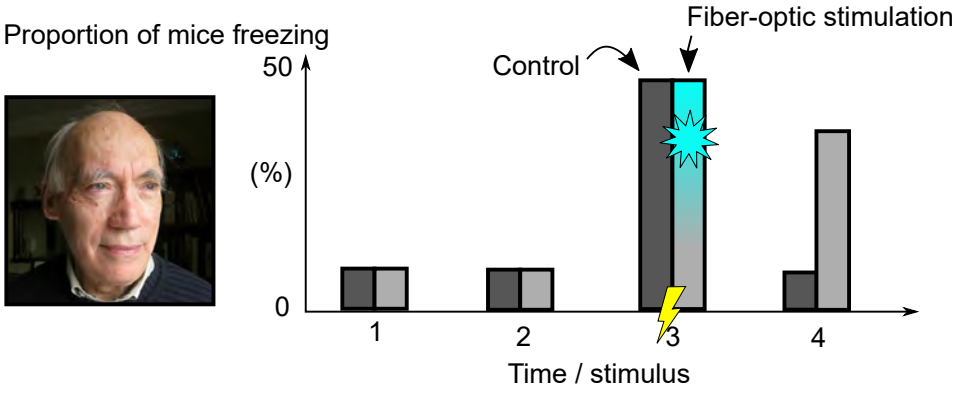
Explanation:
This question was inspired by Kevin Moffat of Warwick University.

The combined influence of receptor signalling, enzyme cascades, and environmental influences on a complex behaviour is analysed.

- Arouser decreases alcohol sensitivity, thus increasing tolerance.
- EGFR activates Arouser. And so stopping Arouser stops the decrease in sensitivity, i.e. it increases it.
- AKT signals through Arouser and so can have no effect in an arouser knock out.
- Social isolation has the same effect as arouser, and so isolation will make mutants more like WT.

OPTOGENETICS

Tim Bliss (1940-present) discovered that stimulating a neuron sensitises it to future stimulation, and that this is a basis of long term memory. This can occur because stimulation leads to transcription of ion channel genes. One of these channels was fused to a light sensitive protein, which allows it to be activated by fiber-optic implants, and cloned into mice.
The mice were treated as follows:
(1) Transcription of this channel was blocked in every cell, until adult mice were first shown a particular, non-threatening stimulus.
(2) Transcription of this channel was re-blocked in every cell.
(3) Mice were given an electric shock, causing them to freeze. In a subset of mice, electrocution was accompanied by a flash of fiber optic light to the brain.
(4) Mice were shown the non-threatening stimulus again.



	True	False
The freezing behaviour in (4) is explained by mice forming an association between flashing lights and electrocution.		X
The same neurons are activated by electric shock and fiber optic lights.		X
Flashed mice have a false memory of the non-threatening stimulus.	X	
Control mice are likely to freeze if they are shown the electric shock apparatus after the experiment.	X	

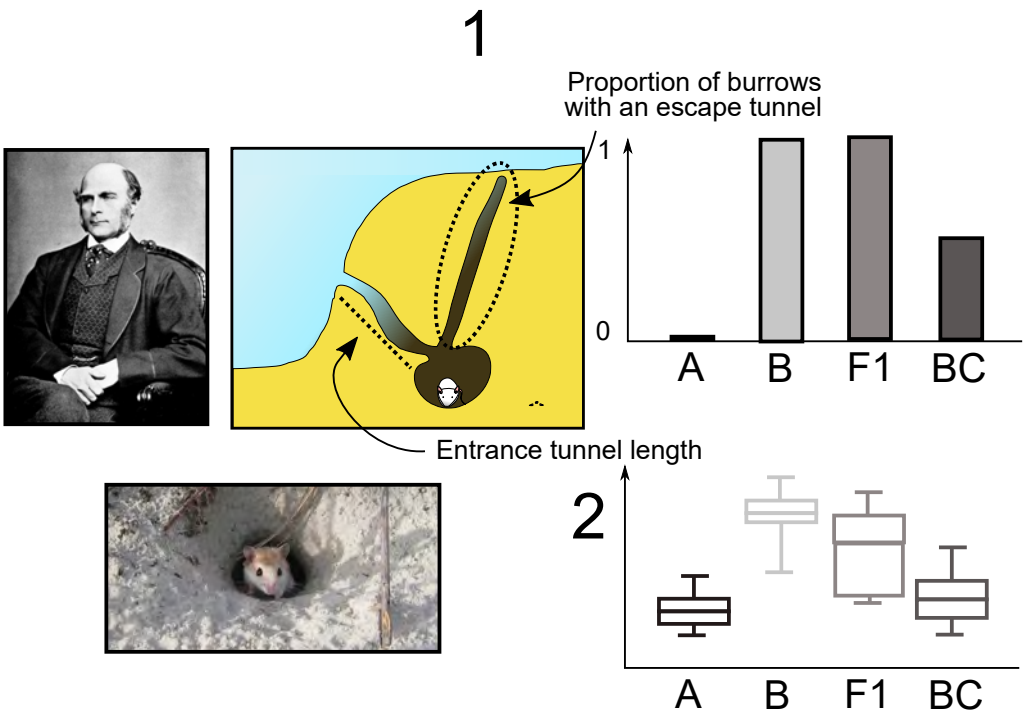
Explanation:
This question was inspired by Remie Janssen of the Dutch olympiad.

This is a complex question that requires careful thought to work out which neurones are being triggered at each step, exploring how neurones are able to process memories.

- In 1, mice express the light sensitive protein specifically in neurons activated by the non-threatening stimulus. In 3, mice are electrocuted. In a subset, light is used to activate a memory of the non-threatening stimulus, as the electrocution occurs. These mice form a false association between the non-threatening stimulus and electrocution. Therefore, the mice become scared of the non-threatening stimulus.
- See above.
- See above.
- They form a true association between the equipment and the shock

BURROWING MICE

Sir Francis Galton (1822-1911) invented the field of behavioural genetics.
Burrowing mouse *(Juscelinomys)* burrows have quantifiable features, including presence or absence of an escape tunnel (1) and length of the entrance tunnel (2).
Species A and Species B were crossed. The first generation of the cross (F1), were back-crossed (BC) with Species A.
In BC mice, there is no correlation between entrance tunnel length and the presence or absence of an escape tunnel.



	True	False
Entrance tunnel length is determined by a single locus (gene).		X
Alleles for the presence of an escape tunnel are dominant to those for absence of an escape tunnel.	X	
F1 hybrids can be distinguished from Species A and Species B by looking at their burrows.		X
Genes determining entrance tunnel length and the presence or absence of escape tunnels are close to each other in the genome.		X

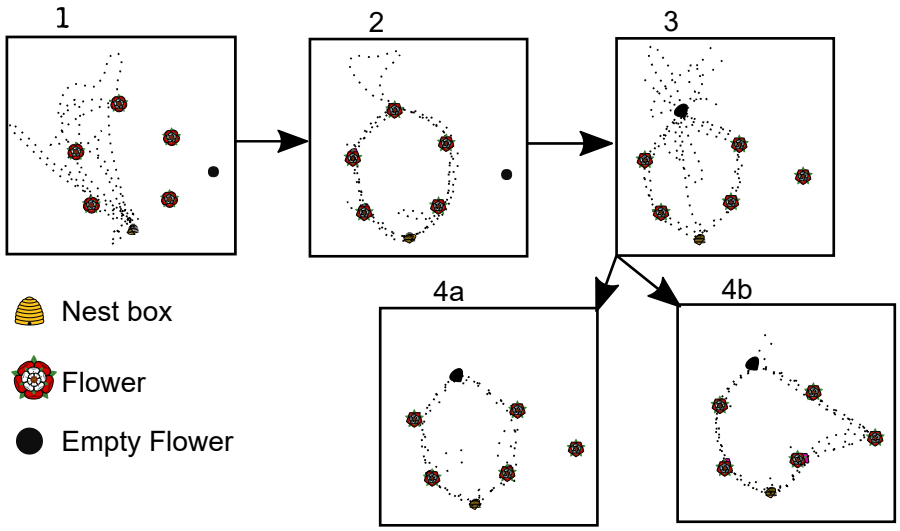
Explanation:
This question was inspired by Katherine Lister of the University of Cambridge.

Understanding of monogenic versus polygenic inheritance is assessed, in the context of natural behaviour.

- There is a large spread in size in the F1, suggesting more than one locus. (2)
- The F1 all have escape tunnels, showing dominance of this allele. (1)
- Due to the variability in tunnel length, F1 and B cannot be distinguished. (2)
- False, as there is no correlation between the two traits in the backcross individuals, they segregate independently. (Stem)

TRAVELLING SALES-BEES

Radar, invented in the UK, was used to track the flight of English bees *(Apis mellifera)*. To investigate how bees forage, the following experiment was performed:
(1) Fake flowers were filled with syrup, and the bees were released from a nest-box.
(2) Bees were given time to build experience of the setup.
(3) Syrup was removed from one flower, leaving it empty, and placed in another.
(4) Bees were allowed to build experience of the new set up. Representative flights are shown (a & b).



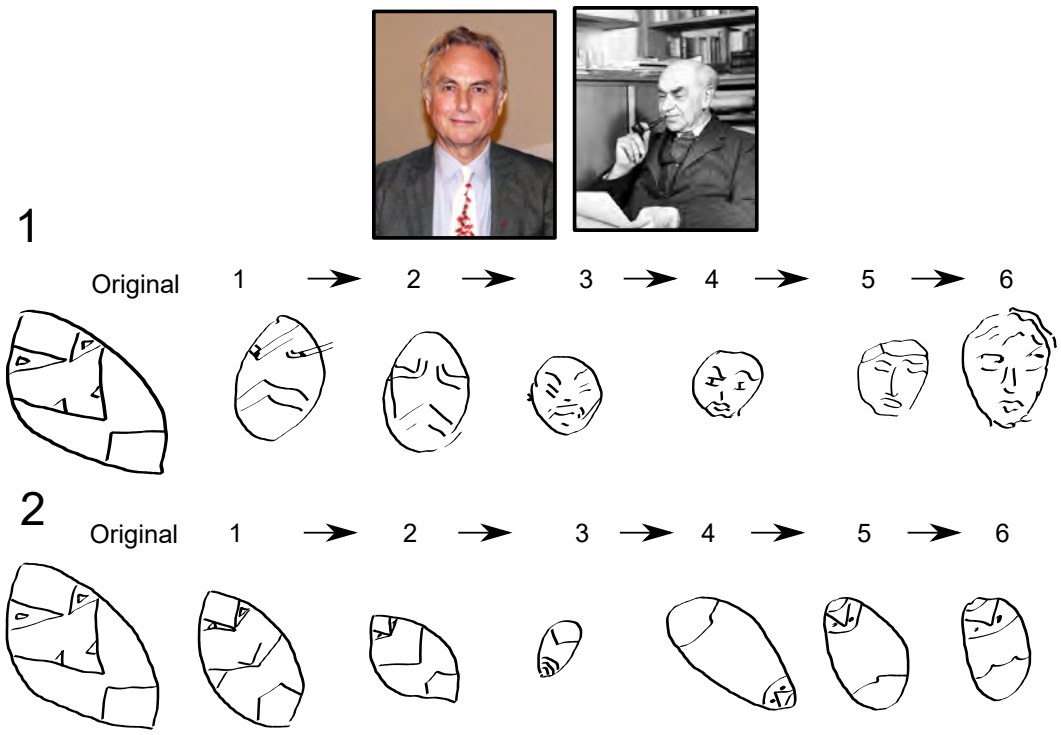
	True	False
Bees continually attempt to optimise the shortest route to harvest syrup from the flowers.		X
Experienced bees do not search for new flowers unless the environment changes.		X
Bees exhibit signs of confusion if they encounter unexpected features on their route.	X	
Bees visit all the nutritious flowers within range of their nest.		X

Explanation:
The question requires creativity to analyse the behaviour of an animal very different to humans, and how they respond and learn.

- They do not move to the shortest route, continuing to visit the deceased flower, even once they know it's empty.
- Exploratory loops are still present even after bees become used to an environment.
- Bees explore wildly about the missing flower when it's first removed.
- Bee 2 never visits one flower, even though it's within range of bee 1

MEMES

Richard Dawkins (1941-present) invented the idea of memes, and Sir Frederic Bartlett (1886-1969) showed previous knowledge alters the processing of new stimuli. Bartlett asked British people to reproduce a drawing of Native American masks from memory. This was passed to a new person to memorise and reproduce, and the reproduction passed on, several times in succession (1). This study has been repeated recently (2). Representative reproductions are shown.



	Experiment (1)	Experiment (2)
Memories tended to simplify the original object.		X
Objects tended to be remembered as more similar to familiar objects than is actually the case.	X	
The participants of the study were instructed to focus on making accurate reproductions.		X
Memories included features not present on the actual object.	X	

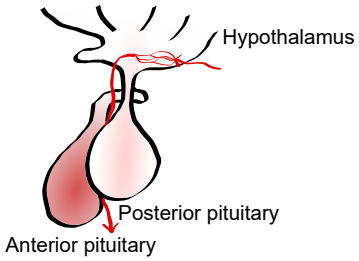
Explanation:

This question finishes the analysis of memory by considering humans.

- The number of strokes, and detail of features (pupils, teeth) decreases versus original. 2 becomes simpler overall at the end of the progression.
- 1 shows a clear tendency to become more like a natural face.
- 2 tends to become less naturalistic, and maintain more of the angular details.
- Hair, noses, eyebrows appear.

THE PITUITARY GLAND

The hypothalamus is the central regulator of homeostasis, whilst diverse hormones from the posterior and anterior pituitary glands orchestrate most bodily functions. To investigate how these three structures coordinate their actions in rats *(Rattus norvegicus)*, each was electrically stimulated, and the effect on hormone secretion from both pituitary glands was observed (1). Secondly, whole rat pituitary glands were transplanted to locations with different blood supplies, to reveal why they usually receive blood from hypothalamic veins (2).



1

		Secretes hormones?	
		Posterior	Anterior
	Hypothalamus	+	+
Electrical stimulation	Posterior	+	-
	Anterior	-	-

2

		Secretes hormones?	
		Posterior	Anterior
	Hypothalamic	-	+
Blood supply	Non-hypothalamic	-	-
	Temporarily non-hypothalamic, hypothalamic supply restored	-	+

	True	False
The hypothalamus controls secretion of hormones from both pituitary glands.	X	
Hypothalamic blood specifically has factors necessary for the survival of the anterior pituitary.		X
Hypothalamic neurons innervate the posterior pituitary.	X	
Hypothalamic hormones cause the posterior pituitary to secrete hormones.		X

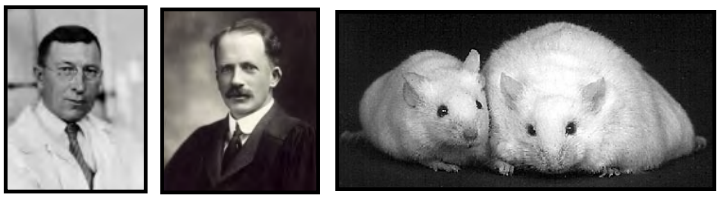
Explanation:
This question was inspired by Scarlet Harris of the University of Oxford.

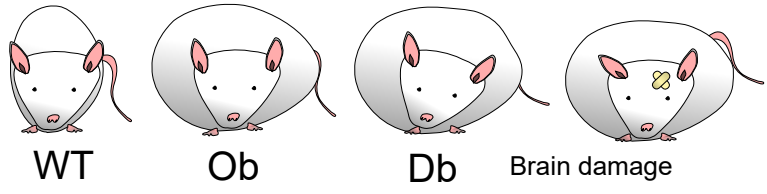
Candidates must use observations to work out how the brain controls hormone production.

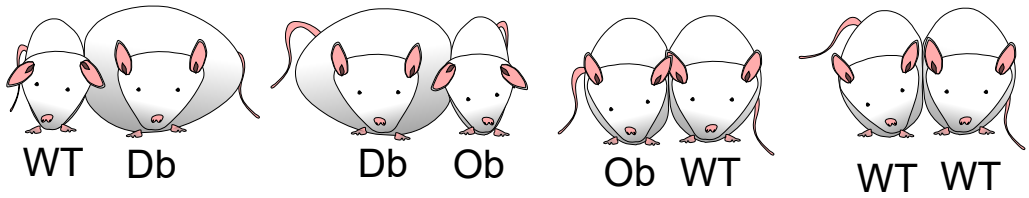
- Electrical stimulation of the hypothalmus stimulates secretion from both. Removing blood from anterior, or signalling from posterior, from the hypothalmus, abolishes secretion from both.
- Function is lost when it is transplanted elsewhere, but regained upon return. This indicates it didn't die whilst elsewhere, but just lacked appropriate signals to drive secretion.
- Electrical stimulation stimulates it, whilst severing this electrical connection abolishes its function.
- See above.

OBESE MICE

Sir Frederick Banting (1891-1941) and John Macleod (1876-1935) discovered insulin and invented modern diabetes treatment. This inspired similar investigations into body weight homeostasis and obesity: two mouse *(Mus musculus)* lines (obese; Ob, & diabetic; Db) have identical over-eating phenotypes. Each is deficient for a single, but different, gene. Damaging the arcuate nucleus in the brain, which is the structure soley responsible for regulating the sensation hunger, produces a similar phenotype. Mice were joined surgically, allowing a small amount of blood to flow between them, and then observed.







	True	False
Surgical connection allows mice to freely share nutrients.		X
Db mutants overproduce an appetite suppressing substance.	X	
The products of genes Ob and Db act in the same pathway.	X	
The WT product of gene Ob promotes a pathway which activates the arcuate nucleus.	X	
Some cases of human obesity can be treated by administering a hormone.	X	

Explanation:
This question presents nobel prize winning data and assesses its meaning.

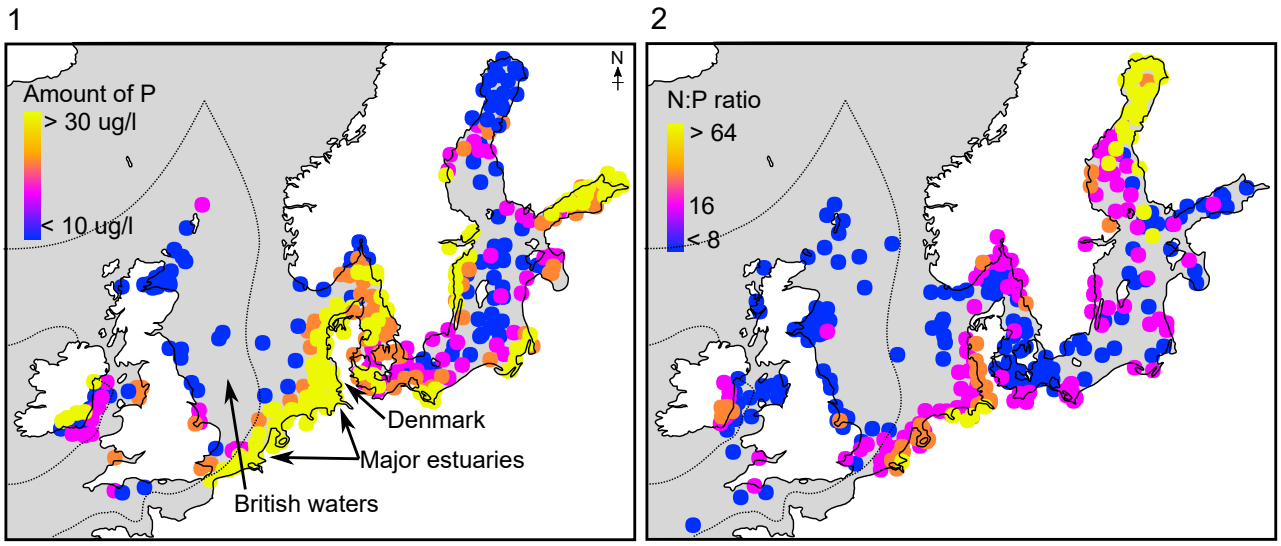
Db = Leptin, Ob = Receptor

- Mice in pairs can adopt different body weights (and told defect is with appetite
- although fat, it can be seen A causes weight loss in other mice. This is overproduced, since WT mice do not produce as severe weight-loss in mutant B as A does. Good students should be aware of feedback loops in homeostasis, and predict obese mice to produce appetite suppressants.
- The phenotypes produced are identical. WT mice are seen to reduce the weight of B, therefore B can respond to satiety signals, but cannot produce them. A does not respond to these signals, but does cause weight loss in B, so can produce them. It is logical to evaluate as true that B lacks a hormone, and A the receptor. This is exactly how Leptin was discovered. The phenotypes show epistasis.
- The product of gene B acts through its receptor (the product of gene A) to stimulate X. Eating is behavioural, therefore ultimately integrated in the brain. It is sensible to suggest a signal stimulates an appetite suppressing brain region. If either component is disrupted, a similar obese phenotype will be produced.
- As insulin is effective a treating type 1 diabetes (but not efficeint for type 2), Leptin can cure some cases of genetic severe early onset obesity.

SHARING THE WORLD

EUTROPHICATION

The use of fertilisers, and release of sewage, have altered the balance of nitrogen and phosphorus based nutrients in the environment. Additionally, nitrogen oxides from pollution dissolve in rain to form acids. Growing algae incorporates an average of 16 nitrogen atoms per phosphorus atom, and eutrophication occurs when algal decomposition depletes oxygen. In 1957, the River Thames was declared 'biologically dead', without oxygen, but is now among the world's healthiest major rivers. Phosphorus availability (1), and nitrogen:phosphorus molar ratio (2) was measured across Northern Europe.



	True	False
The risk of eutrophication would be much lower if European farmers reduce phosphate, but not nitrogen, fertiliser use.	X	
Nitrogen oxide pollution increases the risk of eutrophication around Great Britain.	X	
Based on nutrient levels, British waters contain most of Europe's large, energetic fish species.	X	
European rivers are more polluted with phosphate than nitrogen.		X
Nutrient levels allow algae to grow quicker along the East coast of Denmark, than the West.		X

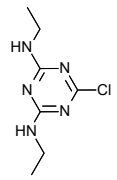
Explanation:
This question was inspired by Mats Carlberg of Sweden.

Candidates must explore the concept of limiting factors in ecology, and how geography influences life.

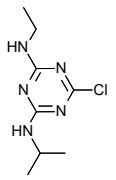
- Around the coast of continental Europe, where farm run-off reaches the sea, the N:P ratio is very high. Therefore, P is more likely to be limiting, so reducing P will inhibit growth more than N.
- Around Great Britain, N:P ratio is below optimum, so nitrous acid could increase algal growth (from a much lower baseline than in European seas).
- Pelagic fish live in cold, oxygen rich, comparatively algal poor, clear waters, where they can hunt prey. P levels are lowest around Britain, and the N:P ratio indicates N is even lower, indicating relatively healthy, algae poor, oxygen rich, clear water. (About 3/4 of all European fish is caught by ships sailing through British water).
- The amount of P in costal european waters is very high, but the N:P ratio is vastly higher, indicating they are still producing a huge excess of N based contamination, compared to P based waste.
- Both are strongly contaminated with P waste, but the N:P ratio is more optimal along the west coast.

ELISA

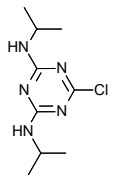
John Marrack (1886-1976) discovered the biochemistry of antibody-antigen interactions. These can be harnessed to detect pesticides contaminating sample water:
1) Wells are coated in the pesticide.
2) A very small amount of antibody against the pesticide is mixed with sample water, and then the mix is transferred to the wells.
3) The wells are washed with clean water several times.
4) An enzyme that produces a blue pigment, which is attached to a secondary antibody that binds the first antibody, is added.
Different antibodies (X, Y & Z) can be used for this process.



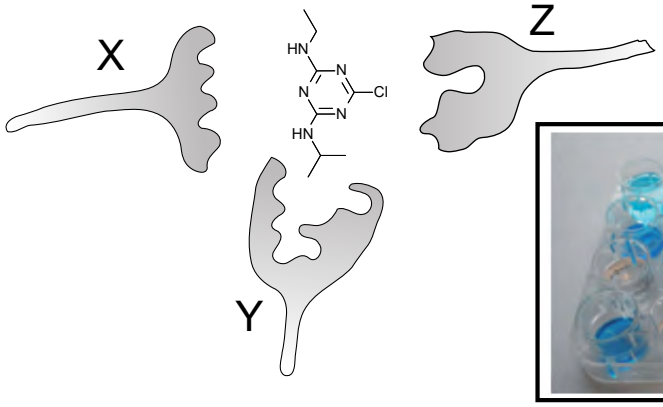
Simazine

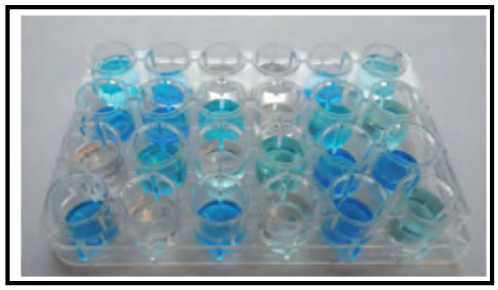


Atrazine



Propazine





	True	False
To test for atrazine specifically, antibody Y should be used.		X
All these pesticides probably have the same mechanism of action.	X	
All these antibodies could come from the same mouse injected with atrazine.	X	
Darker blue wells contained water which was more contaminated with pesticide.		X

Explanation:
This question was inspired by Vibeke Birkmann of the Danish olympiad.

Continuing the theme of water pollution, antibody technologies are explored.

- Antibody Y will react bind both Atrazine and Propazine
- The core molecules are very similar suggesting the same mechanism of action.
- The antigen binding sites of all antibodies would fit Atrazine.
- A sample with pesticide would be clear.

1) -
2) Antibody reacts with pesticide in sample water. Antibodies, therefore, cannot bind to well.
3) All the antibodies are washed out as they cannot bind the well.
4) No blue-making enzyme can bind the well, as no original antibody bound.

Therefore, clear.

MADAGASCAR

The Royal Botanic Gardens, Kew, is responsible for the millennium seed bank, including archiving the 70% of Madagascan plant species that are threatened with extinction. The shape of a plant phylogeny can be used to prioritise species for conservation.
The tree fern (*Cyatheales*) phylogeny is typical of plants (1).
The chance of plants being at risk of extinction is related to the age of the species (the length of its branch on a phylogeny) (2).

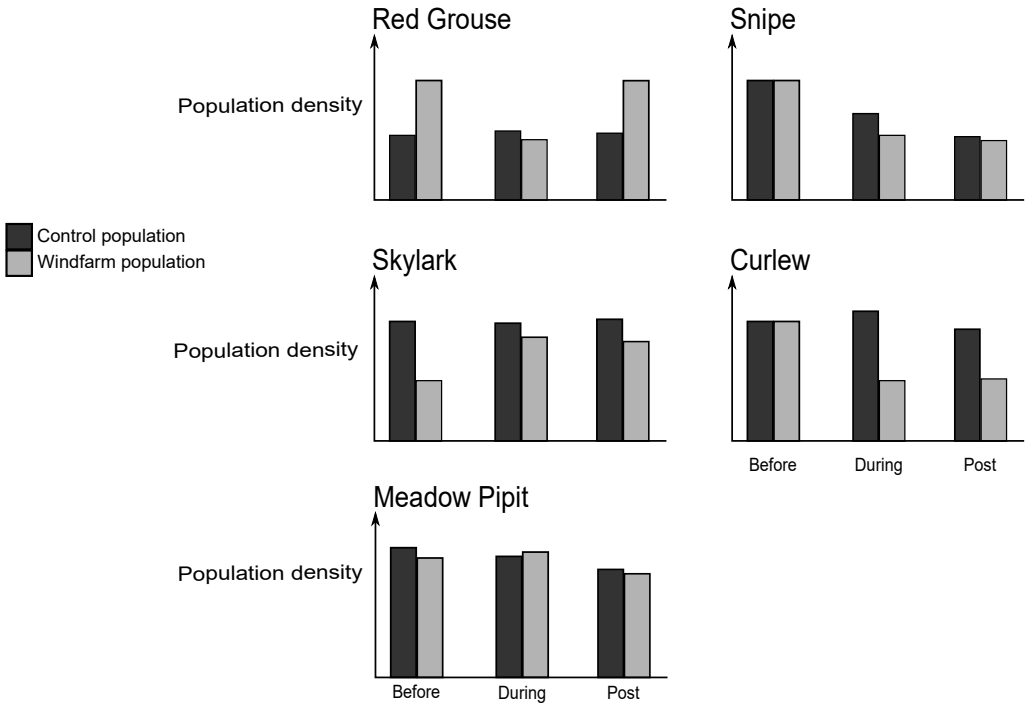
	True	False
The vast majority of Madagascan species are likely found nowhere else.	X	
The age of Madagascan species accounts for their unusually high extinction risk categorisations.		X
Globally, Kew should focus on collecting seeds from old species.	X	
Failure to bank any Madagascan species will result in a greater loss of evolutionary history and genetic diversity than failure to bank African species.	X	

Explanation:
Candidates must consider time and geography of a phylogeny to interpret the ecology of plants. The impacts of this on conservation efforts are explored.

- This is clearly seen on the cladogram - madagascan species are isolated suggesting no breeding into other countries. (1)
- Younger species, like those of Madagascar, tend to be less threatened usually.
- Yes, old species are more at risk (2)
- Yes. As seen in (1) Madgascan clades have long branch lengths and few relatives so a large loss of evolutionary history will occur if they die out.

WINDFARMS

The UK is extending the world's largest offshore windfarm in the North Sea. However, disruption to wind patterns, and other effects, causes difficulties for birds. Several windfarms were previously built in the habitats of red grouse (*Lagopus lagopus scotica*), snipe (*Gallinago gallinago*), skylark (*Alauda arvensis*), curlew (*Numenius sp.*), and meadow pipit (*Anthus pratensis*). The population density of these species was compared to undisturbed habitats before, during and after windfarm construction.



	True	False
The process of windfarm construction reduces the red grouse population.	X	
Of the species studied, finished windmills are only dangerous to curlews.	X	
The study windfarm is located in better red grouse habitat than the control site.	X	
An environmental change, other than windfarm construction, is harming the snipe population.	X	

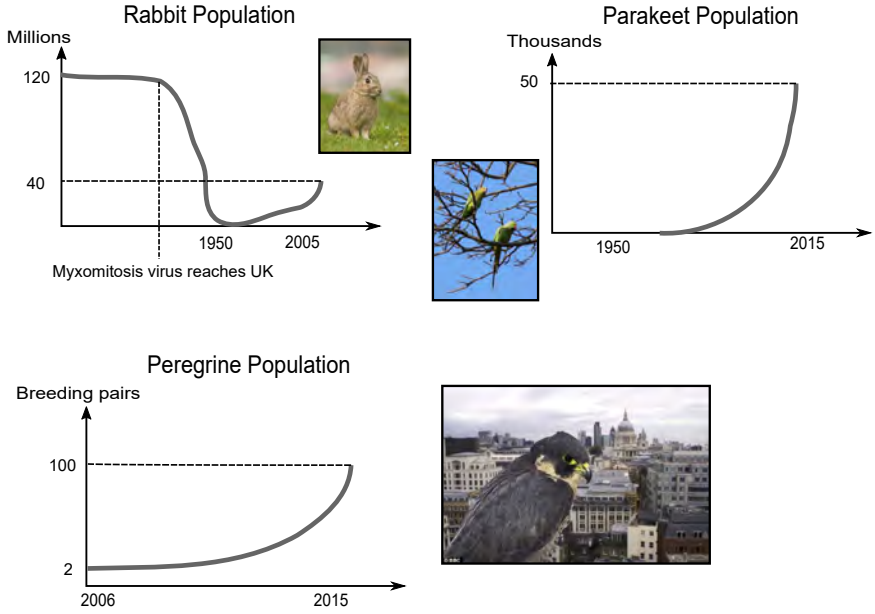
Explanation:

The role of humans in putting different species at risk is also explored. Candidates need to understand how to interpret controls and trends in ecology.

- 4/5 species have marked decreases in Windfarm populations compared to control during construction.
- Only curlews have a significant drop after windfarms are completed compared to control.
- Before construction, Red Grouse levels are higher in the windfarm area than the control (suggesting it is a poor control!).
- Control and windfarm populations are declining (over time).

POPULATIONS

The world's longest conservation efforts have restored the populations of many native British species, but several aliens are also thriving.
(1) Rabbits *(Oryctolagus cuniculus)* were introduced to Great Britain ~ 2000 years ago by the Romans.
(2) A pair of Parakeets *(Psittacula krameri)* was released into London by Jimi Hendrix.
(3) Native Peregrine Falcons *(Falco peregrinus)* nest in London, which as Europe's largest city, provides ample nest sites and prey, including parakeets.



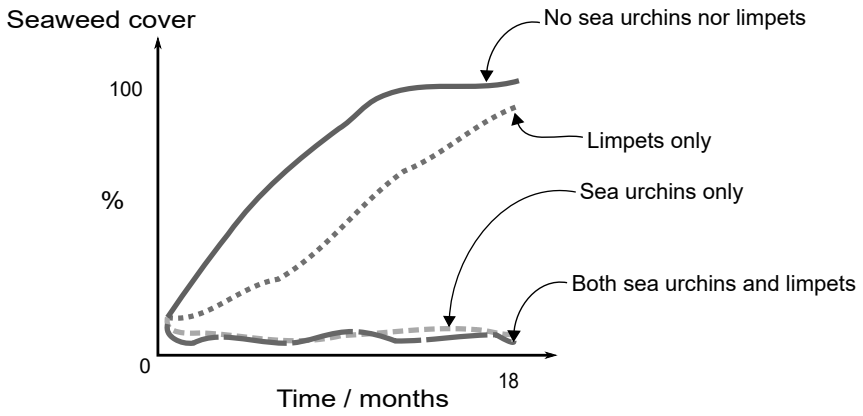
	True	False
Myxomatosis resistance in British rabbits arose around 1950.	X	
Peregrine falcons are effective biocontrols for the parakeet population.		X
Removing rabbits from the UK would help conservation of native species such as Peregrines.		X

Explanation:
Moving on from declining populations, this simple question addresses thriving species and aliens.

- The population will return to the carrying capacity seen in the 1900s.
- The parakeet population is growing exponentially, so falcons are not an effective control. There are also only 100 pairs versus 50 000 parakeets.
- Rabbits have been in the UK so long they are now part of the ecosystem and so removing them would likely not help Kites or Peregrines.

SEA URCHINS

Sea urchins *(Echinoidea)* are a major food source of otters *(Enhydra lutris)*. Sea urchin populations tend to explode where sea beds have been damaged by human activities. Sea urchins, limpets (*Patella vulgata*) and seaweeds can live together. Seaweed coverage was measured in an experimental area where the populations of urchins and limpets was artificially controlled.



	True	False
Limpets affect seaweed growth when sea urchins are present.		X
Sea urchins have a bigger effect on seaweed than limpets have on seaweed.	X	
Sea urchins help damaged seabeds recover.		X
Increasing the number of otters increases ocean primary productivity.	X	

Explanation:
This question was inspired by Quyen Nguyen Van of the Vietnamese Olympiad.

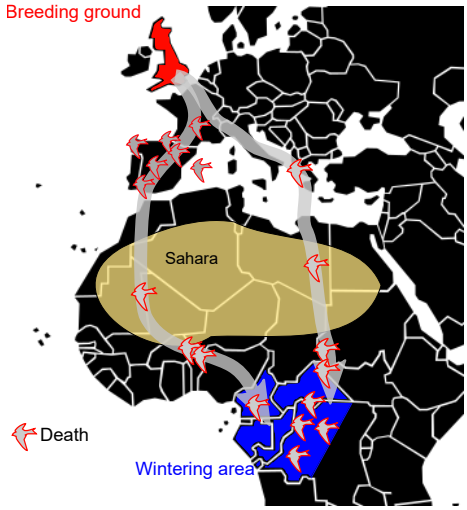
This simple question assesses understanding of food chains.

- Adding limpets in the presence of urchins still results in no seaweed cover.
- Limpets only only mildly reduces seaweed recovery.
- Urchins prevent the growth of seaweeds.
- Otters reduce urchin population, therefore seaweed grows quicker, so there is higher primary productivity.

CUCKOOS

The Island of Great Britain is a critical hub for migrating birds, from as far away as Canada and South Africa, due to its mild climate and well preserved habitats.
Cuckoos *(Cuculus canorus)* are one migratory species which has seen its population decline in recent years. Therefore, many cuckoos had trackers attached to them, and were monitored over several years. Their Autumn migration routes are shown (1). A roughly equal number of birds took the Eastern and Western routes through Africa. Points at which tracked birds died are shown.
The change in cuckoo populations in different regions of Great Britain, and the migration route which these populations take are shown (2).

1



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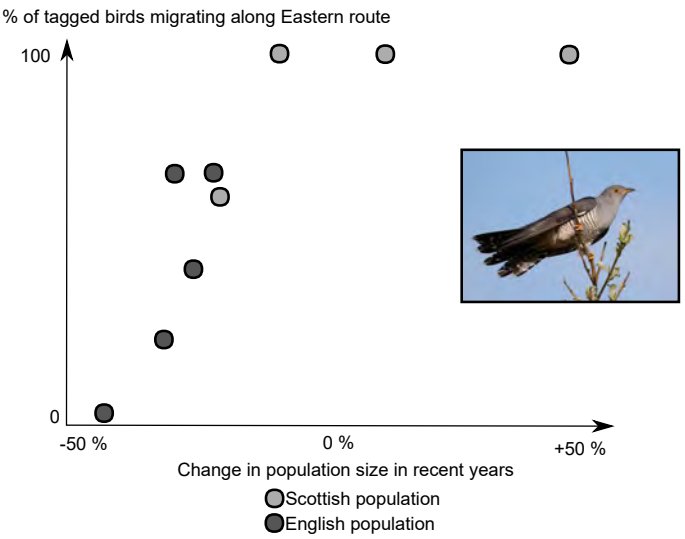


	True	False
Conditions for birds migrating along the western route have changed in recent years.	X	
Crossing the Sahara desert is the biggest challenge for migrating cuckoos.		X
Mortality rates during migration have an important impact on the population sizes of cuckoos in different parts of Britain.	X	
These data would be consistent with Summer conditions within Britain, before migration begins, determining mortality rates during migration.	X	

Explanation:
This complex question requires candidates to consider the role of sample selection, geography, time and migration in ecology.

- Much higher mortality, therefore, for large numbers of birds to still be taking it, must be a new phenomenon.
- Mortality in the Sahara is low.
- Good correlation between birds taking the less dangerous eastern route, and population size.
- Birds from England more likely to take the Western route. Plausibly the Western route isn't inherently more dangerous, but birds from England are just less prepared.

WILDCATS

Lynx *(Lynx lynx)* may be reintroduced to Great Britain in 2017, after being hunted to extinction 1300 years ago. Scottish wildcats (*Felis silvestris grampia*) are Britain's only remaining endogenous cat species, and are distinguished from wildcats (*Felis silvestris silvestris*) by their larger size, thicker fur, and unique hunting technique. Feral domestic cats (*Felis silvestris catus*) are also present in Scotland.
Motion-sensitive cameras in 347 Scottish locations captured 200 000 images, and volunteers used these to identify wildcats, feral cats, and hybrid cats by their markings.
In one year they found 10 different Scottish wildcats. In the next year, they found 9 different Scottish wildcats, one of which had the same markings as a cat photographed in the preceding year.
Additionally, they estimated that there were about 500 feral cats, and 300 hybrid cats in the area.

	50	100	150	200	250
Choose the nearest number to the correct estimate.		X			

Explanation:
The world's rarest cat!

- Using simple mark-recapture formula (which is easy to work out if not memorised) Number in population ~ 10*9/1 = 90

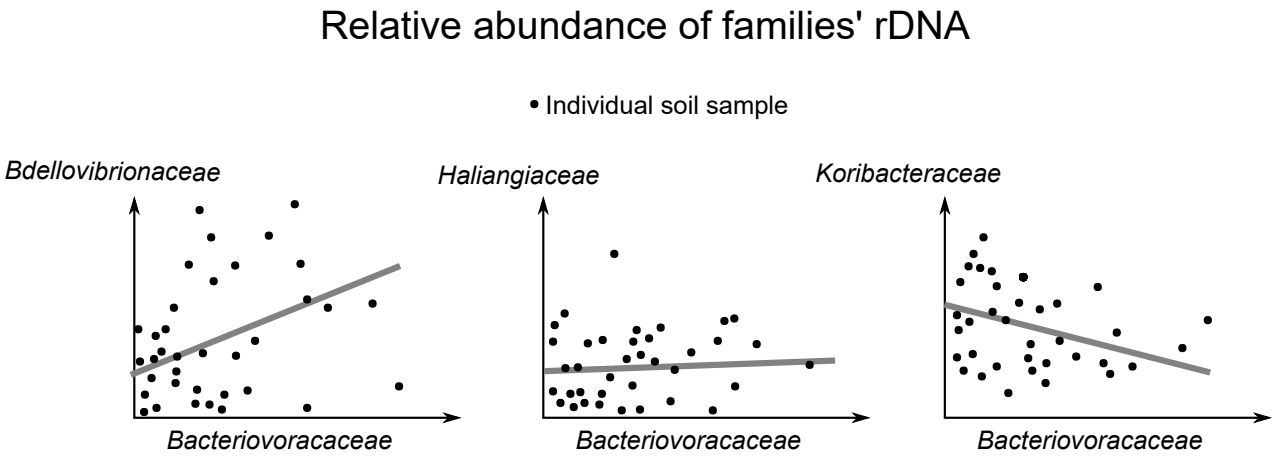
	True	False
Neutering feral cats would help prevent the Scottish wildcat population becoming less fit over time.	X	
Vaccinating feral cats would be an effective way to prevent transmissible disease in Scottish wildcats.	X	
Two species which have coexisted in the same region for thousands of years, are more likely to interbreed than two species which have recently come into contact but are equally genetically divergent.		X

Explanation:
This question requires understanding of the 'mark-recapture' technique, and explores the co-evolution of closely related species.

- Prevents hybridisation, which dilutes the phenotypes that make wildcats successful.
- They vastly outnumber wildcats, so provide herd immunity.
- To maintain pure populations and fitness for so long, they must have very high reproductive barriers. Species newly in contact have not evolved these.

SOIL BACTERIA

Sequencing of ribosomal RNA genes (rDNA), which have very low diversity within families, reveals the identity and relative abundance of bacterial families in different soils.
Bacteriovoracaceae and *Bdellovibrionaceae* are predatory bacteria. Each has a different maximum growth rate in optimal conditions.
Haliangiaceae and *Koribacteraceae* are non-predatory.



	True	False
Bdellovibrionaceae and *Bacteriovoracaceae* occupy different niches.	X	
Bacteriovoracaceae is an important predator of *Haliangiaceae*.		X
Bacteriovoracaceae and *Koribacteraceae* might occupy different habitats.	X	
rDNA sequencing could be used to assess the abundance of individual *Bacteriovoracaceae* species.		X

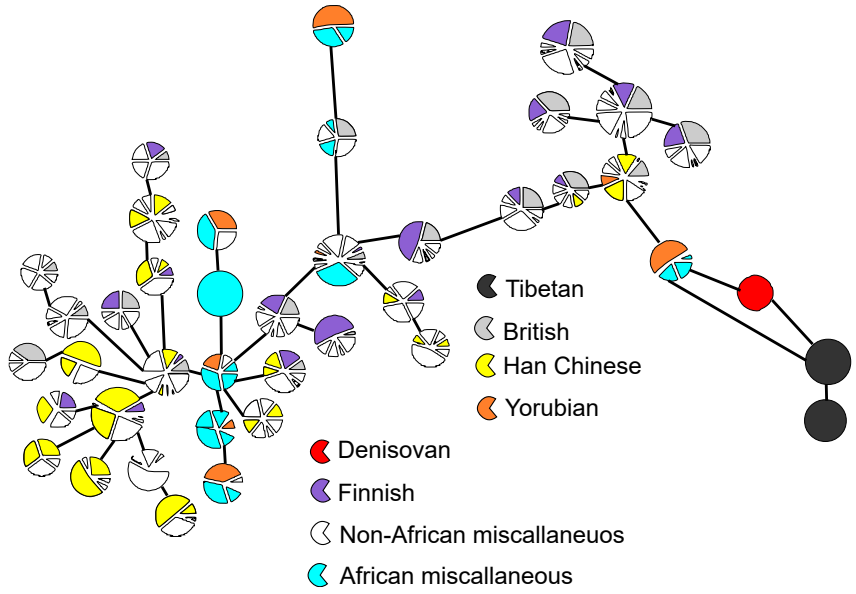
Explanation:
This question was inspired by Dmitar Epihov of the University of Sheffield.

This question assesses understanding of correlations and different kinds of symbiosis.

- Niche exclusion principal states that two species cannot exist in the same habitat (soil sample), if they do not have seperate niches, since one will always out-compete the other. Since they are positively correlated, they must have subtely different niches, and both prosper in the same habitats.
- Haliangiaceae populations are not significantly reduced by Bacteriovoracaceae
- They are inversely correlated, which could indicate direct predation, competition, or that habitats favourable to one, are unfavourable to the other.
- rDNA is very conserved, so cannot be used to distinguish close species.

HAPLOTYPE NETWORK

A haplotype network shows the evolutionary relatedness of haplotypes, and the proportion of individuals from a specific population with each haplotype. The 1000 genomes project, coordinated by the European Bioinformatics Institute in England, allows a global human haplotype network to be produced.
The circles represent one haplotype, each from the same locus.
The colours represent different populations by geographic location.
The lines represent an evolutionary link between two haplotypes.
Denisovans are a non-human subspecies of the genus *Homo*.
Tibetans are an Asian population, closely related to their neighbours across most of their genome.



	True	False
The British are more related to the Finnish than the British are to Yorubians at this locus.	X	
Tibetans have a higher genetic diversity than Han Chinese at this locus.		X
The Yorubian people are found in Africa.	X	
Tibetan ancestors interbred with Denisovans after leaving Africa.	X	

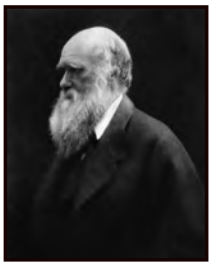
Explanation:
This question was inspired by George Campbell of The University of Manchester, and Mats Carlberg of Sweden.

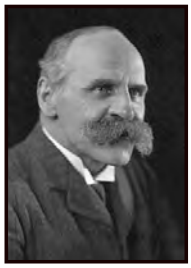
This question requires careful analysis of haplotypes, as described in the crib sheet. Candidates need to interpret the data creatively to find the most logical explanation for the patterns.

- The same haplotypes are often possesed by British and Finnish people, indicating a close relationship. This is not true of the British and the Yorubians.
- Tibetans all share two closely related haplotypes.
- They only have haplotypes shared with other Africans.
- Denisvonian haplotypes only have descendents in the tibetans. Therefore, they bred with this insular group after it had stopped exchanging genetic material with the rest of humanity. The link between Denisvonian haplotypes and African populations (but not the rest of humanity) is best explained as reflecting the presence of a very ancient African ancestral haplotype in the Human-Denisvonian common ancestor's genepool.

EVOLUTIONARY GENETICS

Charles Darwin's (1809-1882) discovery was united with Mendelian genetics by Sir Ronald Fisher (1890-1962) and John Haldane (1892-1964). Equations then allowed biologists to predict evolution, including the evolution of complex traits like behaviour, and quantitative traits like height.





Symbol	Definition
r	The relatedness of two individuals
n_i	The number of alleles the two related individuals share
n_p	The number of alleles shared on average between two members of the population
N_t	The total number of alleles in the genome
w	Inclusive fitness
D	Direct fitness: the number of offspring an individual has, multiplied by the relatedness (r) of each of those offspring to the individual.
I	Indirect fitness: the number of offspring an individual's relative has, multiplied by the relatedness (r) of each of those offspring to the individual.
C	The cost an individual's behaviour has to the number of offspring that it can produce.
B	The benefit an individual's behaviour has to the number of offspring that a relative can produce.
g	Genetic value of an individual: the value a quantitative trait would have, if no environmental influences acted on it.
Δ_s	The change, between generations, due to natural selection.
$\beta_{w,g}$	The correlation between fitness (w) and genetic value (g).
var(g)	The variance in genetic value (g) in a population

Rule for adaptive behaviours/traits	Equation
Relatedness	$\mathbf{r = (n_i - n_p) / N_t}$
Inclusive fitness	$\mathbf{w = D + I}$
Hamilton's rule	$\mathbf{C < r\ B}$
Price equation	$\mathbf{\Delta_s\ g = \beta_{w,g}\ var(g)}$

	True	False
Spiteful behaviours (cost to the actor and recipient) exist when actors are more related to recipients than expected by chance.		X
These equations predict alleles exist which produce a recognisable trait (e.g. appearance or pheromones) and also cause altruism towards unrelated individuals with that trait.	X	
These equations predict stress responses in some organisms include purposeful increases in mutation rate.	X	
Naked mole rat *(Heterocephalus glaber)* colonies of many adults, with a single breeding pair, can be explained by inclusive fitness.	X	
Natural selection acts more quickly when aphids are in their sexual lifecycle, than asexual lifecycle.	X	

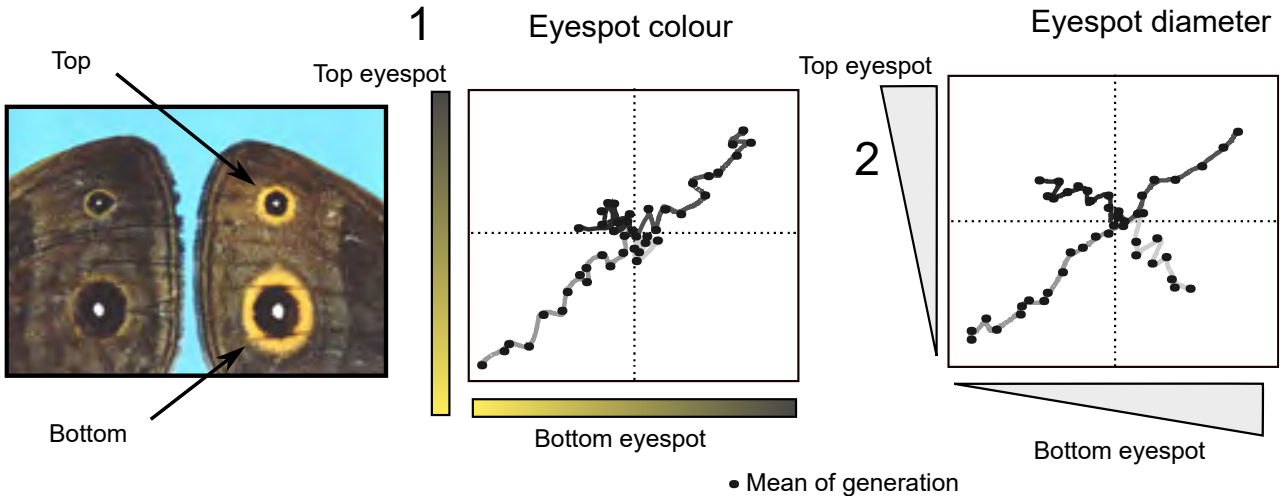
Explanation:
This question was inspired Mike Smith.

This question assesses candidates' ability to understand the implications of population genetics equations that underlie natural selection.

- Hamilton's law states $C < 0$ and $B < 0$ can only occur if $r < 0$. This can be understood from (r) as occuring when the number of alleles the actor and recipient share < number exepected on average. Ergo, Spite (as an adaption) occurs when individuals interact with others which are less related to them than expected by chance.
- Green Beard traits. They bias (r), since, from the allele's point of view, possession of the trait guarantees relatedness at that locus. This allows C to be large since r*B is large.
- Increases var(g), and therefore the change between generations due to natural selection (rate of adaptation). Allows stress to be overcome.
- As long as r is high, individuals can take a large (C) to Benefit the breeding pair.
- Sex creates new allele combinations, to drive up var(g), as above.

BUTTERFLY EYESPOTS

To investigate the evolution of butterfly eyespots, their mean size and colour was measured in each generation. Butterflies were split into 4 groups based on the colour (width of the golden ring) ratio (1), or size ratio (2), of their top and bottom eyespots. The most extreme butterflies in each group were bred together, and this was repeated with each successive generation.



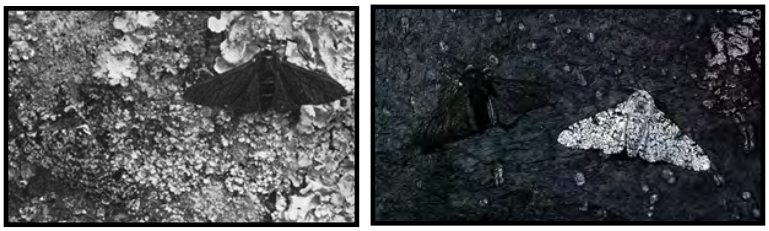
	True	False
Top:bottom eyespot colour ratio has greater genetic variation than top:bottom eyespot size ratio.		X
Simply selecting for top eyespots which are more golden will produce bottom eyespots which are more golden.	X	
Many relatives of this species have very different top:bottom eyespot size ratios.	X	
Eyespot size would remain constant if random butterflies of this experimental population were mated.		X

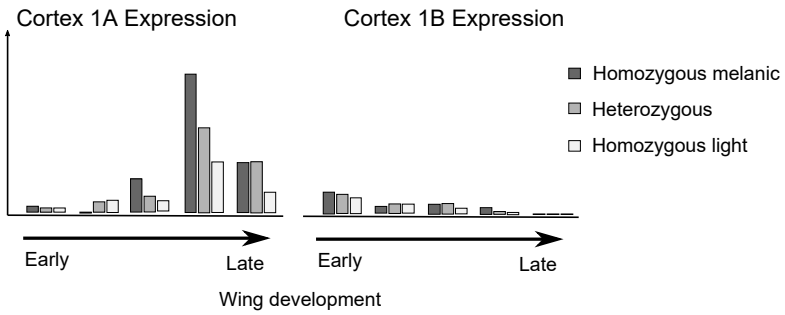
Explanation:
This question builds on the concepts explored previously to explain the results of an artificial selection experiment.

- Selection can only act if there is genetic variation in the trait (see prev. question). (1) shows selection to make spots different colours is very slow, compared to (2) selection to make spots different sizes. Hence there is less genetic variation giving variable colour ratios than variable size ratios.
- The colours of the top spot is genetically linked to the bottom (most genetic variation in one affects the other) which produces the results shown in (1).
- There is a reasonable genetic variation in size ratio evidenced in (2) (although small, it is present). Natural selection works over vastly longer timescales, with vastly larger populations, so the available morphospace is fully explored.
- Genetic drift would occur.

INDUSTRIAL MELANISM

The light-coloured form of the English peppered moth *(Biston betularia)* was replaced by a melanic (dark) form, as camouflaging lichens disappeared due to pollution during the industrial revolution. Clean air, lichens and light moths have now returned.
To discover the genetic basis of this, hundreds of moths were genotyped and haplotypes were determined. Melanism was due to a dominant mutation in the *cortex* gene. *Cortex* mRNA is cut (spliced) into two forms, Cortex-1A and Cortex-1B. Cortex mRNA abundance was measured.





	True	False
There is a greater diversity of haplotypes in the population close to the melanic *cortex* allele, than further away.		X
Cortex-1A is more likely to be driving melanism than *Cortex-1B*.	X	
The expression data are consistent with the melanic phenotype being dominant.	X	
The light form has reappeared because of back-mutation (reversion).		X

Explanation:
This question was inspired by Vasili Pankratov of the Belarus Olympiad.

This question rounds of the paper by analysing gene expression to find the genetic cause of an adaptive phenotype.

- There as been a rapid increase in frequency in the melanic form of cortex, due to natural selection (then a rapid increase in frequency in the white form). Selective sweeps reduce the diversity of the surrounding region, because the positively selected allele spreads through the population more rapidly than crossing-over can dissociate it from linked loci. The whole chunk of genome that the cortex mutation first appeared in has come to dominate the population. Then more recently, the few remaining haplotypes including the white allele have swept through the population, further reducing diversity.
- Cortex 1a has higher expression in developing wings, and shows a more pronounced difference between light and dark forms. (Especially later in development when you expect colour to form).
- Dark form has high expression of cortex 1a, hets medium expression, and light form low expression. Consistent with there being a high threshold level of expression necessary to give a dark phenotype.
- Very unlikely. More likely that some light indivduals persited through the industrial revolution, and then gained a selective advantage and swept back to prominance.