INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

Theoretical Exam 1

2020.8.12.

signature

IBO Challenge 2020

A Substitute for The 31st IBO 2020 Nagasaki, JAPAN

General instructions for theoretical examinations

Exam 1

- Date: August 12th 2020
- Total time of Exam 1 is 3 hours. Follow the instruction by Jury members of your country.
- Exam 1 consists of 50 questions.
- Each correct answer scores 1 marks, each incorrect or missing answer score 0 marks.

Instruction and regulations

- Make sure that you are using the correct answer sheet (Theoretical exam 1).
- Write your **Country code** and **student ID number** (provided by a jury member or supervisor) in the given box of the answer sheets provided, and write down **your name**.
- Make sure to **sign all the answer sheets and the cover page of question sheets**.
- You must mark your answer to the answer sheets properly, using a pen or a pencil.
- You must have the following equipment for this exam.
  ① Pen or pencil to mark answer sheets.
  ② Scratch paper sheets provided by Jury member. (You must not bring any paper into the examination room by yourself.)
  ③ Ruler and eraser.
- The use of a calculator is prohibited, including a calculator application on your PC or a web browser.
- You must not communicate with any other people in the room during the examination.
- You must not access any information that could unfairly help you answer the questions during the examination.
- Stop answering immediately at the end of examination time.
- After the examination:
  ① If you are under **on-site supervision, a jury member / supervisor** will collect your question and answer sheets immediately after each exam. Your country coordinator will later scan and submit the sheets to the IBO2020 Organizing Committee.
  ② If you are under **online supervision**, **you (competitor)** must scan (or take photos of) the answer sheets. Then, digitally send the scanned files/photos and the PDF question sheets (with your signature on the cover page) to your country coordinator as soon as possible. Your country coordinator will submit the file to the IBO2020 Organizing Committee. Make sure the answer sheets are scanned correctly. The IBO2020 office may ask you to resubmit the sheet,

so don’t discard them.

Biochemistry

Q1

The citric acid cycle is central to metabolism, for the supply energy and various key compounds. In citric acid cycle, the enzyme aconitase catalyzes the reversible conversion between citrate and isocitrate. In this reaction, OH group at C3 and H group at C4 of citrate are removed as water, thereafter a water molecule is added back in a reverse manner to generate isocitrate (Figure 1). However, OH group is never added at C2.

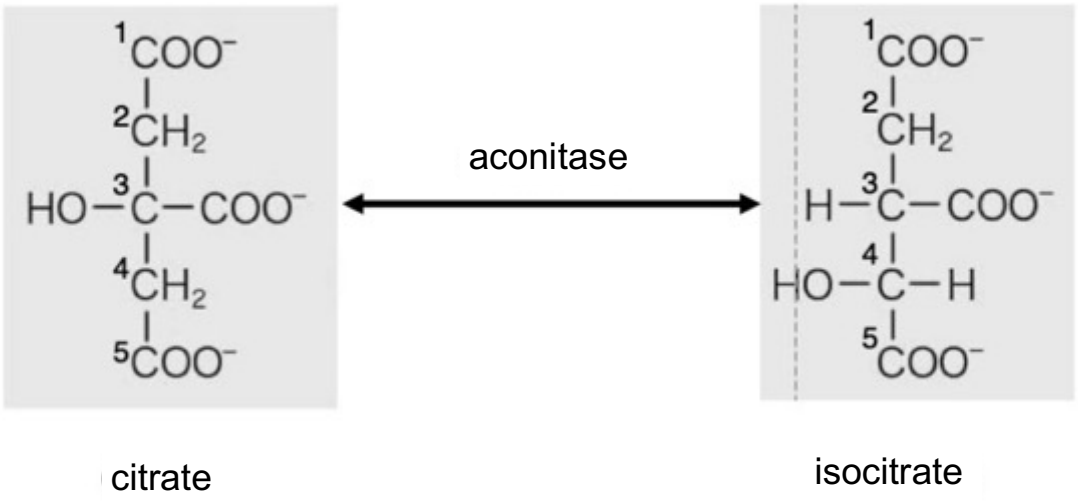


Figure 1

Indicate whether each of the following statements is true or false.

A. Citrate has enantiomers. 1

B. Isocitrate has enantiomers. 2

C. Two $-CH_2COO^-$ groups are stereochemically equivalent when citrate is free in solution. 3

D. Two $-CH_2COO^-$ groups are stereochemically equivalent when citrate is bound to aconitase. 4

Biochemistry

Q2

The figure 1 illustrated below shows oxygen consumption (respiration) in aqueous suspension of intact animal mitochondria with additions of ADP or chemical compounds (dinitrophenol (DNP) or N,N'-dicyclohexylcarbodiimide (DCCD)). The suspension already contains respiratory substrates, oxygen, and inorganic phosphate.

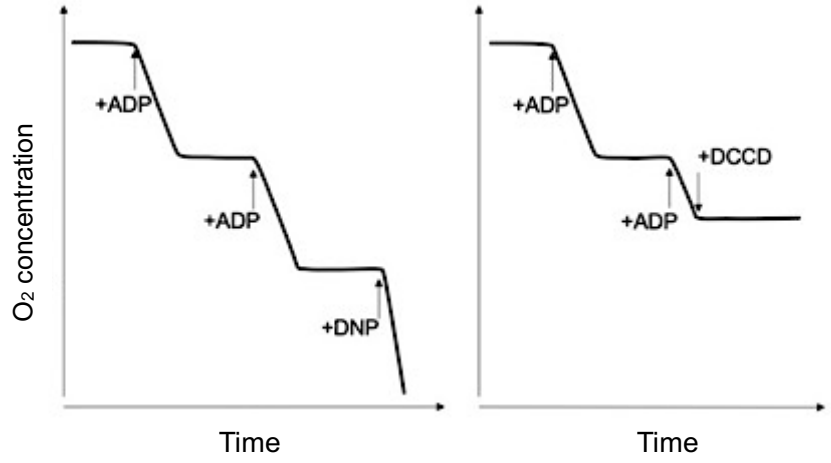


Figure 1. Oxygen consumption of mitochondria in suspension. Identical aliquots of ADP were added in both experiments.

Indicate whether each of the following statements is true or false.

A. The mitochondria are able to incorporate exogenously added ADP. 5

B. Before addition of chemical compounds, the mitochondria respire only when ATP can be produced. 6

C. The reason why DNP stimulates oxygen consumption is that ATP synthesis is stimulated by DNP. 7

D. DCCD inhibits ATP synthesis. 8

Biochemistry

Q3

When carbon isotopes (^{13}C and ^{12}C) are analyzed, plants can be categorized into two groups (Figure 1), based on the isotope fractionation ($\delta^{13}C$, equation 1). This is because of the slight differences in molecular mass between $^{13}CO_2$ and $^{12}CO_2$, although there are no known chemical differences between them. In photosynthesis, two types of carboxylase enzymes fix carbon from CO_2 in the two groups, provided that CO_2 is converted to H_2CO_3 by an enzyme carbonic anhydrase.

Reaction 1: $C_3H_5O_6P + H_2CO_3 \rightarrow C_4H_4O_5 + H_3PO_4$ (C4 plants)

Reaction 2: $C_5H_{12}O_{11}P_2 + CO_2 + H_2O \rightarrow 2\ C_3H_7O_7P$ (C3 plants)

$$\delta^{13}C = \left(\frac{\left(\frac{^{13}C}{^{12}C}\right)_{sample}}{\left(\frac{^{13}C}{^{12}C}\right)_{standard}} - 1 \right) \times 1000 \qquad \text{(equation 1)}$$

Sample: a plant material Standard: the reference represents the typical carbon on the Earth

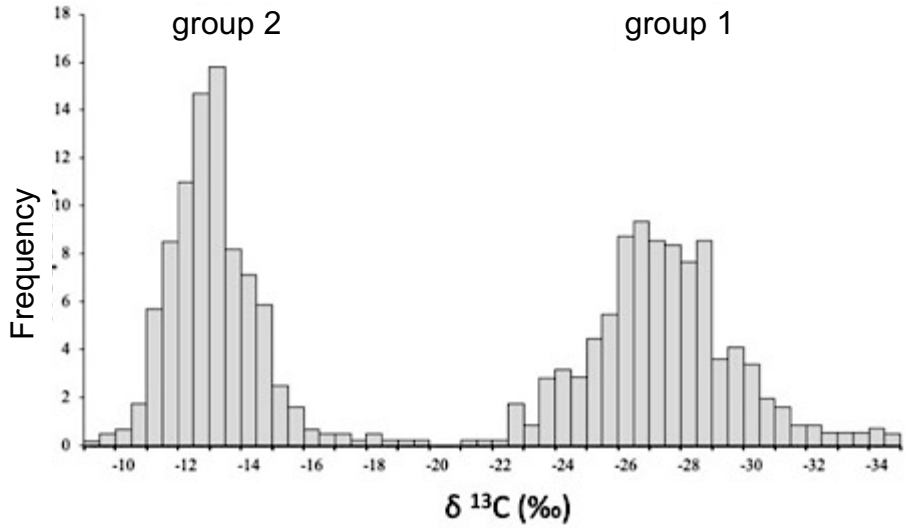


Figure 1 Distribution of the carbon isotope fractionation ($\delta^{13}C$ value) of various plants.

Indicate whether each of the following statements is true or false.

A. The relative difference in molecular mass due to the carbon isotopes is larger in CO_2 than H_2CO_3. 9

B. Reaction 1 is catalyzed by ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO). 10

C. Both groups of plants discriminate between the isotopes. 11

D. Rice belongs to group 1 and corn (maize) belongs to group 2. 12

Cell Biology

Q.4

Centrifugation is one of the most important biochemical techniques in the separation and purification of biomolecules and organelles. The sedimentation speed (v) of specimens during centrifugal operation is proportional to the applied acceleration rate (g_c), as shown in equation (1).

$$v = S \times g_c. \qquad (1)$$

S in the equation is called the sedimentation coefficient and is determined by the ratio between the centrifugal force applied to the object in the solvent (numerator) versus a parameter reflecting the magnitude of viscous resistance against sedimentation (denominator), as shown in equation (2).

$$S = \frac{V_m(\rho - \rho_0)/N_A}{6\pi\eta r}, \qquad (2)$$

Vm : the molar volume of a sedimenting specimen

ρ : the densities of the specimen

ρ_0 : the densities of the solvent

r : the radius of the specimen when it is assumed to be spherical

η : the viscosity coefficient of the solvent

N_A : the Avogadro constant, 6.02×10^{23}.

Indicate whether the following descriptions are true or false.

A. For organelles of the same size and shape, S can be used to estimate differences in organelle density. 13

B. Since many protein molecules have densities between 1.3 and 1.4 (g/mL), we can use S to distinguish sizes of spherical protein molecules. 14

C. Assuming that two ribosomal subunits of similar size are combined to form a large complex, S is approximately doubled. 15

D. Since it is commonly expected that the viscosity of a solvent will increase at low temperatures, S also decreases when chilled. 16

Cell Biology

Q5

ATP is an important energy source for maintaining normal membrane potential in nerve cells. Figure 1 shows the result of an experiment demonstrating Na^+ efflux from an isolated squid giant nerve axon after injecting a buffer solution (artificial cytoplasm) that contains radioactive $^{24}Na^+$.

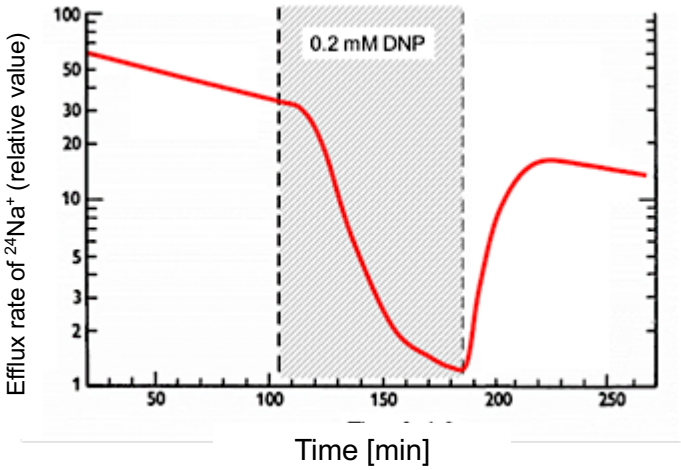


Figure 1 Investigation of the efflux rate of radioisotope ^{24}Na from a squid giant axon to the external solution (seawater). At 0 min, a buffer solution containing $^{24}Na^+$ was injected into the giant axon. For 100-190 min, the external seawater was replaced with a solution (seawater) containing 0.2 mM DNP (dinitrophenol), an uncoupler of oxidative phosphorylation.

Indicate whether each of the following statements is true or false.

A. This experiment should have been carried out under the condition with sufficient oxygen to maintain the activity of ATP production by mitochondria. 17

B. The efflux of $^{24}Na^+$ observed in seawater without DNP indicates the leaking of Na ions out of the cell by nonspecific transportation. 18

C. Delayed decrease of $^{24}Na^+$ efflux after using DNP reflects some amount of ATP storage inside the axon, including that being produced by glycolysis. 19

D. Active transport of sodium ions was estimated to increase internal sodium concentration by 10% in 50 min. 20

Cell Biology

Q6

For the growth of plants, the supply of nutrient inorganic ions is essential. A certain crop was grown in two different soils (X, Y). The concentrations of nutrients (potassium ions and chloride ions) in each type of soil are shown in the table. The estimated cytosolic concentrations of each ion in the root epidermal cells of this crop are also shown. When the membrane potential of the epidermal cell is −150 mV, how is each ion transported into the cell?

Ion movement is determined by electrical and concentration gradients. The membrane potential which would counterbalance the concentration gradient is given by the Nernst equilibrium potential equation:

$$E = -\frac{60}{\mathbf{z}} \log \frac{\mathbf{C_i}}{\mathbf{C_o}} \text{ (mV)}$$

E : the Nernst equilibrium potential

z : the charge of the ion, e.g. z for Ca^{2+} = +2

$\mathbf{C_i}$: the molar concentration of the ion in the cytosol

$\mathbf{C_o}$: the extracellular molar concentration (here, the concentration in the soil) of that ion.

The direction of ion transport is determined by comparing the Nernst equilibrium potential with the membrane potential of the cell. Here, transport against the electrochemical gradient of each ion is called "active transport" and transport according to the electrochemical gradient of each ion is called "passive transport."

	Soil X	Soil Y	The estimated cytosolic concentrations of each ion in the root epidermal cells
K^+	1 mM	0.01 mM	100 mM
Cl^-	0.5 mM	5 mM	5 mM

Indicate whether each of the following statements is true or false.

A. In soil X, potassium ions are absorbed by the active transport system. 21

B. In soil Y, potassium ions are absorbed by the active transport system. 22

C. In soil X, chloride ions are absorbed by the passive transport system. 23

D. In soil Y, chloride ions are absorbed by the passive transport system. 24

Cell Biology

Q7

"Secondary metabolism" in microorganisms and plants is not essential for their survival, but is a metabolic process that plays an important role depending on species or in environmental adaptation. Many secondary metabolites accumulated by plants, such as nicotine and caffeine, play a role in resistance to damage from herbivorous insects.

Glucosinolate, which is accumulated in the leaves of *Arabidopsis thaliana*, is a repellent for herbivorous insects (*Helicoverpa armigera*). The leaves of the wild type (Wild) and the leaves of the mutant (Mutant) incapable of synthesizing glucosinolate are arranged as shown in Figure 1.

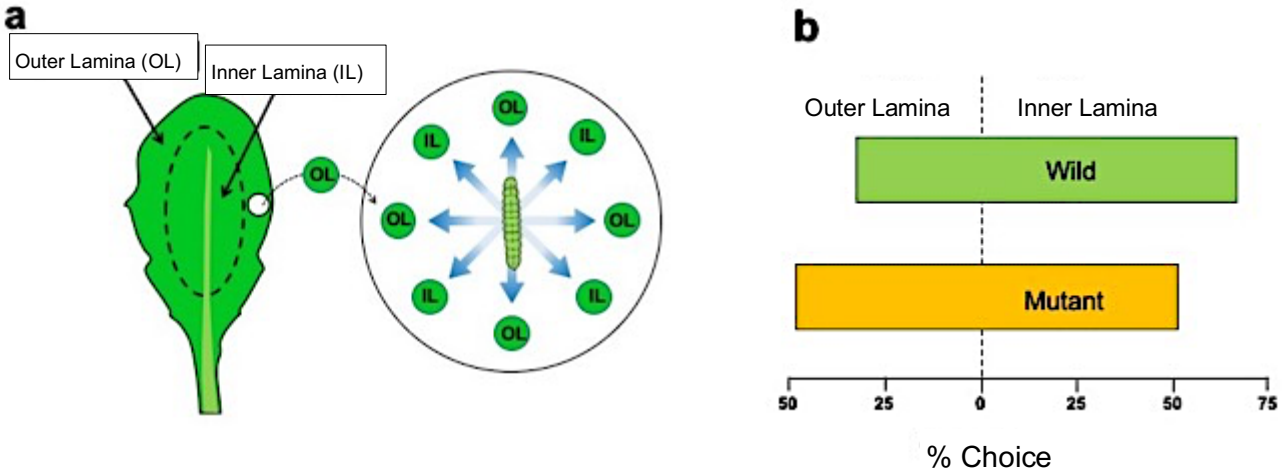


Figure 1 (**a**) Experimental design, (**b**) Result of choice by insect larvae.

The following conclusions can be assumed from this experiment.

Indicate whether each of the following statements is true or false

A. In the *Arabidopsis* wild strain, glucosinolate is accumulated more in the outer lamina of the leaves. 25

B. In this mutant, glucosinolate is evenly accumulated at any region of the leaf. 26

C. *Arabidopsis* accumulates only glucosinolate as a repellent in its leaves. 27

D. For *Arabidopsis*, inner lamina is likely to be more physiologically important than outer lamina. 28

Cell Biology

Q8

Isoetes howelli is an amphibious plant that can live in both aerial and submerged conditions. In a completely submerged condition in shallow fresh water, *Isoetes howelli* shows characteristic metabolism; CO_2 is fixed to malate in a certain time period and released in another period to be used in photosynthetic carbon assimilation. This metabolism is not seen in the aerial condition. There shall be a strong photosynthetic competition in daytime between *Isoetes howelli* and other photosynthetic organisms.

Indicate whether each of the following statements is true or false

A. The malate concentration in the leaves is the highest just before sunrise. 29

B. The characteristic metabolism is adaptive because it reduces water loss. 30

C. This species has characteristic bundle sheath cells with well-developed chloroplasts. 31

D. In the submerged condition to which this species is adapted, it is more difficult to use CO_2 in daytime than in nighttime. 32

Genetics

Q9

Both eukaryotes and prokaryotes have a common feature that mRNA starts translation at the AUG codon. Eukaryotic mRNA is usually a monocistron that encodes only one protein, whereas prokaryotic mRNA is often a polycistron that encodes multiple proteins. The following experiments were performed to investigate the mechanism of the AUG codon that initiates translation. Post-translation decomposition need not be considered.

(1) For several operons of *Escherichia coli*, the promoter was replaced with a yeast promoter and introduced into yeast cells. Although all full-length mRNAs were transcribed for all operons, some operons translated only the first gene correctly, while other operons did not translate any genes.
(2) The promoters derived from *E. coli* were ligated to cDNAs obtained by removing introns from several yeast genes and introduced into the *E. coli* host. Full-length mRNA was transcribed for all operon genes, but there was little translation of any genes.

From these experiments, it is considered that the AUG codon that initiates the translation of *Escherichia coli* and yeast is determined through the following mechanism.

Indicate whether each of the following statements is true or false.

A. In *E. coli*, the translation starts with the first AUG codon of the mRNA as the start codon. 33

B. In yeast, translation starts with the first AUG codon of the mRNA as the start codon. 34

C. In *E. coli*, translation starts with the AUG codon designated by the specific sequence in the mRNA as the start codon. 35

Genetics

Q10

There are four types of bases used for RNA – A, C, G and U – while for DNA there are four types of bases, A, C, G, and T. I wondered why thymine T could only be used for DNA and looked closely at the base-pairing pattern (Figure 1).

It is reported that the mutant strain of the certain gene in *Escherichia coli* sometimes incorporates dUTP in place of thymine to include bases in the DNA strand. This frequently results in a new mutation. In a chemistry lecture, I learned that compounds such as bases could undergo chemical changes (mainly hydrolytic deamination) by reacting with certain water molecules even under *in vivo* conditions.

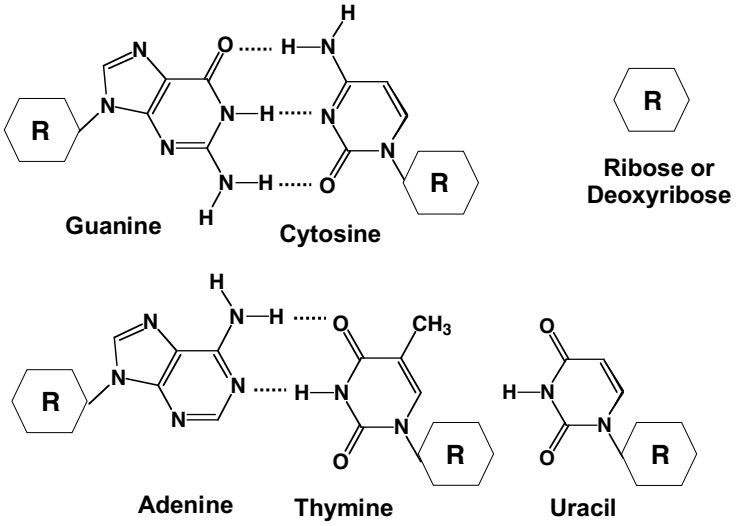


Figure 1

Indicate whether each of the following statements is true or false

A. Chemical changes made to RNA bases are not repaired. 36

B. Chemical changes that occur in cytosine bases are the main reason that thymine bases are used only in DNA. 37

C. *E. coli* mutant strains that incorporate uracil instead of thymine are more likely to mutate the A-T base pair. 38

D. *E. coli* mutant cells containing uracil bases in the DNA chain are susceptible to chemical changes in uracil bases, so that new mutations occur frequently. 39

Genetics

Q11

For the three heritable features, Alfa, Baker, and Charlie, pedigree analysis was performed on pedigree A, pedigree B, and pedigree C, respectively, and the results in Figure 1 were obtained.

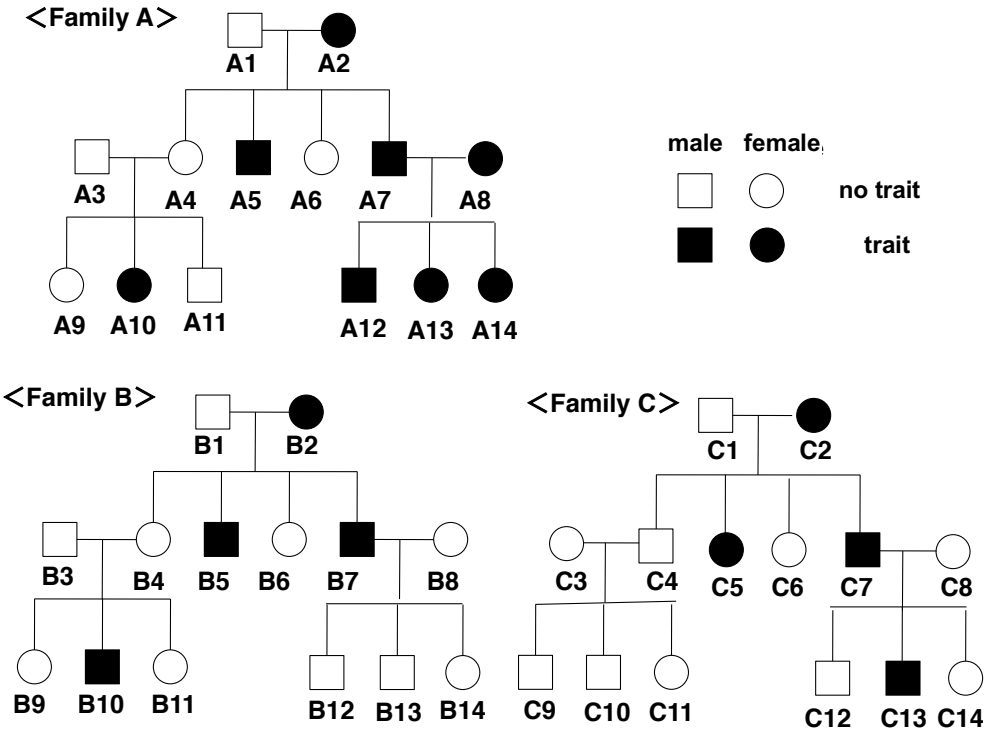


Figure 1

Indicate whether each of the following statements is true or false.

1. An analysis of pedigree **A** suggests that the inheritance pattern of characteristic Alfa could be due to a dominant allele. 40
2. An analysis of pedigree **C** suggests that the inheritance of the characteristic Charlie could be due to a dominant allele. 41

A subsequent detailed analysis revealed that all of the inheritance patterns of Alfa, Baker, and Charlie were due to recessive alleles on the autosome.

3. **B1** and **B3** of family **B** are definitely carriers. 42
4. **C1** and **C3** of family **C** are definitely carriers. 43

Genetics

Q12

In *Escherichia coli*. the *rutA - G* gene cluster activates when pyrimidine is decomposed and used as a nitrogen source. The *rutA - G* genes constitute a single *rut* operon, and a single P_{rut} promoter regulates the expression. The expression of the P_{rut} promoter is regulated by a RutR repressor using uracil as an inducer.

Indicate whether each of the following statements is true or false.

A. As the concentration of uracil increases, the expression level of the *rut* operon decreases. 44

B. When a mutation occurs in the RutR repressor and the affinity for uracil is reduced, the expression level of the *rut* operon is reduced. 45

C. If a mutation occurs in the DNA binding domain of the RutR repressor and the affinity for the DNA sequence decreases, the expression level of the *rut* operon increases. 46

D. When a mutation occurs in the nucleotide sequence of the operator to which the RutR repressor in the P_{rut} promoter binds, the expression level of the *rut* operon always becomes high. 47

Genetics

Q13

Bacteria regulate gene expression through transcription factors that sense environmental changes in order to adapt to the ever-changing environment. One transcription factor often controls multiple genes. Since the expression of a gene consumes energy, the selection of the gene group to be expressed is important for the survival strategy of the bacterium. It is often observed that bacteria move vigorously in search of nutrients in the aquatic environment, while bacteria in biofilms rarely move.

Indicate whether each of the following statements is true or false.

A. Generally, transcription factors, which induce the expression of glucose utilizing genes, suppress the expression of lactose-metabolizing genes. 48

B. Transcription factors activated by phosphate depletion activate the expression of glycogen-utilizing genes. 49

C. Transcription factors that activate the expression of fatty acids-metabolizing genes are generally activated under oxygen depleted conditions. 50

D. Transcription factors that activate the expression of biofilm-forming genes usually suppress the expression of the genes of flagella formation. 51

Genetics

Q14

With the advancement of DNA research, various technologies have been developed, and it has become important to select appropriate research methods according to one's research purpose. Among the research methods M1 to M7, mark (T) if it is appropriate as the method that provides the most direct information on the following research purpose A – D, and mark (F) if it is inappropriate.

Research methods

(M1) DNA microarray

(M2) Quantitative RT-PCR

(M3) CRISPR-Cas9 method

(M4) In situ hybridization

(M5) Reproductive cloning

(M6) Construction of iPS cell

(M7) Metagenome analysis

Purpose of research

A. To examine the site where a specific gene is expressed in a mouse tissue, it is appropriate to perform (M4). 52

B. To analyze the expression level of a specific gene in maple leaves, it is appropriate to perform (M2). 53

C. To search the *Bacillus subtilis* genome for genes the expression of which is induced when the nitrogen source is depleted. (M1) 54

D. To identify microbial species from microbial communities thriving in compost. (M7) 55

Genetics

Q15

The seed germination of plants is mainly controlled by the action of two plant hormones called gibberellin and abscisic acid. Gibberellin promotes germination and abscisic acid suppresses germination. Through the actions of these two plant hormones, plant seeds are regulated so that germination is induced in an appropriate environment.

In plants, gibberellin is biosynthesized from a molecule called geranylgeranyl diphosphate. Geranylgeranyl diphosphate is converted into *ent*-kaurene through the action of *ent*-kaurene synthase. *ent*-Kaurene is then converted into gibberellin through the action of several enzymes such as GA20 oxidase. Biosynthetic intermediates such as *ent*-kaurene do not have germination-inducing activity (Figure 1).

On the other hand, abscisic acid is biosynthesized from carotenoid pigments. Abscisic acid is converted into 8'-hydroxyabscisic acid by an oxidase called CYP707A. Seeds of Arabidopsis mutants lacking the gene encoding CYP707A were observed to have significantly delayed germination as compared to seeds of wild-type plants. In addition, the germination of the seeds of plants in which the CYP707A gene was overexpressed were promoted more than the wild-type seeds. In this experiment, the administered compounds play a similar function of endogenous hormones.

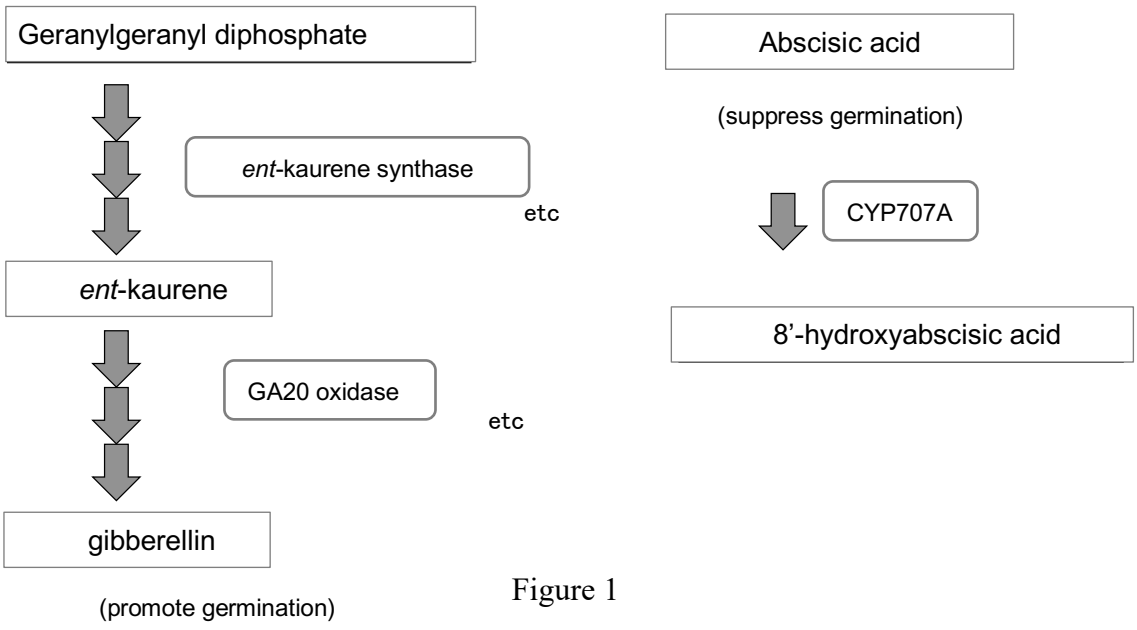


Figure 1

Indicate whether each of the following statements is true or false.

A. In the mutant lacking the *ent*-kaurene synthase gene, germination is delayed compared to the wild-type plants. 56

B. When a mutant lacking the *ent*-kaurene synthase gene is treated with *ent*-kaurene, germination is promoted. 57

C. *ent*-Kaurene treatment to a mutant lacking the gene encoding GA20-oxidase promotes germination. 58

D. 8'-Hydroxyabscisic acid has a stronger germination-inhibiting activity than abscisic acid. 59

Genetics

Q16

Part of the sequence of vector A, which is for protein expression using *Escherichia coli* as a host, is shown. It was planned to express a plant-derived gene X using vector A. Vector A is a plasmid vector that expresses a protein fused to the N-terminus His-tag, which enables efficient purification of the expressed protein. As shown in Figure 1, translation of the protein occurs from the start codon immediately before the His tag with six consecutive His residues. The DNA sequences of the 5' and 3' regions of gene X are shown in Figure 2. We planned to clone gene X using restriction enzyme sites, EcoRI, SmaI, or SalI in vector A. When the gene X is amplified by PCR, a fragment with a restriction enzyme site at the end can be amplified using the primer with a restriction enzyme site. Since the restriction enzyme site is not recognized if it is located at the end of the DNA fragment, three "Cs" were also attached in addition to the restriction enzyme site.
For example, in order to add the EcoRI site to 5'-XXXXXXXXXX----, the primer is designed as below.
5'-CCCGAATTCXXXXXXXXXX----,

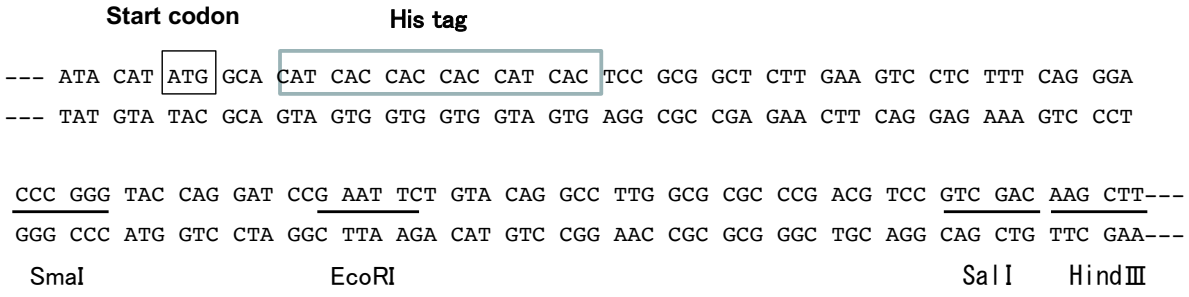


Figure 1. DNA sequence of the cloning region of vector A (double strands).

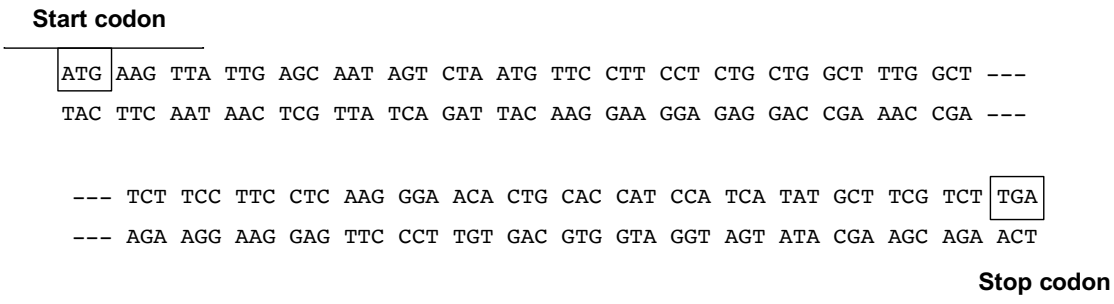


Figure 2. DNA sequence of the gene X showing 5' region and 3' region: 1566 base pair

Choose true if the primer is a correct one to use, if not, choose false.

A. Forward primer（]: the start codon）

5'-CCC GAA TTC [ATG] AAG TTA TTG AGC AAT A-3' [60]

EcoRI site

B. Forward primer（]: the start codon）

5'- CCC CCC GGG [ATG] AAG TTA TTG AGC AAT A-3' [61]

SmaI site

C. Reverse primer（]: the stop codon）

5'-CCC GTC GAC [TCA] AGA CGA AGC ATA TGA T-3' [62]

SalI site

D. Reverse primer（]: the stop codon）

5'-CCC AAG CTT GTA GGT AGT ATA CGA AGC AGA [ACT] -3' [63]

HindⅢ site

Genetics

Q17

In recent years, a genome editing technology called the CRISPR-Cas9 method has been widely used for biology research. In the CRISPR-Cas9 method, an enzyme called Cas9 is guided to the target gene by forming a complex with a guide RNA with a sequence complementary to a part of the target gene. Then, Cas9 cleaves the double-stranded DNA of the target gene specifically with its activity of cleaving double-stranded DNA. Cas9 recognizes a 3-base sequence (NGG) called PAM sequence and cuts the DNA strand 3 to 4 bases upstream of PAM. The cleaved DNA chain is repaired by the DNA repair system, but at that time, a few bases are frequently deleted or inserted.

The CRISPR-Cas9 method was applied by targeting the region close to the translation start codon of the most upstream exon of a gene encoding enzyme A of a certain animal. The base sequence of the target region was determined for each of the four mutants obtained (Figure 1).

Original sequence	TA TCT TAC ATG ATC CTA CAA GTA CCT TAC GCT CGG CAG GAA G
Mutant 1	TAT CTT ACA TGA TCC TAC AAG TAC CTT ACA GCT CGG CAG GAA G
Mutant 2	TAT CTT ACA TGA TCC TAC AAG TAC CTT GCT CGG CAG GAA G
Mutant 3	TA TCT TAC ATG ATC CTA CAA GTA CCT GCT CGG CAG GAA G
Mutant 4	TA TCT TAC ATG ATC CTA CAA GTA CCT TAA CTC GCT CGG CAG GAA G

: Pam sequence recognized by Cas9

Start codon : ATG (underlined)

Stop codon : TAA, TAG, TGA

Figure 1

Indicate whether each of the following statements is true or false.

A. It is highly likely that the activity of enzyme A is retained in mutant 1. 64

B. It is highly likely that the activity of enzyme A is retained in mutant 2. 65

C. It is possible that the activity of enzyme A is retained in mutant 3. 66

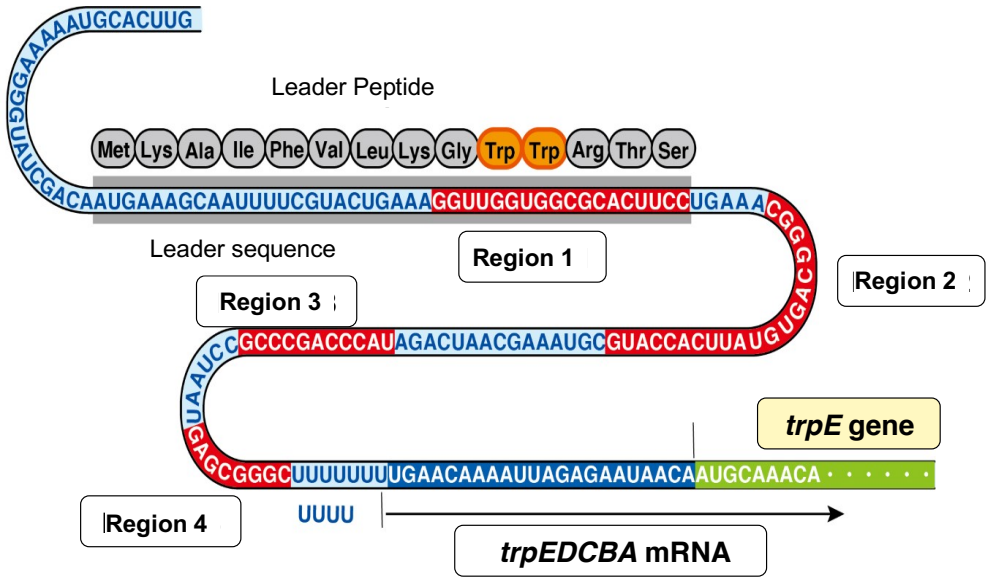
D. It is highly likely that the activity of enzyme A is lost in mutant 4. 67

Genetics

Q18

The tryptophan operon (*trp* operon) of *E. coli* is transcriptionally regulated by a repressor that is activated by the binding of tryptophan. The active form repressor binds to the operator sequence located between the promoter and the transcription initiating point and blocks the RNA polymerase. There is another expression control system called the attenuator linked to transcription and translation in the *trp* operon.

Between the operator sequence and the *trpE* gene, which is the first structural gene of the *trp* operon, there are four sequences of about 15 bases called Region 1-4 (Figure 1). Region 1 and Region 2, and Region 3 and Region 4 have complementary sequences, respectively. When these regions are transcribed as mRNA, they are paired with each other and form stem-loop structures (Figure 2). Furthermore, the sequences of Region 2 and Region 3 are also complementary, so a stem loop structure can be formed.



Region 1/Region 2, Region 3/Region 4; complementary
Region 2/Region 3; complementary
Leader sequence (Region 1) encodes short peptide containing two tryptophan residues (Trp)

Figure 1

A short peptide of 14 amino acids containing two tryptophan codons called a leader peptide is encoded in Region 1 (Figure 1).

If the *trp* operon mRNA is not translated at the same time as it is transcribed by RNA polymerase, Region 1 and Region 2 of mRNA, and Region 3 and Region 4 pair with each other to form stem loop structures, respectively. In this case, a consecutive U bases is located immediately after Region 4. Since the form in which U bases continue immediately after the stem loop structure functions as a transcription termination signal in the

procaryote, the RNA polymerase is released and the transcription is terminated（Figure 2）.

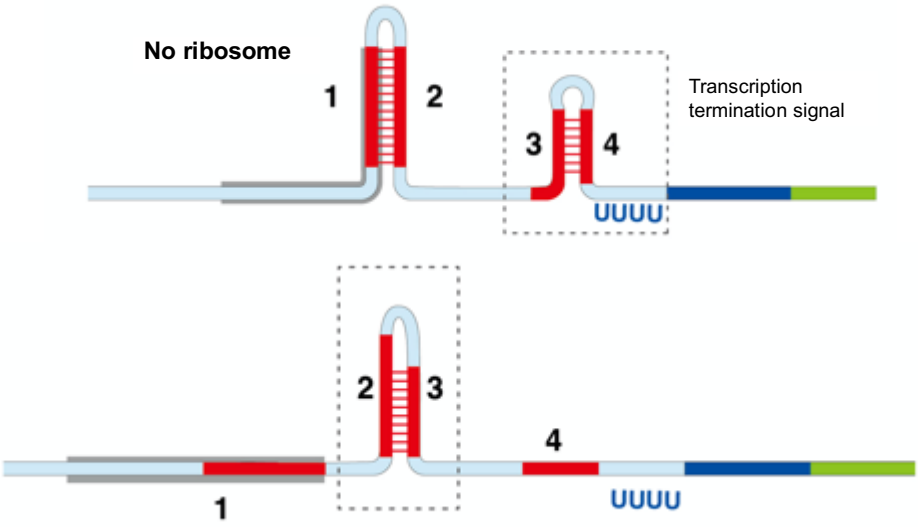


Figure 2

When the translation of the leader sequence occurs at the same time as the transcription of the mRNA, the ribosome can translate the mRNA with the stem loop structure, but the transcription also ends by forming the stem loop structure of Region 3 and Region 4.

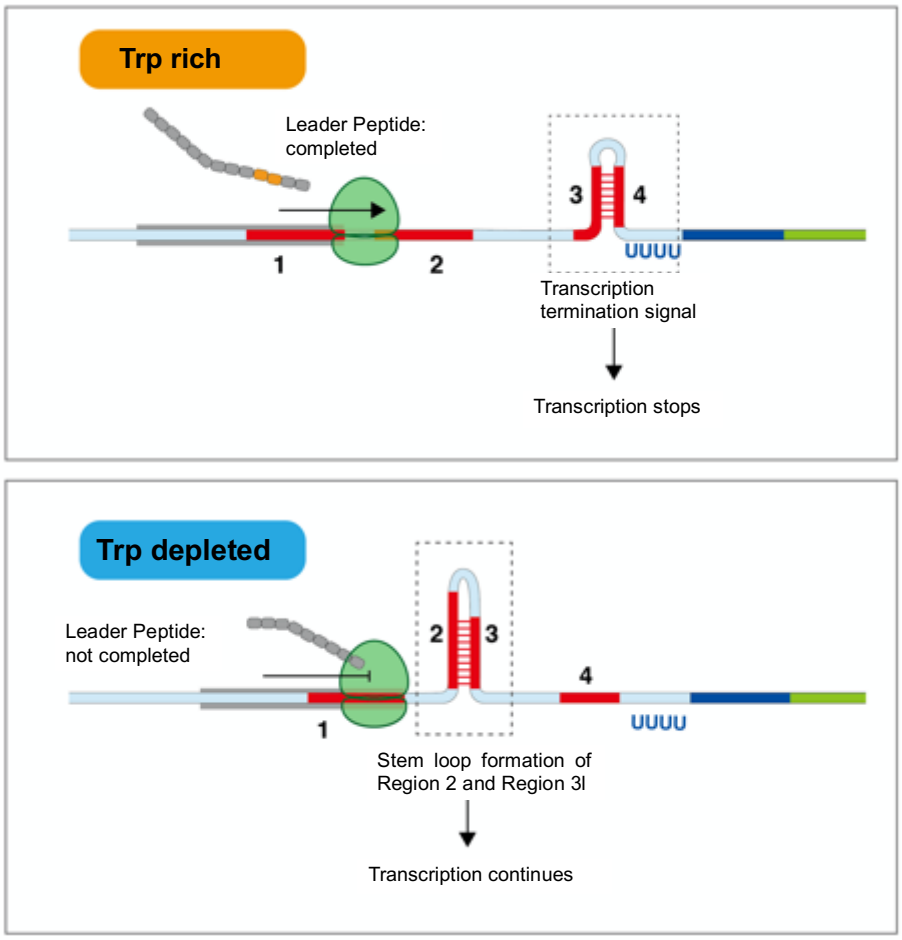


Figure 3

When tryptophan is deficient, it takes time to translate the Trp codons in the leader sequence, and the ribosome temporarily stays in Region 1. The mRNA transcribed during that time will be paired with Region 2 and Region 3 to form a stem loop structure. In this case, since Region 4 does not pair, a transcription termination signal is not formed, and RNA polymerase continues transcription of the *trpEDCBA* operon encoding the downstream Trp biosynthetic enzymes (Figure 3).

Indicate whether each of the following statements is true or false.

A. The transcription rate is much faster than the translation rate in the *E. coli* cell. 68

B. In a mutant strain of *E. coli* lacking the *trp* operator sequence, transcription-truncated mRNA is generated when tryptophan is present in the medium. 69

C. In the mutant strain in which the tryptophan codons in the leader peptide is deleted, the growth is delayed when tryptophan is deficient in the medium. 70

D. The tryptophan concentration in the cells increases in the mutant strain in which 10 tryptophan codons are present in the leader peptide. 71

Animal biology

Q19

Glucagon is secreted from pancreatic A-cells and works as a signal via receptors (GLR) on the cells of target tissues. The amount of GLR expressed on cell surfaces is important in determining the magnitude of the response to glucagon in each target tissue. Figure 1 shows the amount of GLR mRNA in different rat tissues. In the data shown here, the glucagon receptor is not detected in brain tissue, but recent reports have revealed that it is present even in a very small amount, *e.g.*, in the hypothalamus.

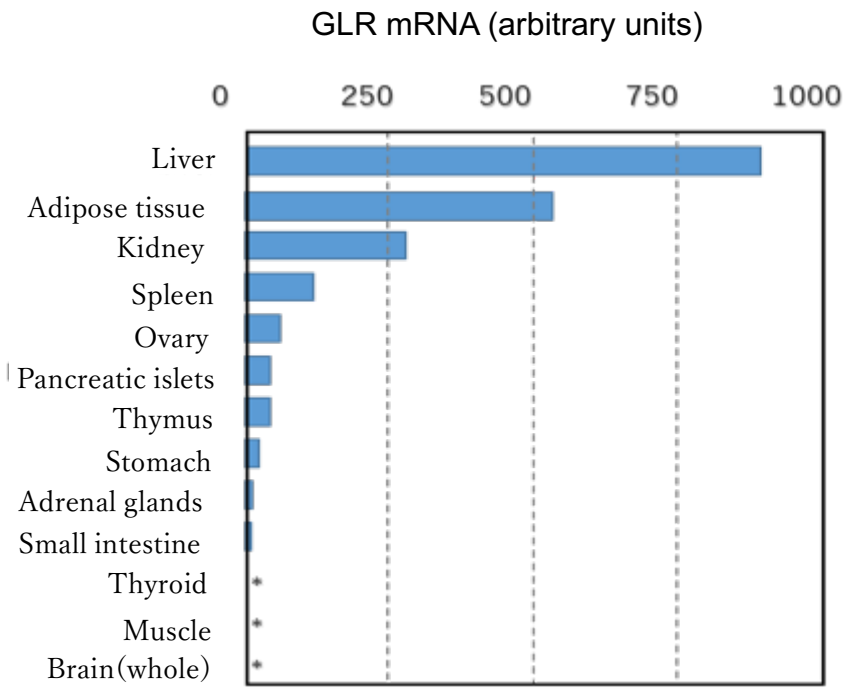


Figure 1 Relative abundance of GLR (glucagon receptor) mRNA in rat tissue. * indicates less than detectable level.

Indicate whether the following descriptions are true or false.

A. Liver expresses the largest amount of GLR because it is working as one of the major organs that uptake and storage glucose in response to glucagon. 72

B. A lack of mRNA detection in brain tissue indicates that neural tissue in the brain does not require much glucose as a nutrient. 73

C. Skeletal muscles hold stores of glucose only used in exercise. This is consistent with the absence of GLR from the results of this experiment. 74

D. Adipose tissue, which has high levels of expression of GLR, is most important energy sources during starvation. 75

Animal biology

Q20

Metabolic substrate	Concentration [mM]	Output power [W]	Expected speed [m/s]	Exercise duration [s]
ATP	8	6400	27	2-4
CP	26	6000	25	10-17
Glycogen	90	1640	6.7	>6000
Fat	7-25	1100	4.6	

Table 1. Types of metabolic substrate and its concentration as an energy source in human muscle cells. The predicted values of output power produced by the muscle tissue, the expected speed at which the athlete ran with that power, and the duration of exercise are shown when only the respective energy sources were used. CP indicates creatine phosphate.

Indicate whether the following descriptions are true or false.

A. Athletes running a 100-meter sprint are supposed to run using ATP originally stored in muscle cells during the former half. During the last half, ATP produced by respiration is used. 76

B. It is possible that marathon runners continue exercising using muscle tissue without ATP. 77

C. A crucial point for middle-distance runners of 1,500 m is switching smoothly from running with CP to that with ATP produced by aerobic breathing. 78

D. Similar to bird migration, stored fat is one of the major energy sources for long-distance runners, although it has some metabolic delay for conversion into ATP. 79

Animal biology

Q21

Huntington's disease (HD) is a genetic disorder characterized by devastating degeneration of nerve tissues that progresses with age. Huntingtin (HTT) is known to be the causative protein of HD. Near the transcriptional initiation point of HTT gene, there is a sequence containing repeated CAG (corresponding to glutamine), which are usually between 9 to 35 repeats in healthy individuals. These repeats are 35 to 75 in HD-population. The symptoms of HD tend to appear at a younger age and are more severe when there are an increased number of CAG repeats.

Recently, scientists in France have revealed that HTT plays an important role in maintaining neuronal fast axonal transport (FAT, Figure 1). By careful observations with fluorescence microscopy, they first showed that HTT was co-localized with motor proteins (kinesin and dynein) that are involved in FAT. HTT was also shown to be co-localized with synaptic vesicles, as well as with glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Interestingly, HTT was not found with mitochondria that were transported by FAT. Next, using cultured neurons, they investigated the effects of oligomycin, an inhibitor of ATP production in mitochondria, and iodoacetate acid, an inhibitor of GAPDH activity (Table 1). Furthermore, when HTT expression was suppressed by RNAi treatment, only the FAT of synaptic vesicles, not that of mitochondria, was significantly reduced. These results indicate that HTT was solely involved in FAT of synaptic vesicles.

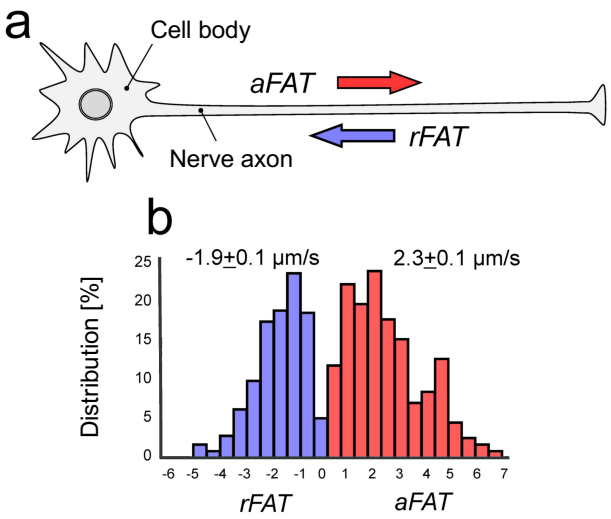


Figure 1 Fast axonal transport (FAT) in nerve cells. **a**, active transportation of synaptic vesicles and mitochondria outward to the nerve ends is called anterograde FAT (*aFAT*). Transportation in the opposite inward direction is called retrograde FAT (*rFAT*). Measured velocity and its distribution (%) is shown in **b**.

	Synaptic vesicles			*Mitochondria*		
	Control	*Oligomycin*	*Iodoacetate*	*Control*	*Oligomycin*	*Iodoacetate*
aFAT	2.3 ± 0.1	2.2 ± 0.2	0.3 ± 0.1	0.9 ± 0.1	0.3 ± 0.1	1.0 ± 0.1
rFAT	-1.9 ± 0.1	-1.9 ± 0.2	-0.2 ± 0.1	-1.2 ± 0.1	-0.4 ± 0.2	-1.0 ± 0.1

Table 1 Effects of oligomycin and iodoacetate on the velocity [μm/s] of anterograde (*aFAT*) and retrograde (*rFAT*) transportation. In the experiments to determine FAT velocity with iodoacetate, pyruvate was included to maintain ATP production by mitochondria. Control experiments were carried out in a buffer medium without inhibitors. Under all experimental conditions, ATP/ADP ratio in axons was maintained >80%.

Indicate whether each of the following statements is true or false.

A. Near the N-terminal end of HTT molecules in HD patients, there is a larger number of glutamine repeats compared to that in healthy individuals. 80

B. It is possible that HTT helps to anchor GAPDH and motor proteins to synaptic vesicles. 81

C. ATP produced by mitochondria is not efficiently used for FAT of synaptic vesicles, even though it can maintain a sufficiently high ATP concentration within axons. 82

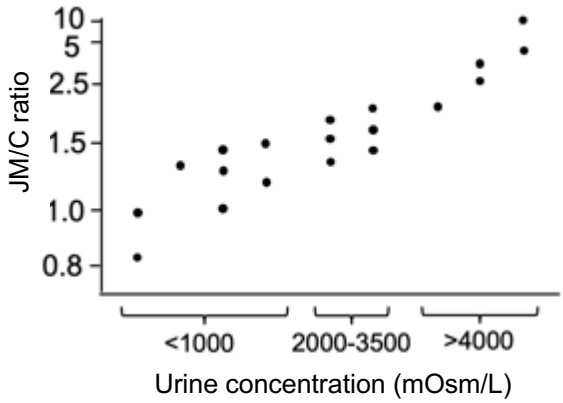
D. ATP produced by glycolysis is crucial for the FAT of mitochondria. 83

Animal biology

Q22

Animals living in deserts like kangaroo rats achieve the ability to sustain themselves on a limited supply of water through incredibly well adapted kidney. To remove waste without losing water, species have developed mechanisms to concentrate their urine. There are two types of nephrons that concentrate urine, a type with a short Henle loop located in the renal cortex (cortex: C) and a type with a long Henle loop located near the renal medulla (juxtamedullary: JM). The ratio of these two types of nephrons differs depending on the animal. The table shows the habitat of each animal species and the urea concentration in urine. The graph plots the juxtamedullary-cortex ratio (the number of JM type loop/the number of C type loop) in each animal species.

Species	Habitat	Urine concentration (mOsm/L)
Rat	moderate	2900
Domestic cat	moderate	3100
Kangaroo rat	dry	5500
Beaver	freshwater/land	520
Human	moderate	1400
Porpoise	marine	1800
Eland	dry	1880
Camel	dry	2800



Indicate whether each of the following statements is true or false.

A. Beavers seem not to possess the cortex type nephron. 84

B. The JM/C ratio of the kangaroo rat is estimated at 1.5 or more. 85

C. Longer Henle loops can efficiently reabsorb salts, resulting in urine concentration. 86

D. Animals living in dry regions have a higher proportion of cortex type nephrons than those living in freshwater. 87

Animal biology

Q23

A researcher recorded neurotransmitter responses from a neurosecretory neuron in the hypothalamus. Gamma-aminobutyric acid (GABA) is well-known as the neurotransmitter at most inhibitory synapses in the brain. The researcher found that the application of GABA to this neuron induced more action potentials (Figure 1). Then, the researcher measured GABA-induced chloride current responses of the neuron under various experimentally controlled membrane potentials (from -50 to 0 mV at 10mV steps; Figure 2). They also plotted maximum current amplitudes (current differences before and after the GABA application) against membrane potentials (Figure 3). A downward deflection of a current trace is referred to as an inward current and reflects the movement of Cl- ions out of the cell (Figure 4). Table 1 shows the intra- and extracellular concentrations and the equilibrium potentials of sodium, potassium, and chloride ions calculated by Nernst's equation.

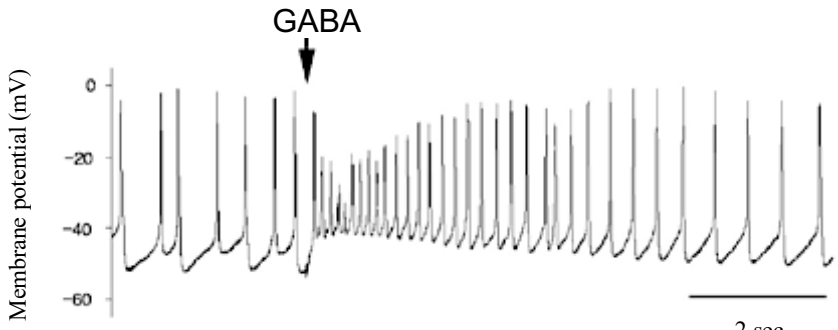


Figure 1

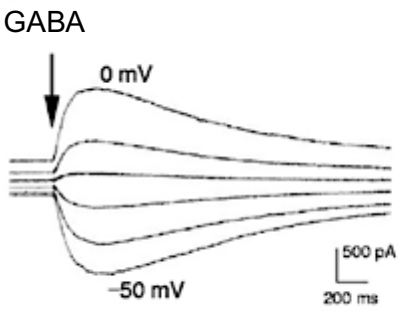


Figure 2

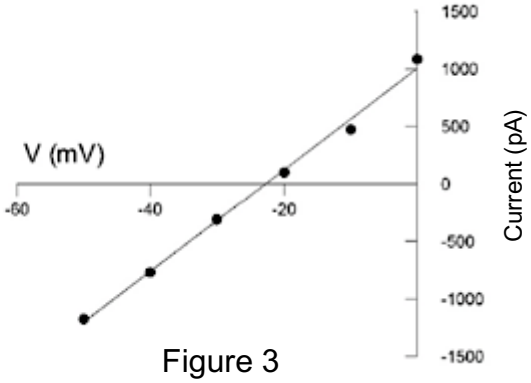


Figure 3

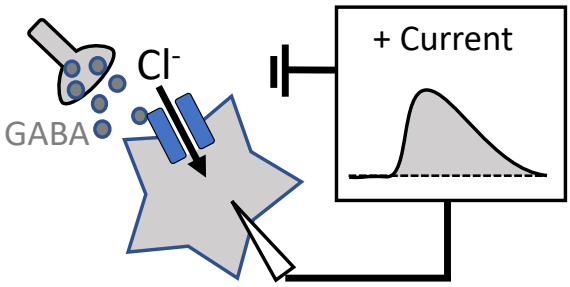


Figure 4

Ion	Concentration (mM)		Equilibrium Potential (mV)
	Inner	Outer	
Na^+	15	150	58
K^+	140	7	-75
Cl^-	40	120	-28

Table 1

Indicate whether each of the following statements is true or false.

A. When the membrane potential was -10 mV, the application of GABA induced the depolarization of the recorded neuron. 88

B. The equilibrium potential of chloride ions was more positive (less negative) than the resting membrane potential of the recorded neuron. 89

C. Under the presence of tetrodotoxin (pufferfish toxin that blocks the generation of action potentials), the higher concentration of GABA depolarized the neuron more positively than 0mV. 90

D. The researcher recorded other neurons. The neurons hyperpolarized their membrane potentials by GABA. If the resting membrane potential of both neurons are the same, intracellular chloride ion concentration of the hyperpolarized neurons is lower than those of the neurons observed in Figure 1-4. 91

Animal biology

Q24

In the African clawed frog, *Xenopus laevis*, the mode of cell division shifts from cleavage to somatic cell division, which has interphase, at the 12th cleavage after fertilization. This is called the mid-blastula transition (MBT).

Microinjection of mRNA of genes that are required for nuclear membrane formation at one-cell stage results in the increase of the nuclear size, but cell size does not change compared with a control embryo. In this experiment, MBT occurs earlier than the 12th cleavage (Figure 1, left). Conversely, when the nuclear size is artificially reduced, the cell size does not again change but MBT occurs later than the 12th cleavage (Figure 1, right). Note: These treatments do not alter the time required for each cleavage.

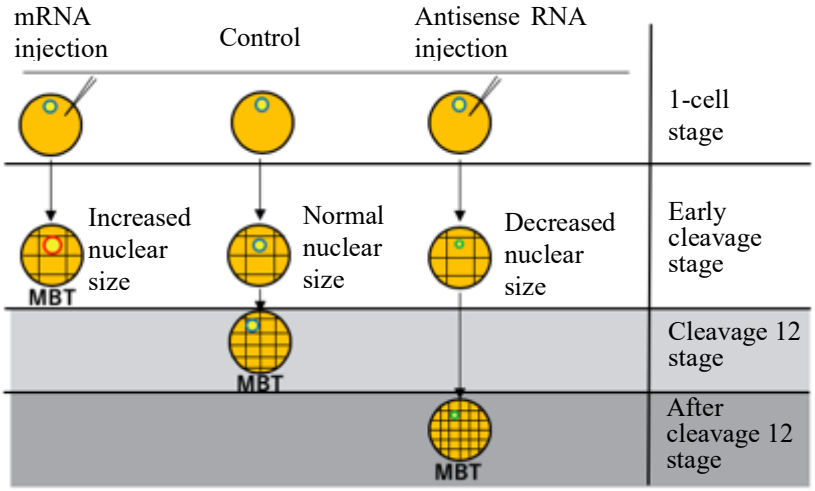


Figure 1

Indicate whether each of the following statements is true or false.

A. This experiment indicates that that MBT occurs when the volume ratio of nucleus/cytoplasm is high. 92

B. When MBT occurs before the 12th cleavage stage, the duration from the fertilization to the 12th cleavage stage is reduced. 93

C. The timing of MBT depends on the number of divisions after fertilization. 94

D. These results indicate that MBT occurs when the amount of histone per nucleus is greater than a certain value (Note: No manipulations performed in this experiment affect amount of histone). 95

Animal biology

Q25

In a *Xenopus* embryo, the dorsal-ventral axis is determined through cortical rotation after fertilization. On the dorsal side of an embryo, the Spemann-Mangold organizer is necessary to determine the body plan of the embryo. When the organizer formation is inhibited, a head defect occurs in embryos. On the other hand, the head is enlarged when the organizer region expands.

β-catenin (β-cat) and GSK3β are involved in organizer formation. The table below shows the results of phenotype of tadpoles microinjected with β-cat, GSK3β, an DN β-cat (β-catenin inhibition factor), and DN GSK3β (GSK3β inhibition factor) into the dorsal or ventral side of the embryo.

mRNA	Dorsal injection	Ventral injection
β-cat	Large head	Secondary head formation
GSK3 β	Head defect	No effect
β-cat + GSK3β	No effect	No effect
DN β-cat	Head defect	No effect
DN GSK3β	No effect	Secondary head formation

Indicate whether each of the following statements is true or false.

A. This experiment shows that GSK3β inhibited organizer formation. 96

B. This experiment shows that GSK3β inhibits β-cat activity. 97

C. This experiment shows that β-cat is not expressed in ventral region. 98

D. This experiment shows that GSK3β works downstream of β-cat. 99

Animal biology

Q26

Animals possess mechanisms for maintaining their body temperature within permissible levels. For example, they show various responses to changes in room temperature. In addition, animals' body shapes are optimized to adapt to various climate changes, and their behaviors also regulate their body temperature.

Indicate whether each of the following statements is true or false.

A. In each ordinary habitat, the body temperature of endotherms is always higher than that of ectotherms. 100

B. In humans, the body temperature is elevated when the temperature of the hypothalamus is artificially increased. 101

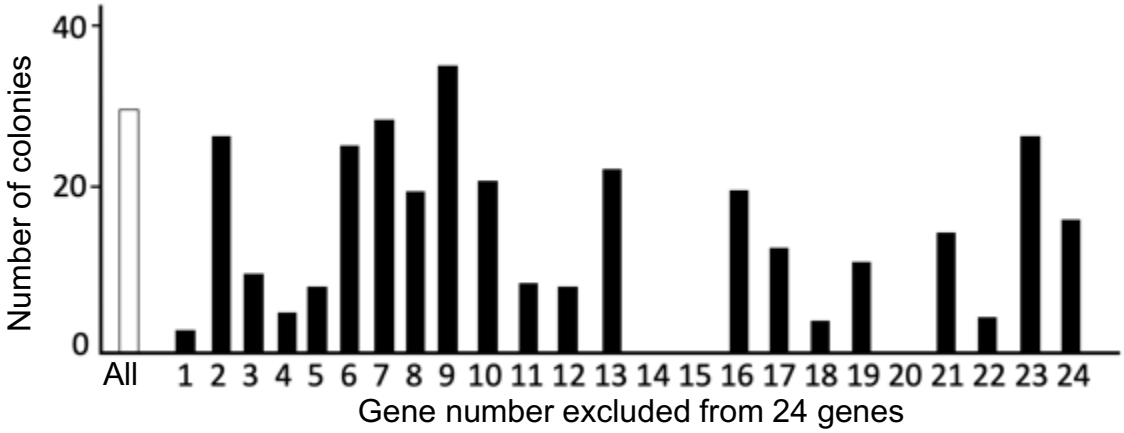
C. When a female Burmese python incubates eggs, her oxygen consumption in a cold room is less than that in warm room. 102

D. Ectotherms require less energy than endotherms for homeostasis. 103

Animal biology

Q27

A researcher aimed to induce undifferentiated cells by expressing multiple genes in human fibroblasts. They focused on 24 genes that were identified as highly expressed in embryonic stem (ES) cells. It was found that when all 24 genes were simultaneously introduced in fibroblasts, the colony formation characteristics of undifferentiated cells occurs. Next the researchers tried to find the minimum set of genes that induce undifferentiated cells. The graph shows the colony formation when 23 genes except one were introduced into fibroblast cells.



("All" on the X axis shows that the 24 genes are all introduced.)

Indicate whether each of the following statements is true or false.

A. These results show that colonies can be formed through the introduction of genes 14, 15, and 20 into the fibroblast together. 104

B. These results show that gene 14, gene15, and gene 20 are required for colony formation. 105

C. These results show that the colony number is the highest when gene 9 is introduced into the fibroblast. 106

D. This experiment alone is not sufficient to find the minimum gene set needed to induce colonies. 107

E. These results show that genes 14, 15, and 20 are expressed in fibroblast cells. 108

Animal biology

Q28

Immature lymphocyte B cells differentiate in an area of the peripheral lymph organ called the embryo center. Myeloma cells are tumor cells that produce one type of mature immunoglobulin. mRNAs for full-length or only 3 'half of the immunoglobulin light chain gene were purified from a myeloma cell and were radioisotope-labeled. Genomic DNA fragments obtained from either the embryo center or myeloma cells were digested with a restriction enzyme and size fractionated by agarose electrophoresis. These DNA were hybridized with radiolabeled mRNA, and radiation was measured after the removal of unhybridized mRNA (the experimental flow is shown in Figure 1). The results are shown in Figure 2.

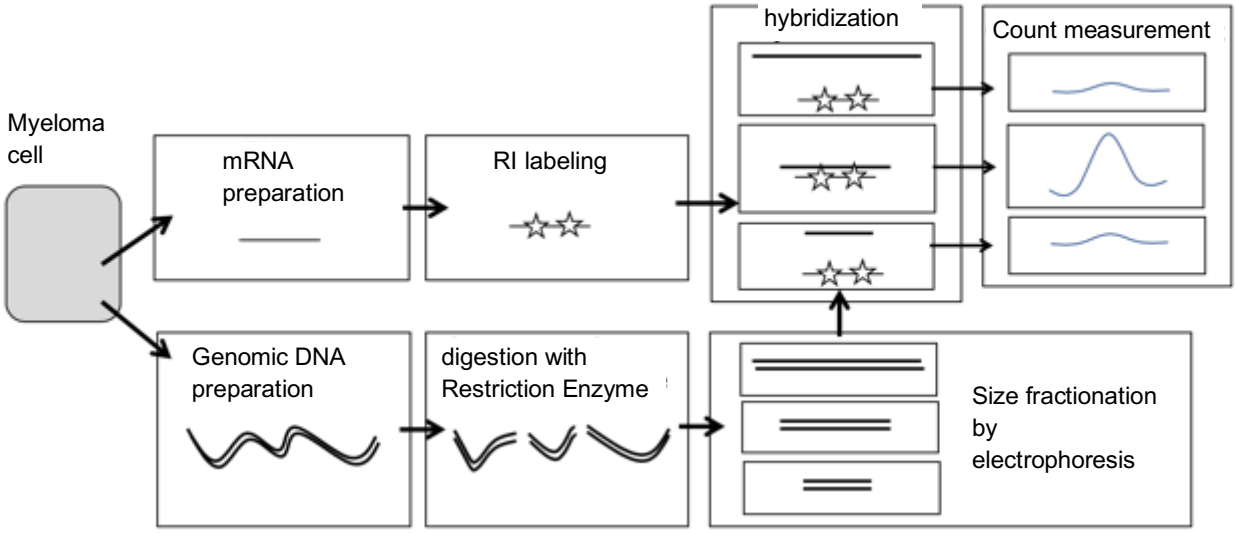


Figure 1

	DNA	mRNA
———	myeloma	Full length
- - - -	myeloma	3'end half
———	embryo	Full length
- - - -	embryo	3'end half

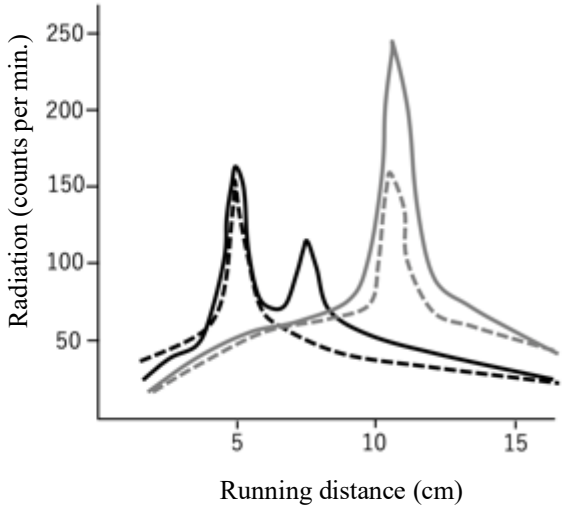


Figure 2

Indicate whether the sentences below are correct or incorrect.

A. The immunoglobulin light chain gene contained in the embryo center cells is shorter than that in the myeloma cells. 109

B. The running distance depends on the length of DNA hybridized with mRNA. 110

C. The nucleotide sequence of DNA region hybridized with 3'-end mRNA is different between the myeloma-derived DNA and the embryo center-derived DNA. 111

D. The full-length immunoglobulin light chain mRNA isolated from myeloma cells contains sequences from two different parts of the DNA genome of the embryo center cells. 112

Plant biology

Q29

In order to prevent an excess water loss, stomata respond rapidly to changes in humidity. Transpiration rate per unit leaf area represents the speed of water loss from the plant body. It is proportional to the diffusion rate of water vapor (d_{water}), the water vapor concentration difference across the leaf epidermis (Δw), and the relative stomatal aperture. Figure 1 shows relative stomatal apertures in normal air and in Helox air (79:21 mixture of He and O_2 with the appropriate concentrations of water vapor and CO_2 added). Relative stomatal apertures were measured in normal air (Air) and in Helox air under three different Δw conditions: the same Δw as that in normal air (Helox), 2/3 of the normal air Δw ($Helox^{2/3}$), and 1/2.33 of the normal air Δw ($Helox^{1/2.33}$). d_{water} in Helox air is 2.33 times higher than that in normal air, while Helox air does not affect any other factors of transpiration. Note that water vapor diffuses only though the stomata and that the water vapor concentration inside the leaf is always saturated.

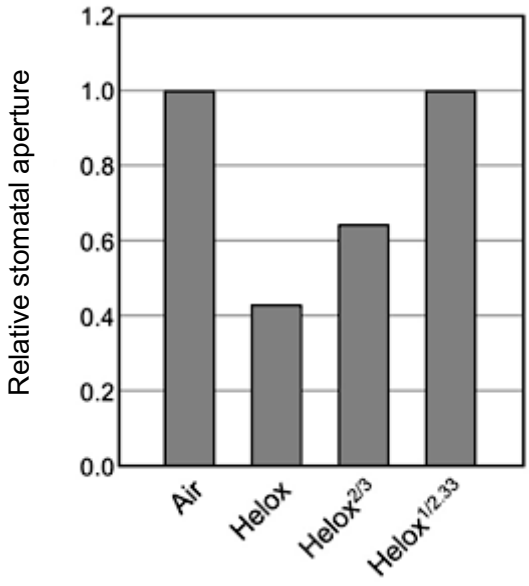


Figure 1 Relative stomatal apertures in various conditions

Indicate whether each of the following statements is true or false.

A. Stomata respond to the absolute humidity of the air. 113

B. Transpiration is higher in Helox air than that in normal air at the same humidity. 114

C. Stomatal response to low humidity decreases the photosynthetic assimilation rate. 115

D. Stomatal response to low humidity keeps the water loss constant. 116

Plant biology

Q30

When tomato leaves are wounded, the expression of protease inhibitor genes is induced and protease inhibitor proteins accumulate in the leaves. This response contributes to defense against insect herbivory as the protease inhibitor proteins suppress the digestive function of insects. Since this response occurs not only in damaged leaves but also in undamaged leaves, it is assumed that some mobile molecules transmit wound signals over long distances.

Jasmonate and systemin, a signaling peptide composed of 18 amino acids, are involved in wound-induced expression of protease inhibitor genes. Indeed, neither systemin-insensitive mutant (*spr1*), jasmonate biosynthesis-deficient mutant (*spr2*), nor jasmonate-insensitive mutant (*jai1*) show expression of protease inhibitor genes after wounding.

To investigate the roles of jasmonate and systemin in the long-distance signaling, experiments with grafts between wild-type and mutant plants were conducted. Leaves of the stock were subjected to wounding and then the expression of protease inhibitor genes were assayed, both in damaged leaves of the stock and in undamaged leaves of the scion (Figure 1). The results are summarized in Table 1.

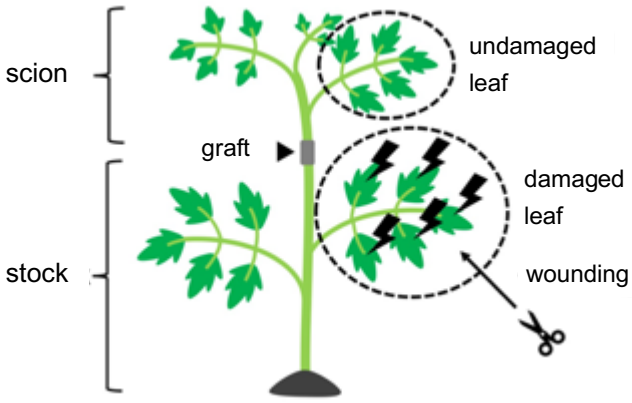


Figure 1. Schematic illustration of graft experiments

Table 1

Genotype		Expression of protease inhibitor genes	
stock	scion	stock	scion
wild type	*spr1*	+	+
spr1	wild type	–	–
wild type	*spr2*	+	+
spr2	wild type	–	–
wild type	*jai1*	+	–
jai1	wild type	–	+

Indicate whether each of the following statements is true or false.

A. Perception of systemin in the proximity of the wound site is required for the expression of protease inhibitor genes in leaves distant from the wound site. 117

B. Jasmonate synthesis required for protease inhibitor gene expression takes place in the proximity of the wound site. 118

C. Perception of jasmonate in the proximity of the wound site is required for the expression of protease inhibitor genes in leaves distant from the wound site. 119

D. Systemin is likely to be the mobile signaling molecule responsible for long-distance wound signaling. 120

Plant biology

Q31

Strigolactone (SL) is a plant hormone that controls shoot branching. In *Arabidopsis thaliana*, several SL-related mutants, such as *max1, max2*, and *max4*, which have loss-of-function mutations in the genes *MAX1*, *MAX2*, and *MAX4*, respectively, have been isolated. While *MAX2* encodes a key component of the SL receptor complex, *MAX1* and *MAX4* each encode an enzyme for SL biosynthesis (Figure 1); *MAX4* for the production of the SL precursor carlactone (CL), and *MAX1* for the conversion of CL into SL. Grafting experiments using these mutants and the wild type (WT) were conducted, and the number of shoot branches were counted (Figure 2 & 3). In this experiment, neither mRNAs nor proteins of the *MAX* genes were found to move across the grafting junction.

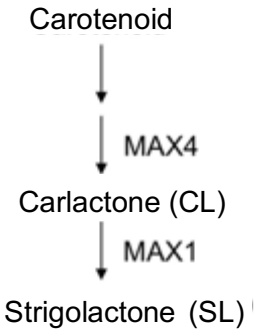


Figure 1.
Biosynthetic pathway of strigolactone

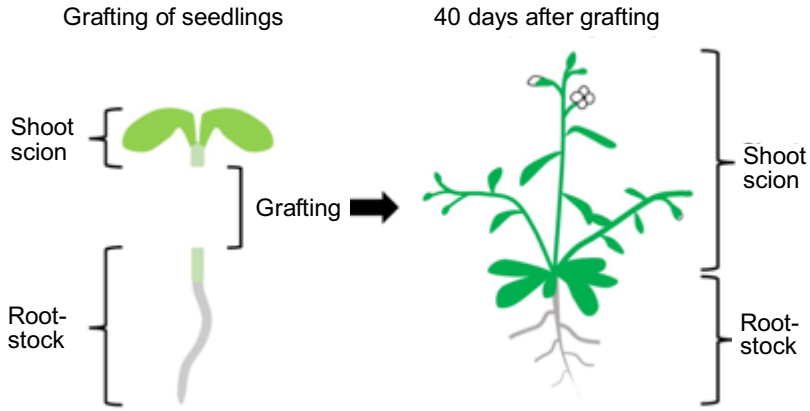


Figure 2.
Schematic illustration of grafting experiments

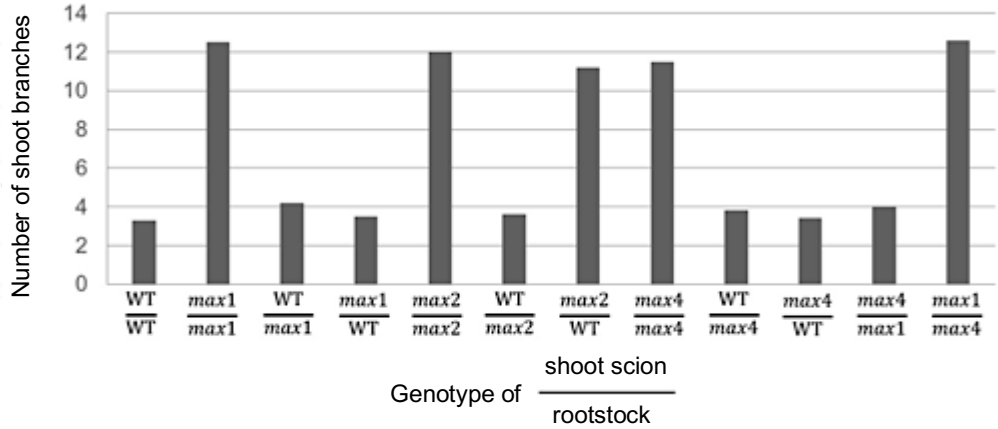


Figure 3. Number of shoot branches in the grafted plants

Indicate whether each of the following statements is true or false.

A. The *MAX2* gene mainly functions in the root. 121

B. SL is synthesized both in the root and shoot. 122

C. CL, the substrate of MAX1, is transported between the root and shoot in either direction. 123

D. If a shoot scion of *max4* is grafted on a rootstock of *max2*, shoot branching will be normal. 124

Plant biology

Q32

Zinc (Zn) and iron (Fe) are both micronutrients for plants. Plants obtain Zn and Fe ions from soil through the root system, and transport them to the shoot. Plant culture media usually contains low concentrations of these micronutrients. Half-strength MS medium, a typical plant culture medium, contains 15 μM Zn^{2+} and 50 μM Fe^{2+}.

Although micronutrients are essential for plant growth, at excess concentrations they inhibit plant growth. To examine the inhibitory effects of excess micronutrients, *Arabidopsis thaliana* plants were grown on half-strength MS media, supplemented with additional Zn^{2+} and/or Fe^{2+}.

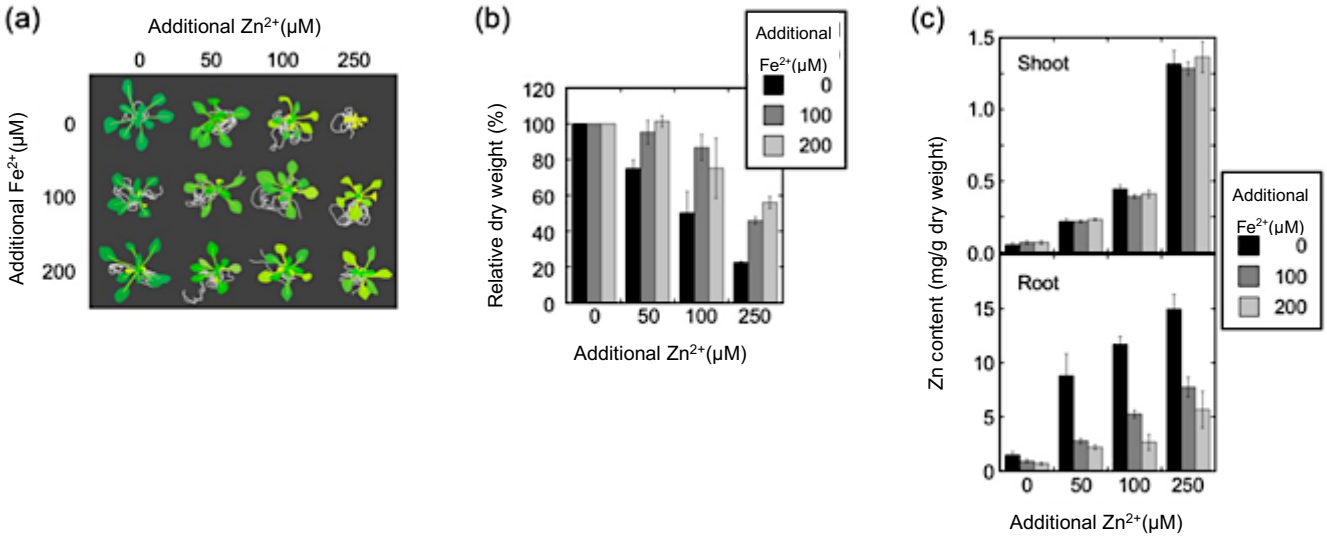


Figure 1. Effects of additional Zn and Fe ions on plant growth

Plants that had been grown on half-strength MS media, supplemented with additional $Zn^{2+}$and/or Fe^{2+}at the indicated concentrations, were pictured from above (**a**), measured for the dry weight of the shoot (**b**), and analyzed for the Zn contents in the shoot and root (**c**).

Indicate whether each of the following statements is true or false.

A. Zn accumulation in the shoot shows a stronger correlation to growth defects than the correlation shown by Zn accumulation in the root. 125

B. The growth defect caused by excess Zn^{2+} in the culture medium is mitigated by the addition of Fe^{2+}. 126

C. High concentrations of Fe^{2+} in the culture medium suppresses Zn^{2+} uptake by the root. 127

D. Total Zn amount in the shoot is not affected by the addition of Fe^{2+} in the medium. 128

Plant biology

Q33

While snapdragon normally has bilateral flowers, flowers of its mutant defective in gene ***G*** lose bilateral symmetry and have radial symmetry, thereby indicating that gene ***G*** confers bilateral symmetry to the flower.

In the inflorescence of wild-type sunflower, the outer region has ligulate florets, whereas the inner region has tubular florets (Figure 1). Variants ***x***, ***y***, and ***z*** of sunflower have DNA insertions in gene ***G'***, a sunflower orthologue of the gene ***G*** from snapdragon (Figure 2). As a result of these insertions, variant ***x*** has only ligulate florets over the entire inflorescence, and variants ***y*** and ***z*** have only tubular florets over the entire inflorescence.

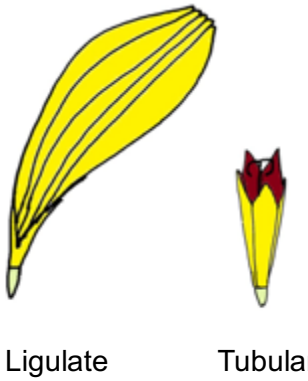


Figure 1 Ligulate and tubular florets of sunflower

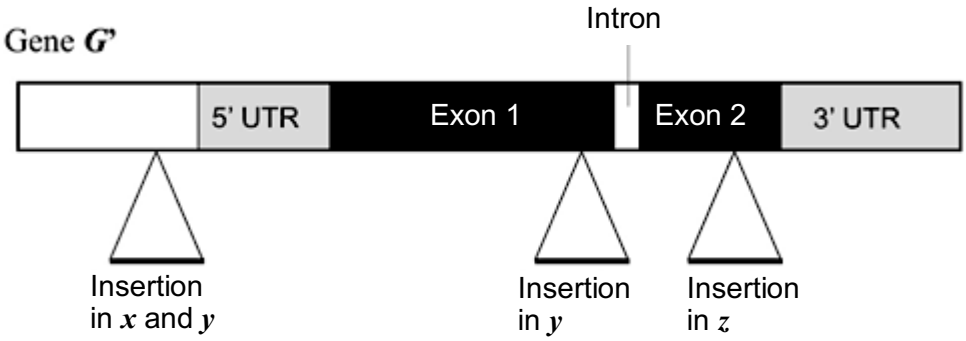


Figure 2 DNA insertions in gene ***G'*** in the sunflower variants

Variant ***y*** has two DNA insertions, while variants ***x*** and ***z*** have one insertion.

Indicate whether each of the following statements is true or false.

A. In the wild-type sunflower, gene ***G'*** is not expressed in the florets that form early during inflorescence development, but is expressed in the florets that form later. 129

B. In variant ***x***, expression of gene ***G'*** is decreased due to the DNA insertion. 130

C. Variant ***y*** is a loss-of-function mutant of gene ***G'***. 131

D. Variant ***y*** is more closely related to variant ***x*** than to variant ***z*** in the lineage of sunflower variants. 132

Plant biology

Q34

Fragaria chiloensis is a stolon*-bearing perennial herb that grows on coastal sand dunes. In coastal sand dunes, nitrogen-fixing shrubs often create small patches of lower photon flux density (PFD) but higher soil nitrogen availability. The presence of such patches frequently makes a difference in the resource availability between stolon-connected ramets**. To examine effects of stolon connection, researchers compared the growth of connected ramets and severed ramets; one ramet in each pair was provided with high PFD but a low level of soil nitrogen, and the other ramet was provided with low PFD but a high level of soil nitrogen (Figure 1). As a result, combined dry biomass of connected ramets was 54% higher than that of severed ramets.

*Stolon: a stem that grows along the soil surface and forms buds and roots at the nodes for clonal propagation.
**Ramet: an individual unit of a clonal colony.

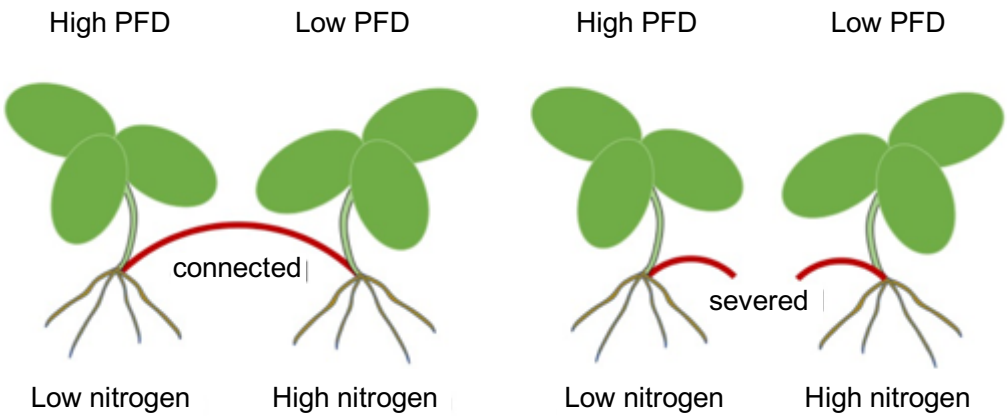


Figure 1 Schematic cartoon of experimental setup

Indicate whether each of the following statements is true or false.

A. Shoot/root ratio of the connected ramet provided with high PFD and low nitrogen was higher than that of the severed ramet provided with high PFD and low nitrogen. 133

B. In the severed ramet provided with low PFD and high nitrogen, PFD was not a limiting factor for plant growth. 134

C. Severing of stolons does not affect the combined dry mass of ramets when resources (i.e. PFD and nitrogen) are distributed uniformly. 135

D. Assimilation products and nitrogen can be translocated via stolons in *Fragaria chiloensis*. 136

Plant biology

Q35

Soil salinity (NaCl) affects the growth of plants. As the increase of osmotic pressure induced by soil salinity reduces the ability of plants to take up water and minerals, soil salinity elicits osmotic stress. Additionally, because cytosolic Na^+ interferes with the activities of metabolic enzymes, soil salinity also elicits ionic stress. Thus, NaCl elicits two primary effects on plant cells, which both trigger a signaling cascade that start with the elevation of the intracellular Ca^{2+} concentration ($[Ca^{2+}]i$). In contrast, sorbitol, a sugar alcohol often used as an osmoticum, elicits only osmotic stress because sorbitol is non-ionic. *x* and *y* are mutants of Arabidopsis isolated as defective in NaCl-induced increases in $[Ca^{2+}]i$. Figure 1 illustrated below shows the dose-dependent $[Ca^{2+}]i$ increases induced by NaCl or sorbitol in the seedlings of the wild type (WT) and mutants *x* and *y*.

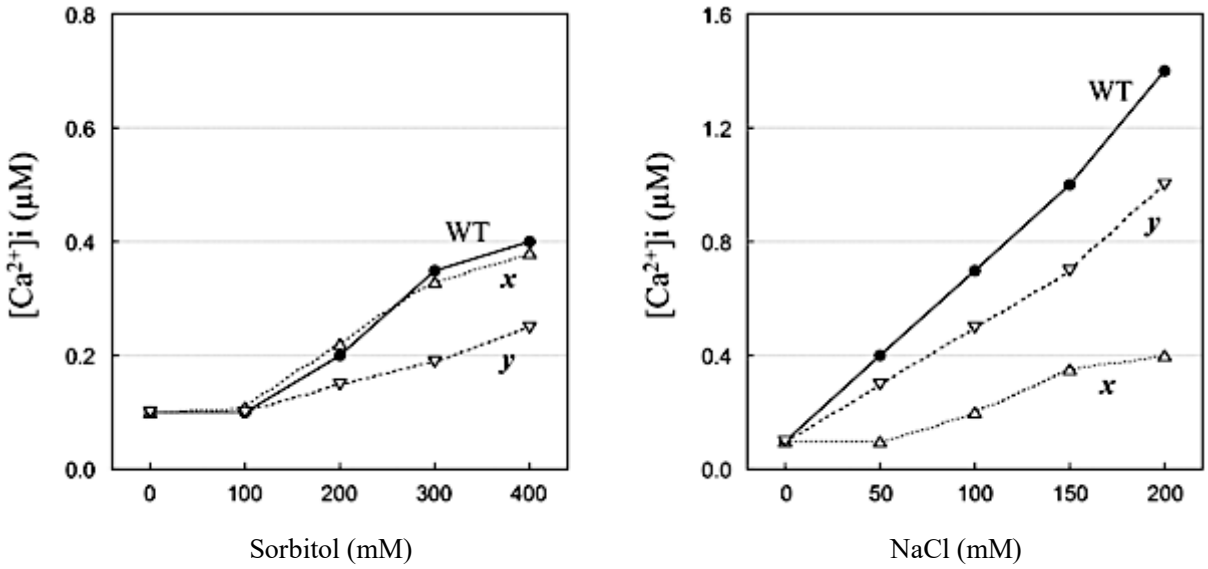


Figure 1

Indicate whether each of the following statements is true or false.

A. Mutant *x* is defective in sensing osmotic stress. 137

B. Mutant *y* can sense ionic stress. 138

C. The dose-dependent $[Ca^{2+}]i$ increases induced by NaCl of the *x y* double mutant are expected to be equivalent to those of the *x* single mutant. 139

D. The dose-dependent $[Ca^{2+}]i$ increases induced by sorbitol of the *x y* double mutant are expected to be equivalent to those of the *y* single mutant. 140

Q36

Following is a description regarding a population of a diploid organism, species A, with a special focus on the locus *C* that is involved in body color.

Based on the given information, indicate whether each of the following statements is true or false.

A. Information: Species A consists of two color morphs, black and yellow, controlled by a single locus C: the allele C^B for the black type and the allele C^Y for the yellow type.

Statement: If the allele C^B is completely dominant to the allele C^Y and the frequency of the yellow type individuals is 9%, the genotype frequency of C^BC^B is about 70%. Note that the population is assumed to be under Hardy-Weinberg equilibrium. 141

B. Information: When the body colors of ten species belonging to the same genus with species A were examined, they were all yellow.

Statement: In this case, the body color of the ancestral species A just after splitting from these closely related species must have been yellow under a parsimony principle. 142

C. Information: A small portion of individuals in the population of species A was isolated due to diastrophism (large-scale deformation of Earth's crust) and formed a new population A'.

Statement: The drastic inter-generation changes of allele frequency of locus *C* in population A' can be best explained by natural selection. 143

D. Information: A slightly deleterious mutation with exactly the same effect on the fitness of an individual independently occurred in both the small population A' and the larger parental population A.

Statement: The fixation probability of this mutation is the same in both populations. 144

Evolution

Q37

The following figure is a phylogenetic tree of *ECP* and *EDN* genes in primates. EDN shows strong ribonuclease activity. By contrast, ECP shows strong anti-bacterial function, although its ribonuclease activity is weak.

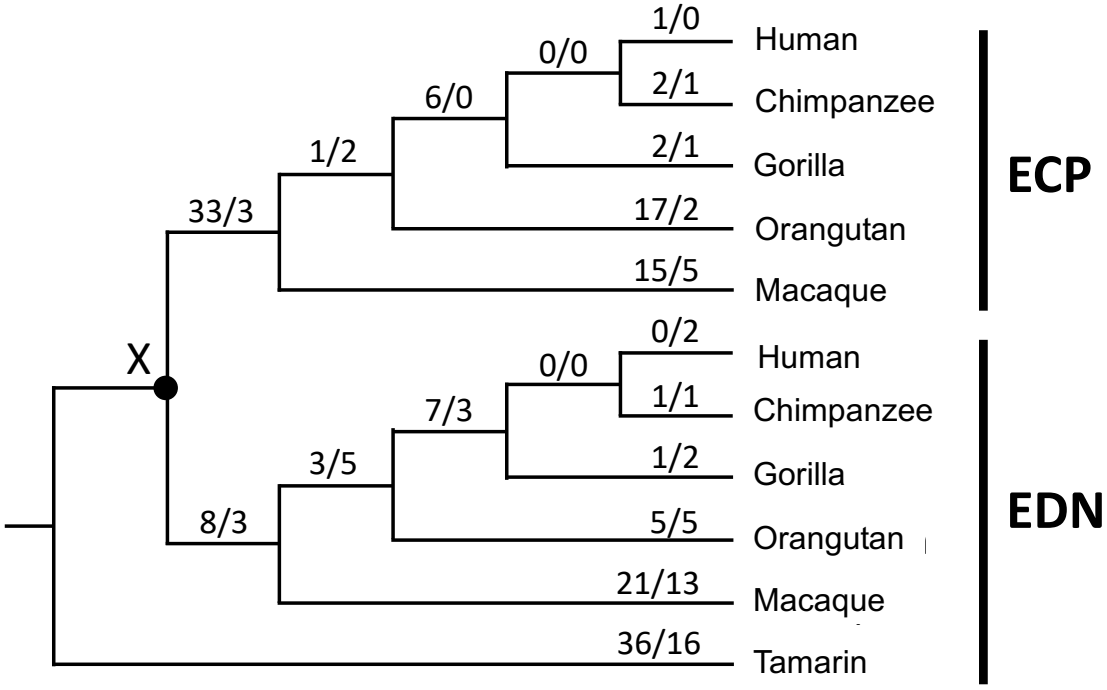


Figure 1. A molecular phylogenetic tree of *ECP* and *EDN* genes in primates based on amino-acid sequences. The numerator and denominator along each branch show the numbers of nonsynonymous and synonymous nucleotide substitutions (substitutions that cause and do not cause amino-acid changes), respectively. Branch length is not proportional to sequence divergence nor time.

Indicate whether each of the following statements is true or false.

A. The most recent common ancestor of these primate species only had the *EDN* gene. 145

B. It is likely that the Human, Chimpanzee, Gorilla, Orangutan, and Macaque independently obtained the *ECP* gene by gene duplication. 146

C. The number of synonymous substitutions in branches between common ancestor X and human *ECP* is smaller than that between X and human *EDN*. 147

D. During the early evolution of the *ECP* gene, positive selection likely operated on mutations that enhance anti-bacterial activity. 148

Evolution

Q38

Following is the phylogenetic tree based on the amino-acid sequences of all opsin genes in the human and zebrafish genomes.

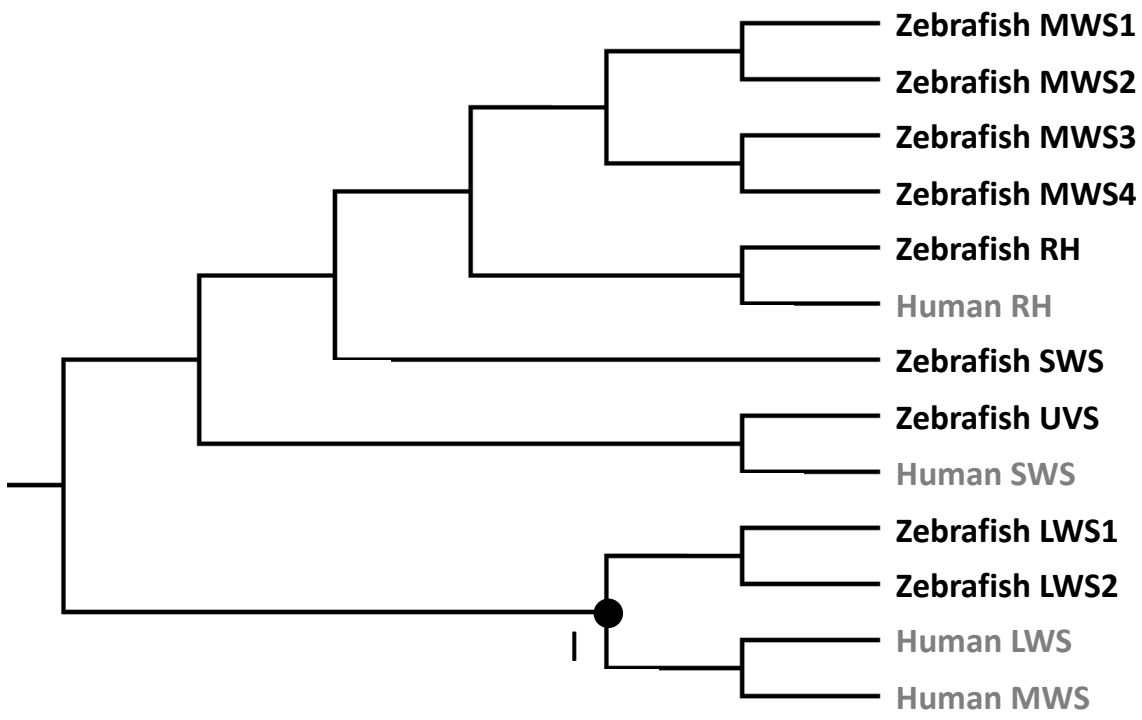


Figure 1. A phylogenetic tree based on the amino-acid sequences of all opsin genes in the human and zebrafish genomes. LWS: Long Wavelength Sensitive opsin, MWS: Middle Wavelength Sensitive opsin, SWS: Short Wavelength Sensitive opsin, UVS: Ultra Violet Sensitive opsin, RH: Rhodopsin type opsin. Branch length is not proportional to sequence divergence nor time.

Indicate whether each of the following statements is true or false under a parsimony principle.

A. In the phylogenetic tree, zebrafish SWS is most closely related to RH. 149

B. The common ancestor of the human and zebrafish had four opsin genes in the genome. 150

C. The opsin gene at node I was supposed to encode an LWS. 151

D. The zebrafish has acquired five opsin genes after splitting from the human. 152

E. The human has lost two opsin genes after splitting from the zebrafish. 153

F. The common ancestor of the human and zebrafish did not have any SWS in the genome. 154

Evolution

Q39

The *Peromyscus polionotus* inhabits the mainland of the Florida peninsula (Figure 1 ④) and has a dark-colored coat (Figure 1). In contrast, *P. polionotus,* inhabiting the light-colored sandy coastal dunes (Figure 1 ①-③), which are estimated to be 6,000 years old, has a lighter-colored coat (Figure 1). These mice show obvious differences in color patterns according to their habitat. The researchers compared the melanocortin 1 receptor gene (*MC1R*), a key gene for melanogenesis, and revealed the existence of two alleles, of which 65th amino acid residue is R or C, among these mice populations.

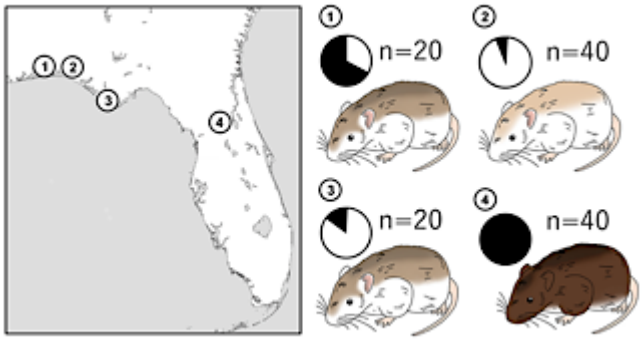


Figure 1

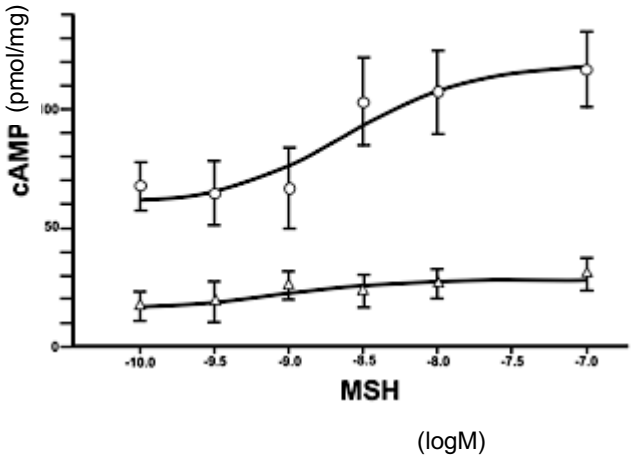


Figure 2

Figure 1. (left) Four localities of habitat of *P. polionotus* in the Florida peninsula. (right) Cartoons represent the color patterns of the mice in each locality. Pie charts indicate the frequencies of the R allele (black) and C allele (white). n indicates the number of individuals surveyed.

Figure 2. Plot of the cAMP response to the MSH (melanocyte stimulating hormone) stimulation for MC1R expressing cultured cells. The X and Y axis indicate the concentration of the MSH and cAMP, respectively. The MC1R proteins encoded by R or C alleles were examined in this experiment.

Indicate whether each of the following statements is true or false.

A. In addition to the *MC1R* gene, other genes are likely to be involved in the body color of these subspecies. 155

B. The dark color coat population is likely to have emerged from the light color coat population. 156

C. It is likely that the C alleles (65th amino acid residue is C) of each population ① to ③ results from an independent mutation. 157

D. In Figure 2, the white circle and white triangle represent R and C alleles, respectively. 158

Evolution

Q40

Pundamilia pundalilia and *P. nyererei* are a closely related sister species pair of cichlids in Lake Victoria. These two species are distinct in male nuptial body colors, in that the former and latter are blue and red, respectively. By contrast, the females of the two species are not distinct, both possessing cryptic body coloration. *P. pundamilia* and *P. nyererei* inhabit shallow and deep environments, respectively. The light component in Lake Victoria is oriented to be blue (short wavelength) in shallow and red (long wavelength) in deep environments. The opsin protein of the two species are shifted to the same wavelength of their habitat light components. In addition, inter-species hybridization occurs under the specific light condition, where red and blue lights cannot be distinguished.

Indicate whether each of the following statements is true or false.

A. The speciation of the two species is considered to have been caused by mating preference of males to females. 159

B. During evolution, each of the two species is considered to have adapted their visual cues to their habitat light environment. 160

C. The consistency between the male nuptial colorations and the light components of their habitats are explained by natural selection for camouflage. 161

D. The sequences of the opsin gene differ between males and females in each species. 162

Evolution

Q41

Molecular phylogenetics is a powerful tool for inferring phylogenetic relationships among extant species. The following are methodological statements on molecular phylogenetics.

Indicate whether each of the following statements is true or false.

A. We must choose gene(s) with faster evolutionary rate(s) when inferring a phylogenetic tree of species with older divergence. 163

B. In order to infer phylogenetic relationships between species, paralogous gene(s) that were duplicated during the evolution of the subject group should not be analyzed. 164

C. To root a phylogenetic tree with an outgroup, we should choose a species, which is as distantly related to the subject species as possible. 165

D. Two species (sp. **X** and sp. **Y**) are described based on morphological characteristics. Here, we sequenced a gene from five individuals of each species. As a result, we found that the gene sequence of an individual of sp. **Y** is more similar to those of five individuals of sp. **X** than those of other four individuals of sp. **Y**. This result contradicts the biological species concept. 166

Evolution

Q42

Figure 1 shows the evolution of cetaceans. Studies using stable isotopes indicate that *Pakicetus* and *Amburocetus* ate freshwater fish, while *Remingtonocetus*, *Miacetus* and *Basirosaurus* ate seawater fish. *Indhyus* was, like most extant artiodactyls, a terrestrial herbivorous animal.

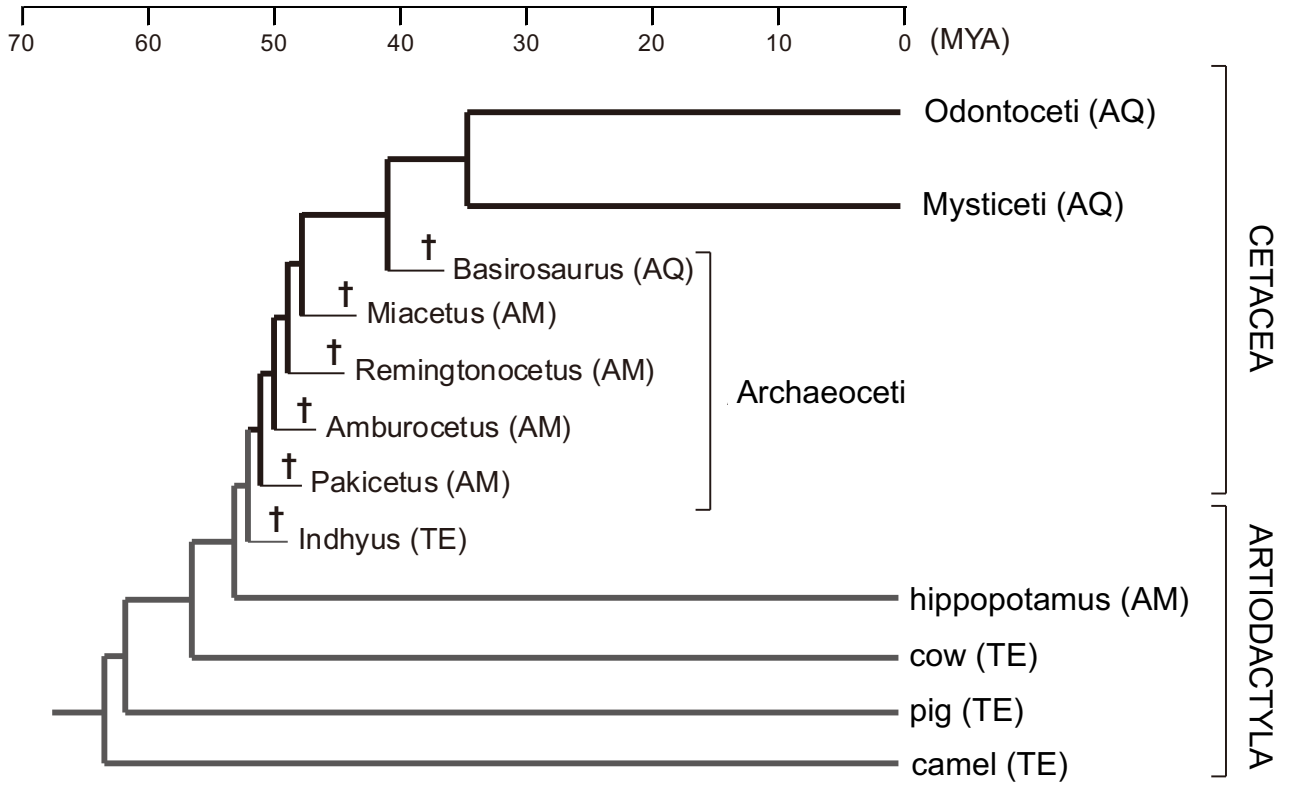


Figure 1. A phylogenetic tree of cetaceans and artiodactyls. Extinct fossil species are shown with †. All extant cetaceans are classified into two subfamilies: Mysticeti (baleen whales, which do not possess enamel-based teeth but possess baleen plates) and Odontoceti (toothed whales, which possess teeth). The lifestyle (AQ: aquatic, AM: amphibious, TE: terrestrial) of each group is also shown. Lifestyles of fossil species are inferred based on morphological characteristics. MYA: million years ago

Judging ONLY from this phylogenetic tree, indicate whether each of the following statements is true or false.

A. Under the maximum-parsimony criterion, the most recent common ancestor of the hippopotamus and modern cetaceans is amphibious. 167

B. There were no fully-aquatic cetaceans 50 MYA. 168

C. Based on these studies, the evolutionary scenario of cetaceans is speculated as follows: 1. becoming

carnivorous, 2. becoming fully aquatic, 3. invasion to oceanic environments, 4. divergence into baleen whales and toothed whales. 169

D. The *enamelin* gene, which encodes an essential protein for the formation of teeth enamel, was lost during the evolution of cetaceans before 35 MYA. 170

Evolution

Q43

Proteins encoded by *Hox* genes share a 60-amino-acid DNA-binding motif, the homeodomain. In the fruit fly *Drosophila melanogaster* genome, eight *Hox* genes are assembled in one cluster on the same chromosome (Figure 1A). The segmental expression pattern of *Hox* genes along the anterior-posterior axis of the fruit fly embryo shows collinearity with the gene order on the chromosome (Figure 1B). Fruit flies normally possess a pair of wings that develop from the second thoracic segment (T2) of the embryo, and a pair of balance organs (halteres) that develop from the third thoracic segment (T3). When the gene expression of *Ubx* gene is lost by mutations, T3 transform into T2 and two pairs of wings are formed. Beetles and grasshoppers have two pairs of wings although the most anterior segment of the UBX protein expression of their embryos is found in T3, the same as that of the wild fruit fly (Figure 1C).

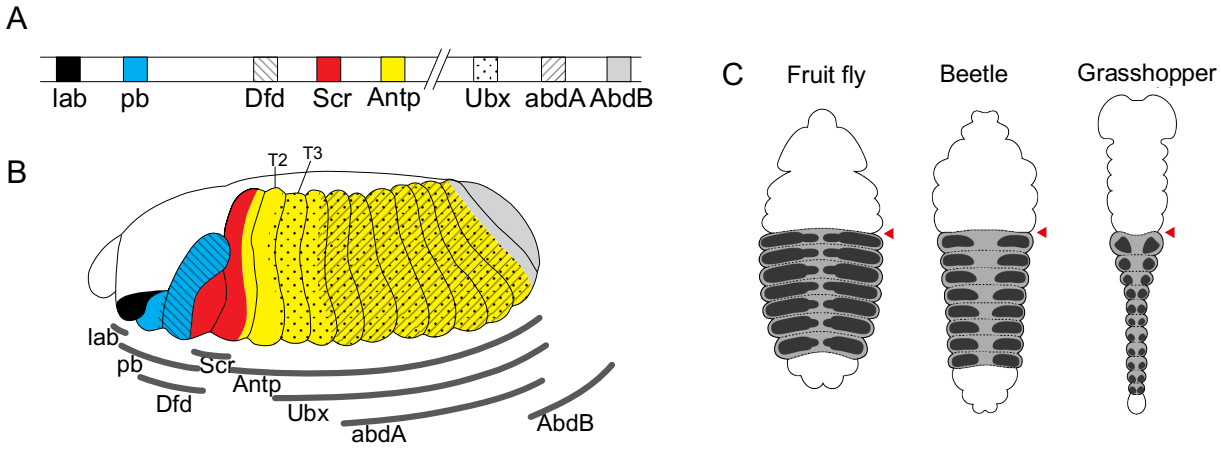


Figure 1. (A) Eight *Hox* genes on the fruit fly genome: *lab*, *pb*, *Dfd*, *Scr*, *Antp*, *Ubx*, *abd-A*, and *Abd-B*. (B) Segmental expression pattern of the *Hox* genes in the fruit fly embryo. Anterior is the left. The expression patterns for each gene are illustrated by labels that correspond to (A). Arced bars shown below indicate the range of the expression of each gene. (C) Schematic drawings for UBX protein expression in three species of embryos. Anterior is up. Red arrows indicate the boundary between T2 and T3. Area with gene expression is painted dark.

Indicate whether each of the following statements is true or false.

A. Proteins encoded by *Hox* genes act as transcription factors that regulate gene expression. 171

B. The segmental expression pattern of the *Hox* genes determines the identities of each segment in fruit fly embryos. 172

C. In the *Ubx* gene mutants, the extension of the *abd-A* expression to the anterior region leads to the transformation of thoracic segment. 173

D. Beetles and grasshoppers have two pairs of wings because their *Ubx* genes control a different set of genes

in T3 from that of fruit flies. 174

Evolution

Q44

Three-spined stickleback *Gasterosteus aculeatus* are widely distributed in both marine and freshwater areas across the world. Adaptive radiation has led to morphological differences between marine and freshwater populations. Of such differences, all of the marine population have a pair of pelvic spines that evolved from the pelvic skeleton, while some freshwater populations of various localities have lost their spines (Figure 1). Genetic analyses revealed that the causative genomic region for this pelvic difference is located around the *Pitx1* gene. This *Pitx1* plays an important role in the development of the ventral spine, thymus, and neuromast. Although the amino acid sequences of *Pitx1* transcripts are identical in both populations, the expression patterns of *Pitx1* in the pelvic fin buds of embryos are different: *Pitx1* is expressed in the marine population (purple), while it is not in the freshwater population (Figure 1 insets).

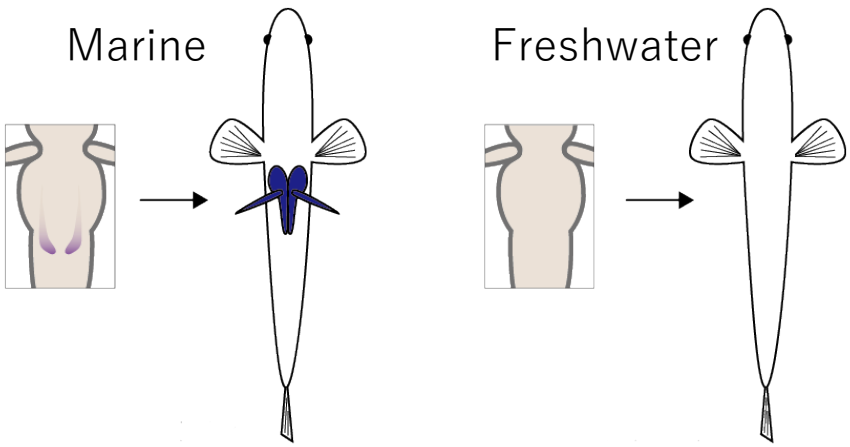


Figure 1. Ventral view of marine (left) and freshwater (right) sticklebacks, showing the presence/absence of pelvic spines (shown by dark blue). Anterior is to up. (Boxes) Magnified ventral view of stickleback embryos showing *Pitx1* expressions in the pelvic fin buds.

Indicate whether each of the following statements is true or false.

A. The freshwater population without pelvic spines independently have likely evolved from the marine population with pelvic spines. 175

B. Pelvic spines can function to protect the marine population against predators. 176

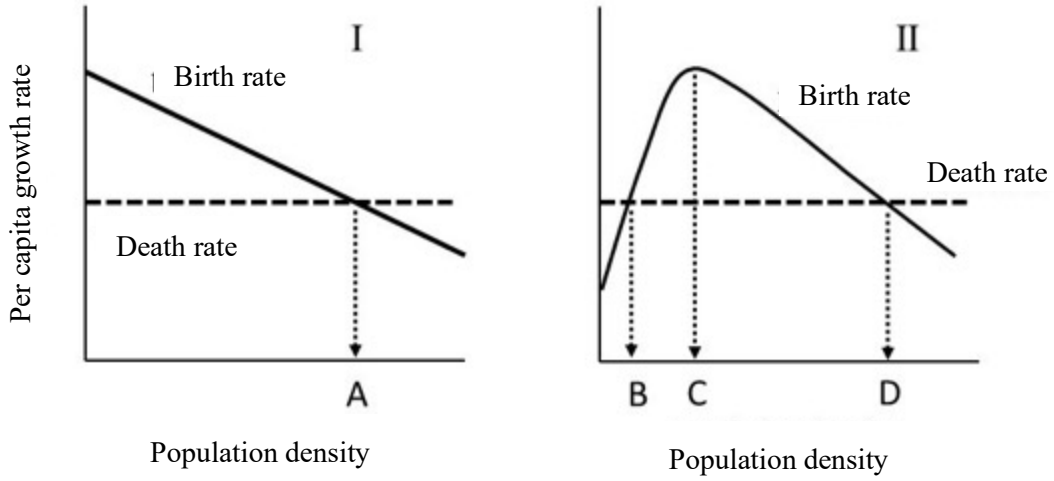
C. The *Pitx1*-knockout individual of the marine population are likely to show similar phenotypes to those of the freshwater population. 177

D. The presence/absence of *Pitx1* expression in the pelvic fin buds of embryos may result from the difference of enhancer sequences that control the gene expression. 178

Ecology

Q45

Density dependence is the fundamental process governing the population dynamics of organisms. The graph below describes per capita (per-individual) birth rate and death rate as a function of population density in two types of species (I and II).



Indicate whether each of the following statements is true or false.

1. Asexually reproducing species are more likely to be type I than sexually reproducing ones. 179

2. Population density is kept constant around all points of A, B, and D with a density-dependent manner. 180

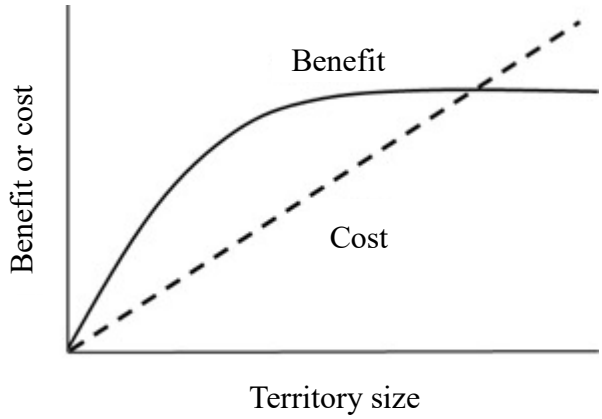
3. The aggregation of individuals is advantageous, rather than detrimental, below the density threshold of C. 181

4. Type I species are more likely to go extinct when the population is severely decreased, than type II species. 182

Ecology

Q46

An animal's territory is an exclusive area defended by an individual to keep resources, such as food, and mates. Territory is different from home range, because home range simply represents an area over which an animal regularly moves and may overlap with those of neighboring animals of the same species. The size of the territory is determined by the cost and benefit obtained from the area, in a way that maximizes the net benefit gain of individual animals. The graph below shows how cost and benefit change with the size of the territory.



Indicate whether each of the following statements is true or false.

A. The benefit curve shows saturation at a large territory size due to the depletion of resources. 183

B. The optimal territory size is the intersection point between the cost line and the benefit curve. 184

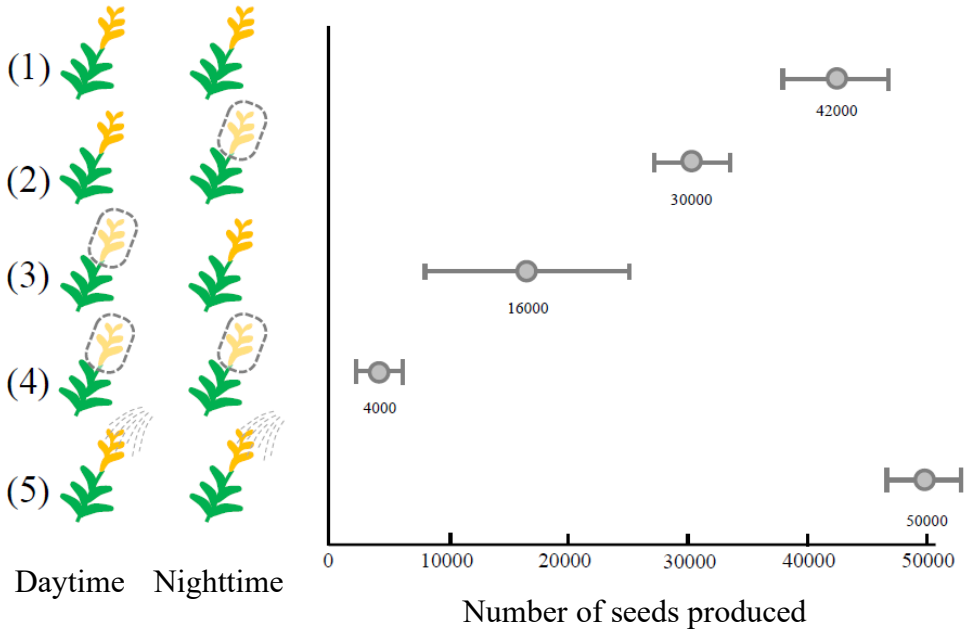
C. When resources become scarce while the cost line is unchanged, the optimal territory size becomes larger. 185

D. When the population density increases and intraspecific competition becomes intense, territorial behavior could disappear. 186

Ecology

Q47

An experiment was conducted to examine the relative effect of pollinators during the night and in the daytime on the reproductive success of golden rod flowers. Pollinators cannot visit the bagged flowers. The figure shows the number of viable seeds produced (mean ± standard deviation) by flowers that were not bagged (1), those bagged during the night (2), those bagged in the daytime (3), those bagged during both day and night (4), and those that underwent enforced pollination by an experimenter (5).



Indicate whether each of the following statements is true or false.

A. Nighttime pollinators contribute to about 60% of the total seed production. 187

B. The flowers may be capable of self-pollination. 188

C. The contribution of daytime pollinators has a greater variability than nighttime pollinators. 189

D. There are no limitations to pollination under natural conditions. 190

Ecology

Q48

Mosquitos are vectors for transmitting human diseases, and the application of insecticides to water bodies is occasionally conducted to control mosquito populations. In a mosquito population, there are two alleles of a locus that affect susceptibility to pesticides, (s): susceptible, and (r): resistant. The resistance is completely recessive. The table below shows the change in the number of individuals with different genotypes before (Pre-1990), during (1990-2000; shown by an arrow), and after (2005-2015) pesticide application.

	s/s	s/r	r/r
Pre-1990	222	3	0
1990	31	12	4
1995	26	35	41
2000	2	12	126
2005	74	64	44
2010	165	45	20
2015	210	12	1

Indicate whether each of the following statements is true or false.

A. No resistance allele was present before insecticide application. 191

B. During pesticide application, natural selection favored the resistance allele. 192

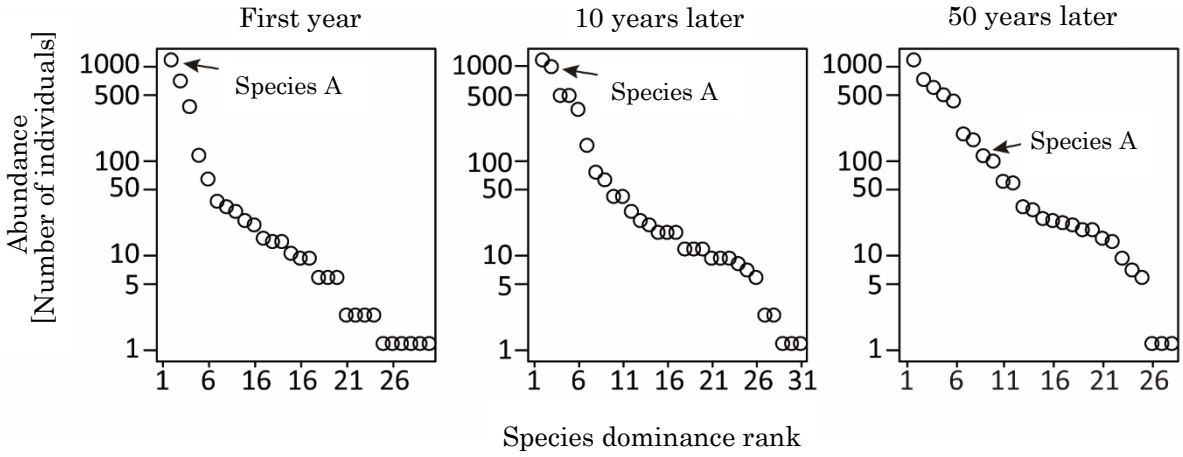
C. Resistant individuals (r/r) are likely to have lower fitness than others (s/r, s/s) in the absence of pesticide application. 193

D. From 1990 to 1995, the frequency of the resistant allele increased more than 10 times. 194

Ecology

Q49

Scientists monitored the number of individuals for ant species in a 5 hectare plot of land for 50 years. The below figures represent the dominance rank of observed species in terms of their abundance, that is, the number of individuals for each species. Each open circle represents the value for each species. Note that the most abundant species is given rank 1.



Indicate whether each of the following statements is true or false.

A. The total number of species does not change in the three periods observed. 195

B. Species A has gradually outcompeted other species over time. 196

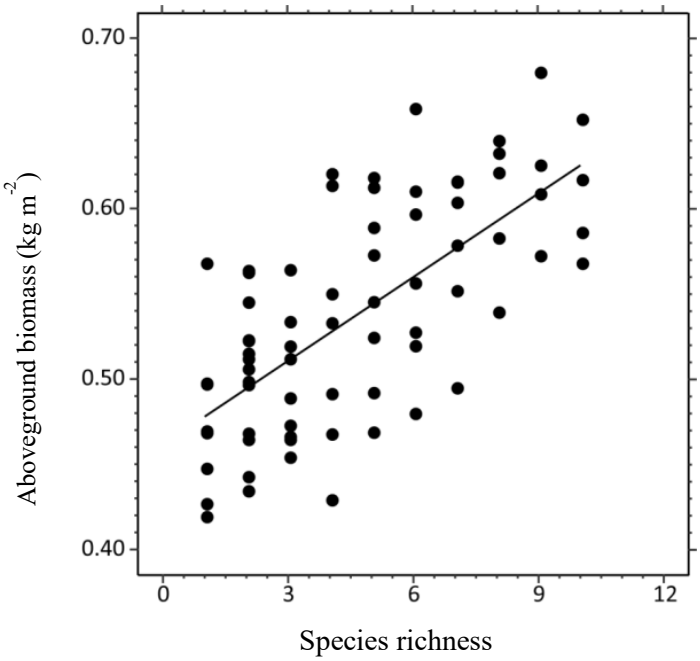
C. The top three species account for more than 75% of the total number of individuals in the first year. 197

D. During the 50 years, evenness among species has decreased. 198

Ecology

Q50

Understanding how plant species richness affects community biomass production is important for biodiversity and ecosystem conservation. In a grassland, scientists created 72 experimental plots (1 m^2 each) with different numbers of vascular plant species (from 1 to 10 species), with species combinations assembled randomly. Both local light and soil conditions were similar among the plots before establishing vegetation. After three years of this experiment, they harvested aboveground vegetation to measure aboveground biomass in each plot. The figure shows the relationship between species richness (number of species) and the dry weight of aboveground biomass (kg m^{-2}) of plant communities in each plot. The line indicates the linear relationship obtained from the least square regression.



Indicate whether each of the following statements is true or false.

A. Niche difference among species is one reason for producing a positive association between species richness and aboveground biomass. 199

B. The plot showing the largest aboveground biomass also has the highest species richness. 200

C. On average, increasing aboveground biomass of 0.1 kg m^{-2} in a plot requires an additional eight species. 201

D. The greater chance of including more productive species in species-rich plots is one reason for producing the positive association between species richness and aboveground biomass. 202

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IBO Challenge 2020

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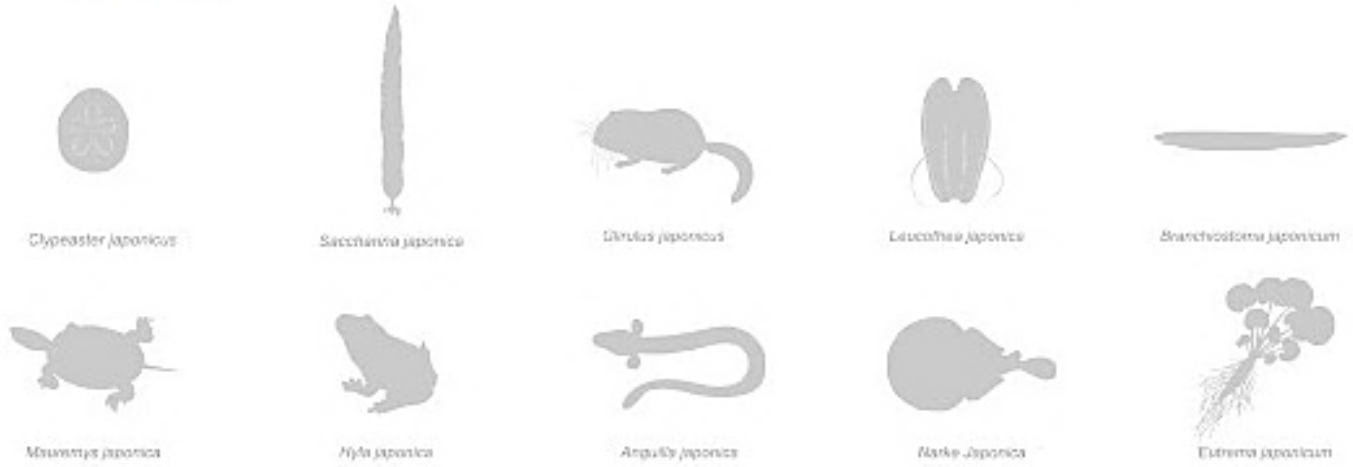
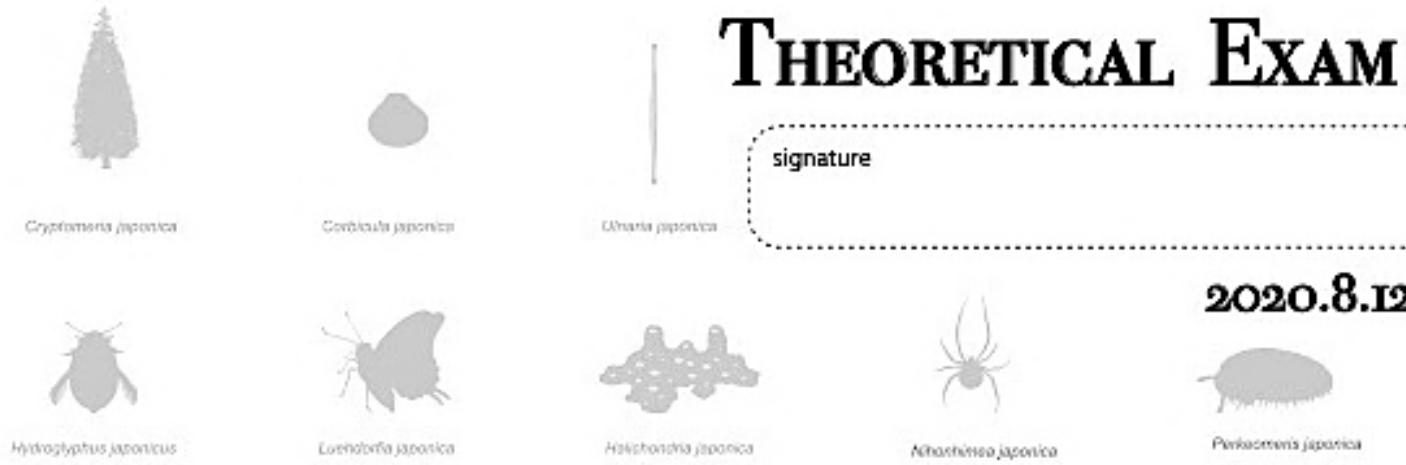


Theoretical Exam 2

signature

2020.8.12.



General instructions for theoretical examinations

Exam 2

- Date: August 12th 2020
- Total time of Exam 2 is 3 hours. Follow the instruction by Jury members of your country.
- Exam 2 consists of 45 questions.
- The score for correct answer is indicated in each question.

Instruction and regulations

- Make sure that you are using the correct answer sheets (Theoretical exam 2-1 and 2-2).
- Write your **Country code** and **student ID number** (provided by a jury member or supervisor) in the given box of the answer sheets provided, and write down **your name**.
- Make sure to **sign all the answer sheets and the cover page of question sheets**.
- You must mark your answer to the answer sheets properly, using a pen or a pencil.
- You must have the following equipment for this exam.
  ① Pen or pencil to mark answer sheets.
  ② Scratch paper sheets provided by Jury member. (You must not bring any paper into the examination room by yourself.)
  ③ Ruler and eraser.
- The use of a calculator is prohibited, including a calculator application on your PC or a web browser.
- You must not communicate with any other people in the room during the examination.
- You must not access any information that could unfairly help you answer the questions during the examination.
- Stop answering immediately at the end of examination time.
- After the examination:
  ① If you are under **on-site supervision, a jury member / supervisor** will collect your question and answer sheets immediately after each exam. Your country coordinator will later scan and submit the sheets to the IBO2020 Organizing Committee.
  ② If you are under **online supervision**, **you (competitor)** must scan (or take photos of) the answer sheets. Then, digitally send the scanned files/photos and the PDF question sheets (with your signature on the cover page) to your country coordinator as soon as possible. Your country coordinator will submit the file to the IBO2020 Organizing Committee. Make

sure the answer sheets are scanned correctly. The IBO2020 office may ask you to resubmit the sheet, so don’t discard them.

Biochemistry

Q1

Glycogen (and amylopectin) is a glucose polymer with some branching. Linear chains of these polymers consist of α(1→4) linkages and occasional branching is formed by α(1→6) linkage (Figure 1). For degradation in cells, glucose residues are released one-by-one from the end of the chains by phosphorylase up to the residue at the branching site. Then, the α(1→6) branching site is removed by a debranching enzyme.

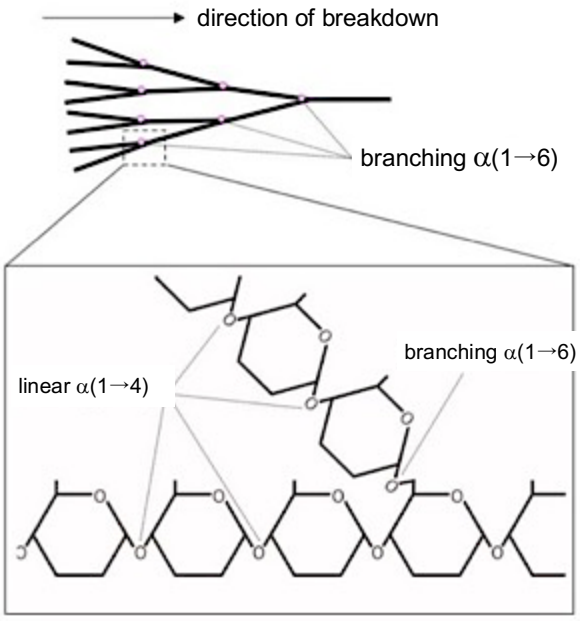


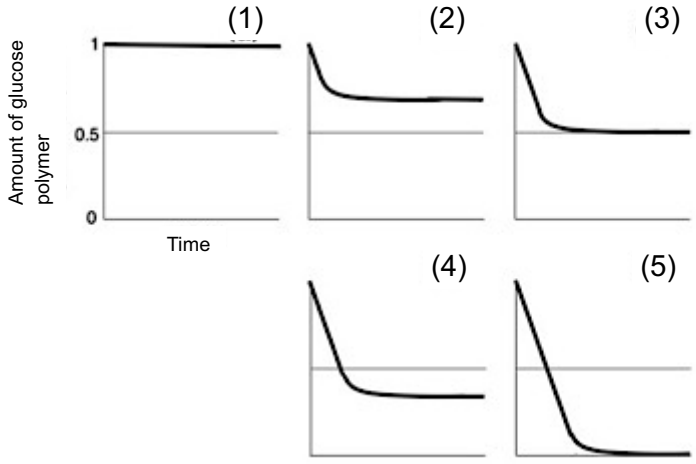
Figure 1 Breakdown of glycogen in cells.

Q1-1 Given that a certain glycogen consisting of 10000 glucose residues is branched at every 10 residues, **how many terminal chains are available for phosphorylase?** [1] (1 point)

(1) about 10 (2) about 50 (3) about 100 (4) about 500 (5) about 1000 (6) about 5000

Q1-2 For degradation of this glycogen by excess phosphorylase or by excess debranching enzyme, **choose an appropriate graph for its breakdown from below.** Assume that the phosphorylase releases all glucose residues from a linear chain without branching. (1 point each)

phosphorylase: [2] debranching enzyme: [3]



Q1-3 Plant amylopectin is similar to glycogen but branching occurs much less frequently. Given that the branching of an amylopectin of similar size of glycogen is formed at every 25 residues, **indicate the combination of correct descriptions about degradation of amylopectin by phosphorylase.** 4

(1 point)

(a) Breakdown speed is slower than that of glycogen.

(b) Breakdown speed is similar to that of glycogen.

(c) Breakdown speed is faster than that of glycogen.

(d) Final breakdown extent is smaller than that of glycogen.

(e) Final breakdown extent is similar to that of glycogen.

(f) Final breakdown extent is larger than that of glycogen.

(1) (a), (d)	(2) (a), (e)	(3) (a), (f)
(4) (b), (d)	(5) (b), (e)	(6) (b), (f)
(7) (c), (d)	(8) (c), (e)	(9) (c), (f)

Biochemistry

Q2

Hydrolases that degrade biopolymers can be categorized into two types: (1) endo-type that hydrolyzes the interior bonds of the polymer, and (2) exo-type that releases the end unit from the polymer. These exo-type and endo-type hydrolases are often linked to their biological roles.

Choose (1), if the enzyme mentioned below (A-D) is endo-type, and choose (2) if it is an exo-type.
(1 point each)

A. Digestive proteases in stomach such as pepsin [5]

B. Proteases that cleave off the translocation signal peptide [6]

C. Proofreading nuclease in the DNA polymerase that removes misincorporated nucleotides during DNA replication. [7]

D. Cas9 nuclease of the CRISPR-Cas9 system for genome editing. [8]

Biochemistry

Q3

Alcohol dehydrogenase is known to convert ethanol to acetaldehyde, which is eventually metabolized to CO_2 and H_2O in humans and many other organisms. The enzyme also catalyzes the conversion of methanol to poisonous formaldehyde, but with less efficiency. This normally means that ethanol is the physiological substrate for the enzyme. However, we may regard that ethanol is an efficient competitive inhibitor for the enzyme against the reaction with methanol under certain conditions. For example, intake of ethanol may prevent the conversion of methanol, when a small amount of methanol is taken up erroneously. Here, you can **calculate the concentration of ethanol** which suppresses 90% of the initial formaldehyde production in a test tube containing 5 mM methanol and alcohol dehydrogenase, based on the equations and assumption of kinetic constants of methanol and ethanol that are 10 mM and 1 mM, respectively.

Ethanol concentration [9] [10] . [11] mM (3 points if 3 digits are correct)

The initial velocity (v_0) of methanol conversion can be obtained using equation 1.

α is defined by equation 2.

[S]: the methanol concentration K_M: kinetic constant for methanol

[I]: the ethanol concentration K_I: the kinetic constant for ethanol

$$v_o = \frac{V_{max}[S]}{\alpha K_M + [S]} \quad \text{equation 1}$$

$$\alpha = \left(1 + \frac{[I]}{K_I}\right) \quad \text{equation 2}$$

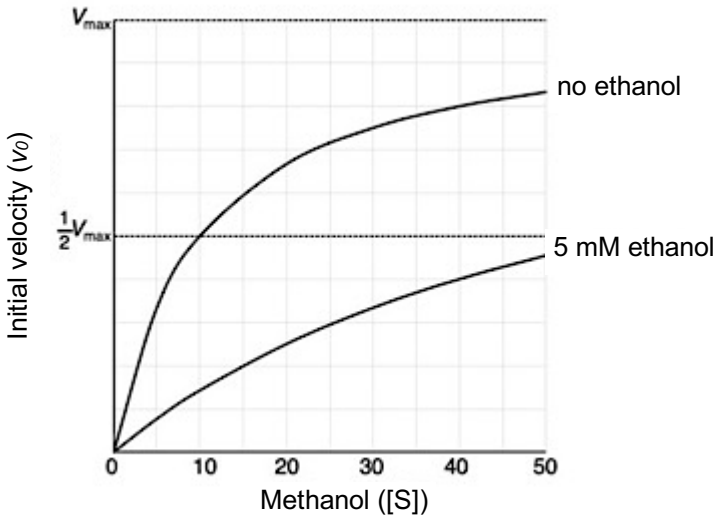


Figure 1 Methanol conversion with or without ethanol

Cell Biology

Q4

Here is a mixture containing viruses, globular proteins, and cell nuclei, which are all assumed to have similar densities of approximately 1.3 g/mL. We would like to separate them by using three different centrifugation methods as shown in Figure 1. The first method entails centrifugation of the mixture (Mix) after placing it on the top of a medium (Med) that has a uniform density (*Exp. A*). The second method (*Exp. B*) entails centrifugation of the mixture using a medium that has a density gradient ranging from 1.0 to 1.6 g/mL (from the top to the bottom). The final method entails the use of a centrifuge tube with the same density gradient as that in *Exp. B*, but the mixture is placed at the bottom of the tube (*Exp. C*). ***g*** indicates the direction of centrifugal forces given to the specimens.

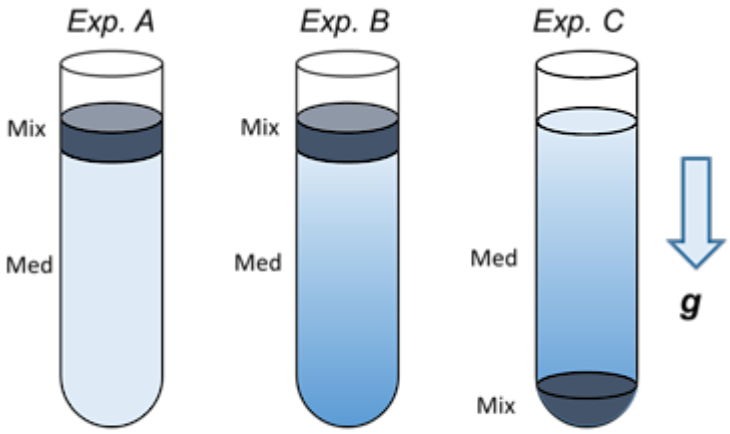


Figure 1

In *Exp. A*, how are viruses, globular proteins, and nuclei supposed to sediment? Choose the most appropriate diagram from (1) **I**, (2) **II**, (3) **III**, (4) **IV** in Figure 2 that shows the sedimentation time courses of specimens. [12] (1 point)

Additionally, choose appropriate lines from (1) **a**, (2) **b,** or (3) **c** in the selected diagram that indicate the time courses of sedimentation of viruses [13], globular proteins [14] and nuclei [15], respectively. (1 point if 3 correct answers)

In *Exp. B,* how are viruses, globular proteins, and nuclei supposed to sediment? Choose the most appropriate diagram from (1) **I**, (2) **II**, (3) **III**, (4) **IV** in Figure 2 that shows the sedimentation time courses of the specimens. [16] (1 point)

Additionally, choose appropriate lines from (1) **a**, (2) **b,** or (3) **c** in the selected diagram that show the time courses of sedimentation of viruses [17], globular proteins [18] and nuclei [19], respectively. (1 point if 3 correct answers)

In *Exp. C*, how are viruses, globular proteins, and nuclei supposed to float? Choose the most appropriate diagram from (1) **V**, (2) **VI**, (3) **VII**, (4) **VIII** in Figure 3 that shows the floating time courses of specimens.

20 (1 point)

Additionally, choose appropriate lines from (1) **a**, (2) **b,** or (3) **c** in the selected diagram that show the time courses of floating of viruses 21, globular proteins 22 and nuclei 23, respectively. (1 point if 3 correct answers)

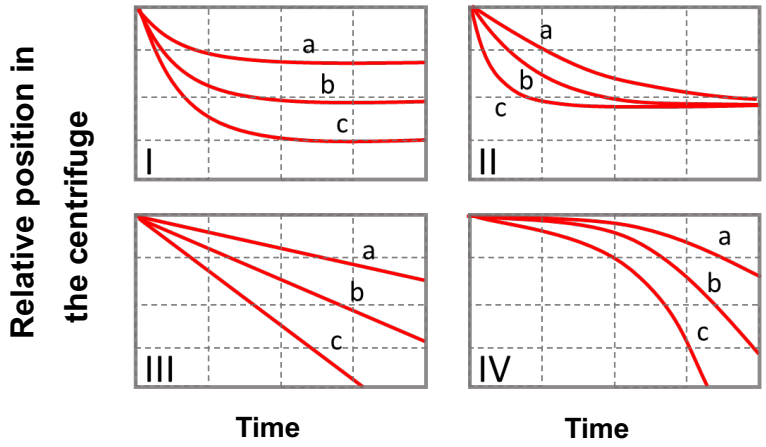


Figure 2

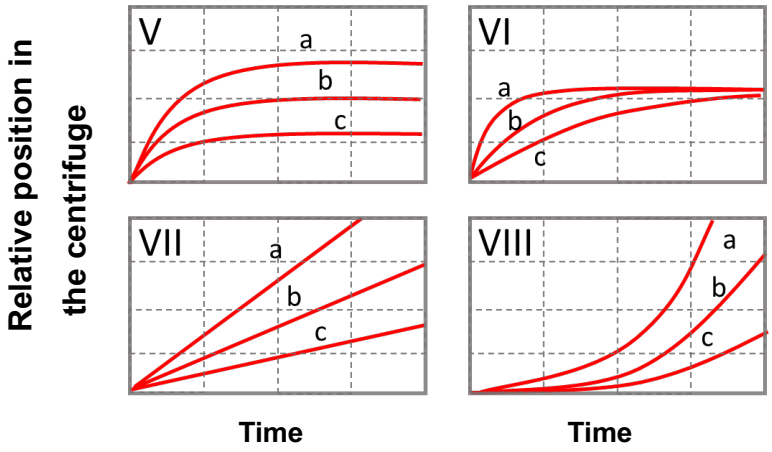


Figure 3

Cell Biology

Q5

Cytoplasm is generally occupied by very high concentrations of biomolecules and condensed organelles. This property is called "molecular crowding", which affects the rate of intra-cytoplasmic diffusion and enzymatic reactions. Mammalian red blood cells (RBCs, Figure 1) are a typical case that demonstrate molecular crowding.

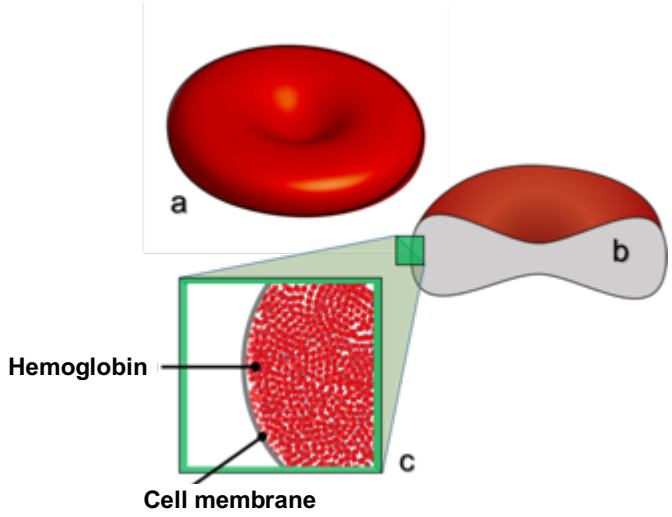


Figure 1 Schematic drawing of red blood cells (RBCs). c, Hemoglobin molecules assumed in a RBC cross section (b).

The concentration of hemoglobin molecules (molecular mass, 64,000 g/mol) inside RBCs is called "mean corpuscular hemoglobin concentration (MCHC)". It is as high as about 320 mg/mL in humans. From this concentration, we can estimate the mean cytoplasmic volume in a RBC occupied by a single molecule of hemoglobin. If hemoglobin molecule has a density as usual protein molecules of about 1.35 g/mL, we can also estimate how large is the molecular volume of hemoglobin. Using these values, the hemoglobin molecules are estimated to occupy about [24] % of the total cytoplasmic volume in RBCs.

Q5-1 Choose the closest number to enter in [24]. Use the Avogadro constant, 6.02×10^{23} for the calculation if needed. (3 point)

(1) 3 (2) 6 (3) 12 (4) 24 (5) 48

Q5-2 How does this hemoglobin concentration affect the rate of diffusion in the actual RBCs? Scientists succeeded in measuring the diffusion rate of hydrogen ions. They first put RBCs in saline with different osmolarity and examined how the cell volume changed (Figure. 2a), and then measured the diffusion rate of hydrogen ions (Figure. 2b). Diffusion rates were also examined for red blood cells from different species (human,

chicken, alpaca; 320, 305, and 450 mg/mL of MCHC, respectively) as shown in Figure. 3.

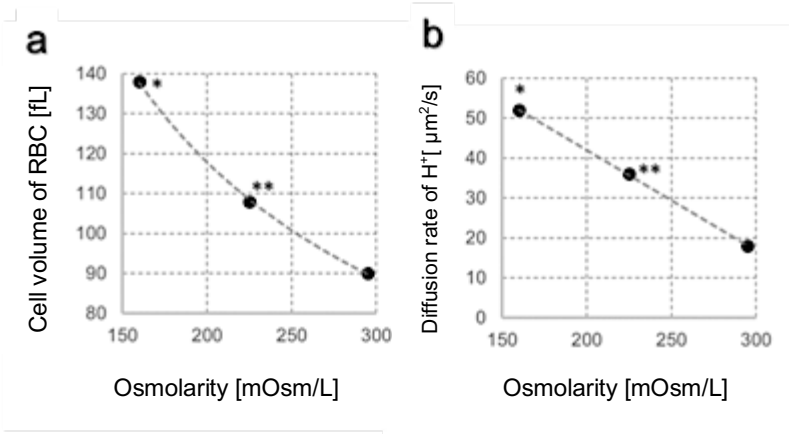


Figure 2 Examination of the relationship between (**a**) the cell volume of RBC (fL = 1×10^{-15} L) and (**b**) the measured diffusion rate of hydrogen ions [$\mu m^2/s$] versus osmolarity [mOsm/L] of saline solutions. 300 mOsm/L corresponds to the osmosis of the body fluid in a healthy human.

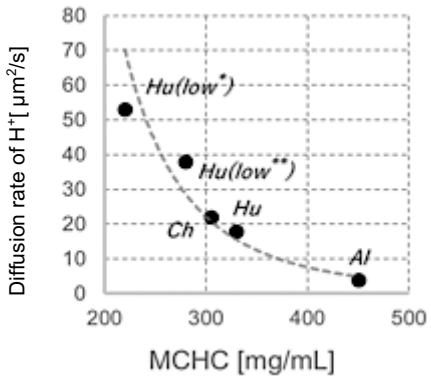


Figure 3 Diffusion rate of intracellular hydrogen ions measured using RBCs from different animal species. *Hu*, *Ch*, and *Al* represent humans, chickens, and alpacas from which RBCs were derived. *Hu (low*)* and *Hu (low**)* indicate the results obtained at 155 and 225 mOsm/L (* and ** in Figure. 2), respectively.

Indicate whether each of the following statements is true (1) or false (2). (1 point each)

A. Alpaca RBCs, which have a hemoglobin concentration about 1.5 times higher than that of humans, have an internal ion diffusion rate of less than 50% of that of human RBCs. 25

B. Ion diffusion rate is low inside human RBCs at low osmolarity, due to the reduced volume of RBCs. 26

C. There is a proportional relationship between the concentration of hemoglobin and the rate of ion diffusion in RBCs. 27

D. Alpaca RBCs have been evolutionally optimized to increase hemoglobin concentration and transport large amounts of oxygen, while promoting O_2 and CO_2 diffusion. 28

Cell Biology

Q.6

Animal cells generally have three types of cytoskeletons: (1) microtubules, (2) actin filaments, and (3) intermediate filaments. Figure 1 shows the morphology of a cytoskeleton during the mitotic metaphase or in interphase. For each statement below A-E, **indicate the corresponding type of cytoskeleton from (1) to (3) in the first box (e.g. 29), and the schematic diagram from ① to ⑥ in Figure 1 in the second box (e.g. 30)** (1 point if 2 correct answers)

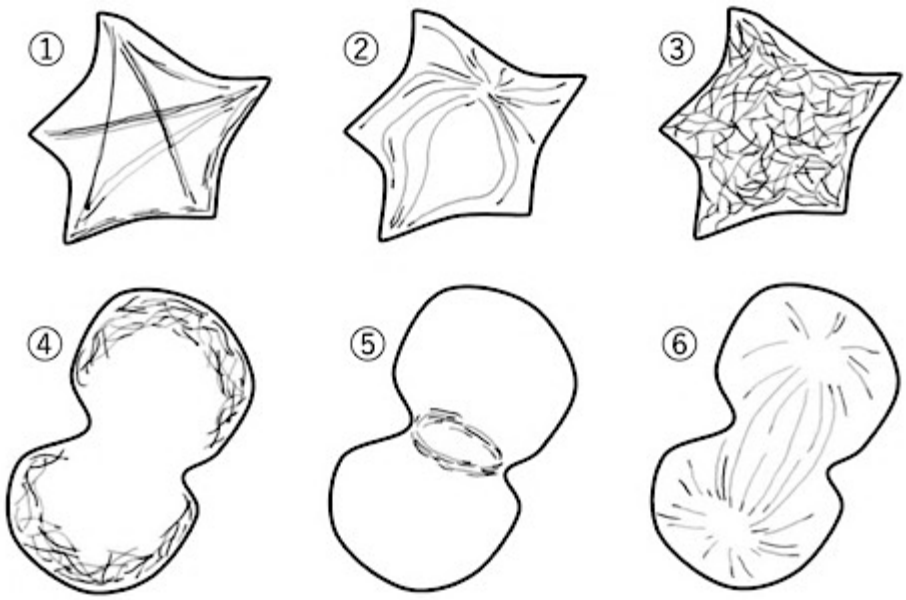


Figure 1

Statements	Type of cytoskeleton	Schematic diagram
A	29	30
B	31	32
C	33	34
D	35	36
E	37	38

A. They are entangled inside interphase cells and exist in a meshwork. They enhance the elasticity of cells and provide a mechanically supportive structure. 29 30

B. It is called a stress fiber. It builds a support beam inside cells and works to maintain the shape of the cell in interphase. 31 32

C. This spindle-shaped structure is formed during cell division. It plays a role in separating replicated chromosomes accurately into daughter cells. 33 34

D. After chromosomal segregation, it forms a ring structure and mechanically separates two daughter cells. 35 36

E. Having radial distributions starting near the nucleus, the fibrous structure is assumed to have structural polarity or directionality. 37 38

Cell Biology

Q7

GLUT1, a protein present in the membrane of red blood cells, is a transporter that transports glucose into cells. The relationship between the extracellular glucose concentration (S) and the rate of glucose uptake (V) into red blood cells is shown in Figure 1. This relationship between V and S can be described by the following equation (1)

$$V = \frac{V_{max}}{1+\frac{K_M}{S}} \quad \ldots. (1)$$

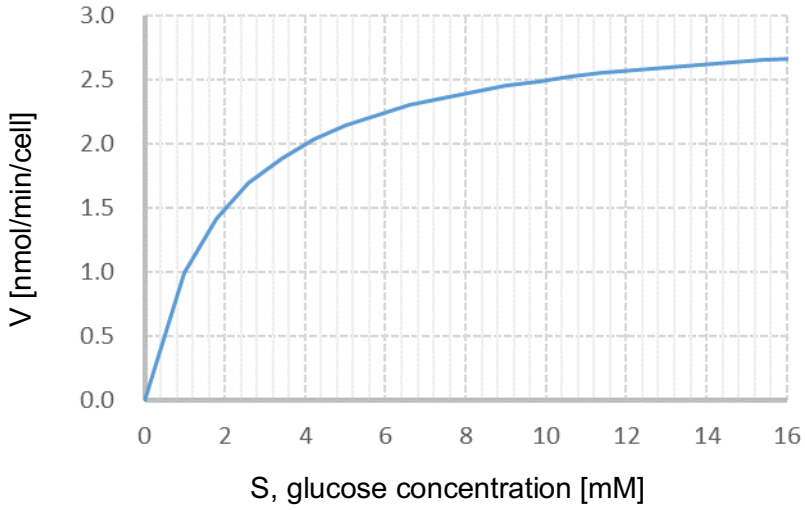


Figure 1 Relationship between S (extracellular glucose concentration, mM) and V (the rate of glucose uptake into red blood cells).

Q7-1 Estimate the approximate integer values for V_{max} and K_M in this equation from the curve shown in Figure 1. (1 point each)

V_{max}: [39] (1) 1 (2) 2 (3) 3 (4) 4 (5) 5 (6) 6 (7) 7 (8) 8 (9) 9

K_M : [40] (1) 1 (2) 2 (3) 3 (4) 4 (5) 5 (6) 6 (7) 7 (8) 8 (9) 9

GLUT2 is a glucose transport protein expressed in hepatocytes in an insulin-independent manner, and V_{max} and K_M are 2 nmol/min/cell and 9 mM, respectively. GLUT4 is another transporter expressed in muscles or hepatocytes functioning in an insulin-dependent manner, and V_{max} and K_M are 0.85 nmol/min/cell and 0.8 mM, respectively.

Q7-2 Indicate whether each of the following statements is true (1) or false (2). (1 point each)

A. Healthy humans that typically has 4 to 6 mM of blood glucose. The rate of glucose transport per molecule by GLUT2 is considered to be approximately equal to that by GLUT4. 41

B. Although the transport rate of glucose by GLUT1 and GLUT4 is almost saturated in healthy humans, GLUT2 has an additional capacity to increase the transportation rate. 42

Cell Biology

Q8

Carbon assimilation in photosynthesis begins when Ribulose-bisphosphate carboxylase/oxygenase (Rubisco) binds one molecule of CO_2 to Ribulose 1,5-bisphosphate (RuBP) to form two molecules of 3-phosphoglycerate. Rubisco is considered to be one of the most important enzymes on the planet due to its ability to produce organic carbon compounds that support almost all organisms.

O_2 can bind to the active site of Rubisco instead of CO_2, in which case one molecule of 3-phosphoglycerate and one molecule of 3-phosphoglycorate are formed. Thus, CO_2 and O_2 function as antagonists. The following values show the enzymatic properties of Rubisco of a seed plant and the environmental condition in vivo.

(a) Kinetic characteristics of Rubisco (substrate concentration at 50% of saturation at 25 ° C)

K_M [X]: the affinity of the enzyme for substrate X.

K_M [CO_2] = 9 μM, K_M [O_2] = 535 μM, K_M [RuBP] = 28 μM

(b) Maximum activity (number of repetitions of enzyme reaction per second)

kcat [X]： the maximum reaction rate when the enzyme catalyzes the reaction of substrate X.

kcat [CO_2] = 3.3 / s, kcat [O_2] = 2.4 / s

(c) Concentration in water in equilibrium with air (assuming 0.035% CO_2 and 21% O_2) at 25 ° C

CO_2 = 11 μM, O_2 = 253 μM

RuBP concentration in chloroplast stroma is 4 to 10 mM.

Which properties from (a) to (c) above are necessary to explain the following facts from A to D? Choose the most suitable set from the following ones. (1 point each)

(1) (a) (b) (c), (2) (a) (b), (3) (a) (c), (4) (b) (c), (5) (a), (6) (b), (7) (c)

A. The carboxylase activity of Rubisco increases as the oxygen concentration in the air decreases. 43

B. In the current global environment, the carboxylase activity of Rubisco is higher than the oxygenase activity. 44

C. Plants must have large amounts of Rubisco to maintain the full capacity of photosynthesis. 45

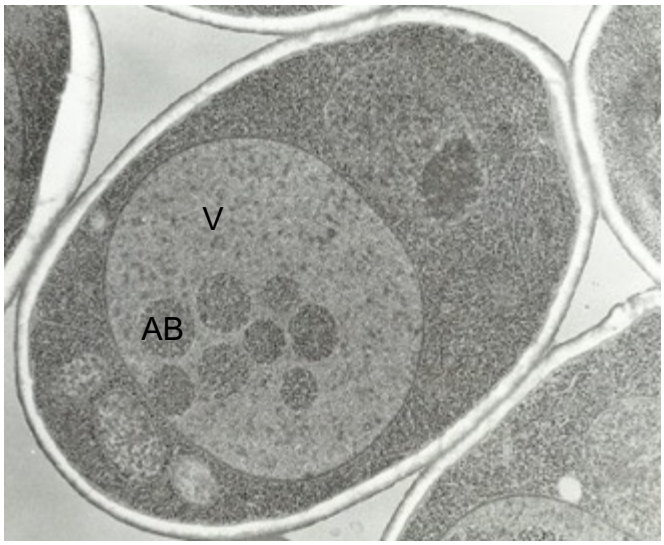
D. Increasing the concentration of CO_2 in the air increases the carboxylase activity of Rubisco. 46

Cell Biology

Q9

All cells must constantly synthesize and degrade intracellular substances and structures, one of processes is autophagy. In autophagy, the intracellular structure is non-specifically or somehow specifically decomposed by lysosomes and vacuoles. The first molecular analysis of autophagy was carried out by Nobel Prize winner, Dr. Ohsumi, by using yeast mutant, as follows.

1. His group cultured a yeast mutant under nitrogen starvation.
2. After a certain period, many round structures (autophagic bodies) (right figure AB) appeared in the vacuoles (right figure V).
3. When observed with an electron microscope, ribosomes were found in the autophagic body.
4. Mutants of this process were isolated and many genes that work in the autophagy system were found out.



Q9-1 What kind of gene had a mutation in this experiment? 47 (1 point)

(1) Phosphatase
(2) Protease
(3) Cellulase
(4) DNA polymerase

Q9-2 What was the organelle found in the autophagic body? 48 (1 point)

(1) Chloroplast
(2) Mitochondrion
(3) Melanosome
(4) Cell wall

Cell Biology

Q10

The growth patterns of plant cells assume the following types.

A. Diffuse growth: the whole cell more or less grows on entire facets of the cell.

B. Tip growth: only the tip of the cell grows.

C. Inclusive growth: combination of A and B.

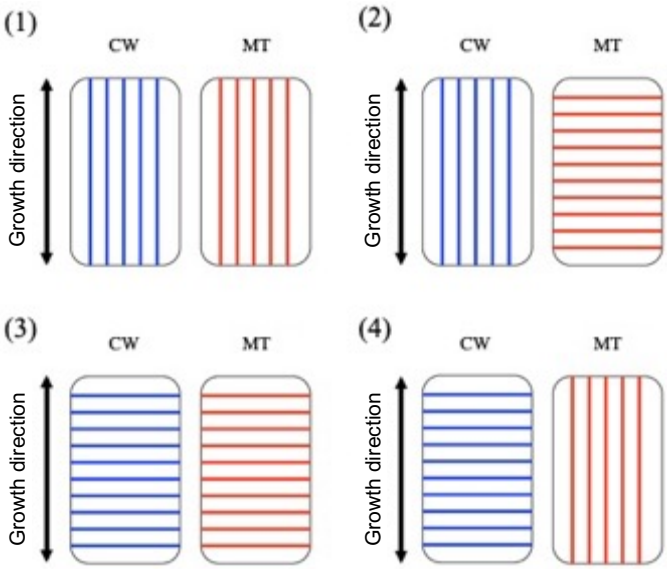
When diffuse growth occurs in plant cells, the cell wall must be loosened, and the growth direction is affected by the orientation of the cellulose microfibrils constituting the cell wall. In cells undergoing diffuse growth, a cellulose synthase complex synthesizes cellulose microfibrils while moving on the cell membrane along the orientation of cortical microtubules inside the cell membrane.

Q10-1 Select a combination of the following (1) to (6) that correctly matches the types of growth (A to C) and the types of plant cells. 49 (1 point)

(1) A—Pollen tube, Root hair,	B—Leaf epidermal cells,	C—Root cortical cells
(2) A—Pollen tube, Root hair,	B—Root cortical cell,	C—Leaf epidermal cell
(3) A—Root cortical cells,	B—Leaf epidermal cells,	C—Pollen tube, Root hair
(4) A—Root cortical cells,	B—Pollen tube, root hair,	C—Leaf epidermal cells
(5) A—Leaf epidermal cells,	B—Pollen tube, root hair,	C—Root cortical cells
(6) A—Leaf epidermal cells,	B—Root cortical cells,	C—Pollen tube, Root hair

Q10-2 The following schematic diagrams (1) to (4) show the orientation of cellulose microfibrils of the cell wall (CW) and the orientation of cortical microtubules (MT) in plant cells extending in the longitudinal direction.

Choose the most appropriate combination. 50 (1 point)



Cell Biology

Q11

A cultured cell of somatic cell A and a cultured cell of somatic cell B of an animal were prepared. A culture dish containing an appropriate amount of cells was prepared, and the number of cells after a certain period of time (at the start of the experiment) and the number of cells after 48 hours were counted. The results are shown in Table 1.

Table 1: Cell numbers of somatic cell A and somatic cell B.

	Cell number（x10^5）	
Time from start of experiment（hours）	0	48
somatic cell A	7.2	115.2
somatic cell B	9.7	77.6

Q11-1 How long are the cell cycles of somatic cell A and somatic cell B, respectively? **Write the letter of your answer in the space provided.** (1 point each)

(1) 3, (2) 4, (3) 6, (4) 8, (5) 10, (6) 12, (7) 16, (8) 24, (9) 32

somatic cell A: [51] hours

somatic cell B: [52] hours

Q11-2 When somatic cells A and B were mixed at a certain ratio and a culture was started in a culture dish, the ratio of the cell numbers of A and B after 4 days was 2 : 1. What was the ratio of somatic cell A and B cell numbers when the culture was started? **Write the letter of your answer in the space provided.** (It is assumed that the cell cycle of the somatic cells A and B progresses independently. The nutrients required by the cells during the cultivation are well-supplied.) [53] (1 point)

(1) A : B = 1 : 1 (2) A : B = 2 : 3 (3) A : B = 1 : 2

Cell Biology

Q12

Yeast can metabolize glucose using aerobic respiration and alcohol fermentation depending on environmental conditions. Each reaction formula is as follows.

Aerobic respiration

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ (32 ATP production)

Alcohol fermentation

$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$ (2 ATP production)

Yeast was cultured in a glucose solution under conditions A and B, and gas inflow and outflow from the incubator were measured to obtain the results shown in the Table 1. Answer the following questions (it is assumed that the same amount of glucose was completely metabolized under conditions A and B).

Table1

Conditions	O_2 absorption (mL)	CO_2 emissions (mL)
A	0	20
B	30	40

Q12-1 How was glucose metabolized under condition A and condition B, respectively? (1 point each)

(1) aerobic respiration only
(2) alcohol fermentation only
(3) aerobic respiration and alcohol fermentation

Condition A: 54
Condition B: 55

Q12-2 Assuming that 100 equivalents of ATP were generated under condition A, **how many equivalents of ATP were generated under condition B?** 56 (1 point)

(1) 50 (2) 100 (3) 300 (4) 500 (5) 750 (6) 850 (7) 1000 (8) 1200 (9) 1400

Genetics

Q13

Lisa is the daughter of Carl with ABO blood-type B and Jane with AB type. Lisa's blood type is O. Normally, there is no parent-child relationship between AB type and O type. Detailed examinations revealed that Lisa is a rare Bombay O type (Figure 1).

The ABO blood-type is determined by the outermost antigen of sugar chains on the cell membrane of red blood cells. The gene for this antigen is located on chromosome 9. Type A has A antigen, type B has B antigen, type AB has both, and type O has neither. Since the A and B antigens bind to the sugar of H antigen, the phenotype will be O regardless of the genotype in the absence of the H antigen. A person who has a homozygous *h* allele with a defect in the H antigen gene (H) on chromosome 19 cannot synthesize H antigen and expresses Bombay O-type (Figure 2).

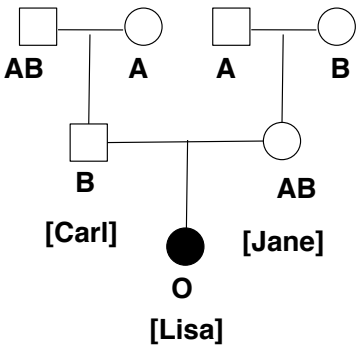


Figure 1

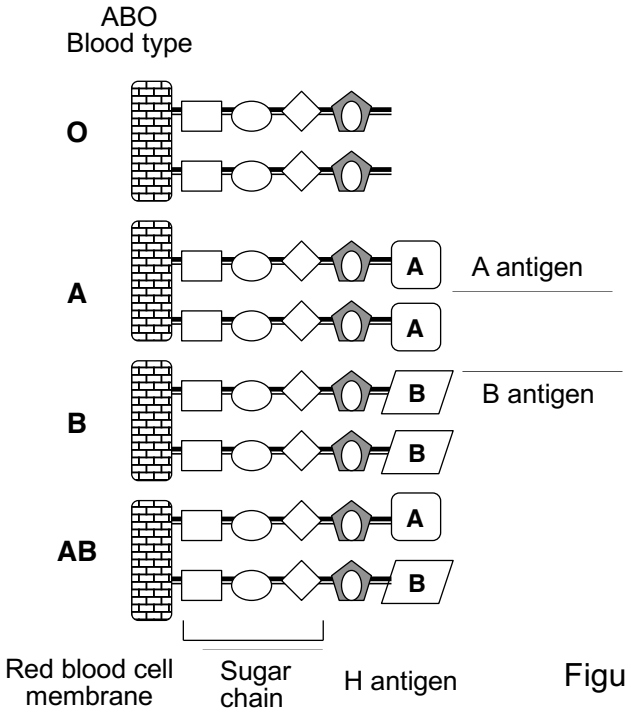


Figure 2

When Carl and Jane have another child, the chance of their child's blood type being B

is [57][58] . [59] %. (3 points)

Genetics

Q14

One cycle of the PCR reaction doubles the number of DNA fragments. Further, each time one cycle of the PCR reaction progresses, the primer pair, the substrate dNTP, and the DNA polymerase molecule are required double amount, so the amount of these components limits the overall amount of DNA that can be synthesized in PCR.

The length of the DNA fragment to be amplified was 100 base pairs including the primers, and the PCR reaction was started with the primer length of 20 bases. The four types of bases A, C, G, and T are evenly distributed in the sequence to be amplified, and the amplification efficiency of PCR is 100%. As the PCR reaction progresses, the reaction will not be completed due to running out of one of the components in a certain cycle.

Choose the correct No. of the reaction stop cycle and the limiting component. [60] (3 points)

Template DNA fragment : 4 copies

Primer : 1,000 sets

dNTPs (dATP, dTTP, dGTP, dCTP) : 48,000 molecules (12,000 molecules each)

DNA polymerase : 1,200 molecules

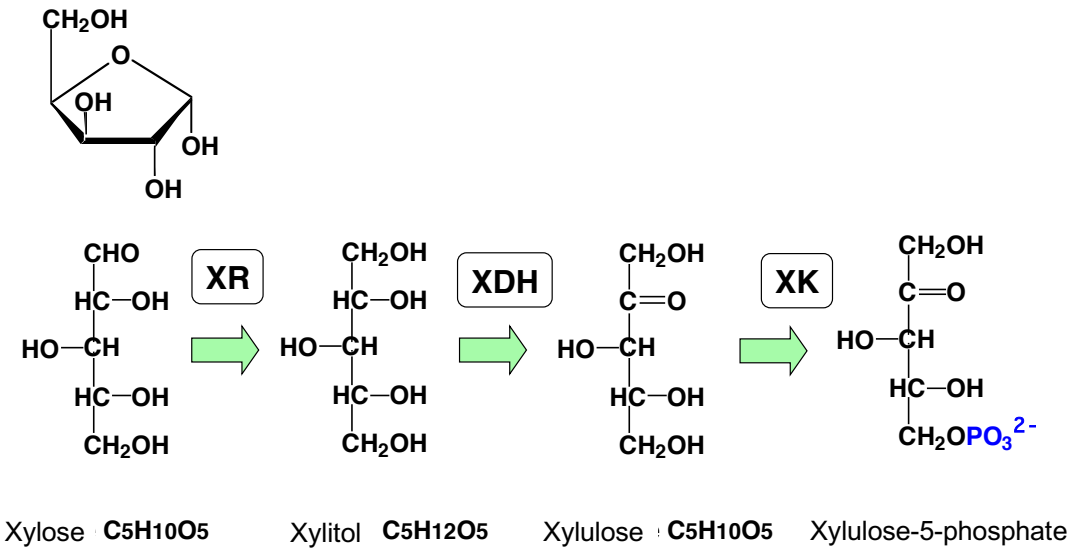
No.	Cycles	Limiting component
(1)	7	Primer pairs
(2)	7	dNTPs
(3)	7	DNA polymerase
(4)	8	Primer pairs
(5)	8	dNTPs
(6)	8	DNA polymerase
(7)	9	Primer pairs
(8)	9	dNTPs
(9)	9	DNA polymerase
(0)	Others	

Genetics

Q15

Streptococcus mutans, which causes tooth decay, cannot utilize xylitol ($C_5H_{12}O_5$). Therefore, xylitol is used as a sweetener to prevent tooth decay. Xylitol is produced by microbial conversion from xylose contained in hemicellulose.

The diploid yeast strain *Candida tropicalis* AT36 can grow with xylose as the sole carbon source 1(Figure 1). In this strain, the enzyme activities of XR, XDH, and XK are almost proportional to the copy number of each gene.



XR: Xylose reductase(XYL1 gene)
XDH: Xylitol dehydrogenase(XYL2 gene)
XK: Xylulokinase

Figure 1

The AT36 strain was cultured by adding 40 g of xylose and 5 g of glucose as a carbon source in the culture solution (1 L). As the result, about 25 g of xylitol was produced, as shown in the graph (A) in Figure 2. Therefore, the following gene-disrupted strains were constructed and cultured in the same manner in order to increase xylitol production.

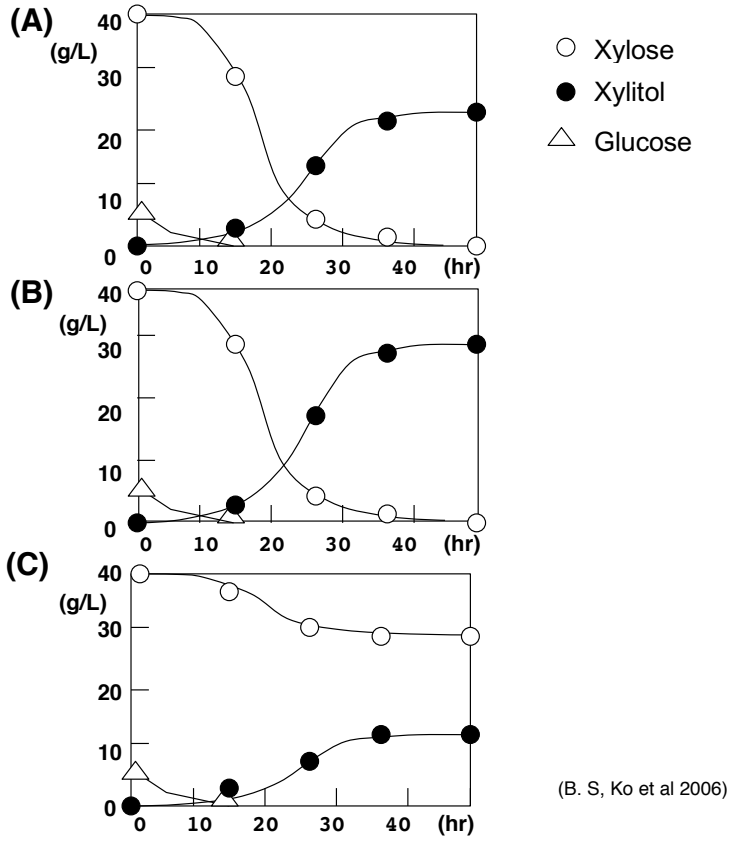


Figure 2

(Disruptant A) One of the *XYL1* genes of the AT36 strain was disrupted.

(Disruptant B) Both of the *XYL1* genes of the AT36 strain were disrupted.

(Disruptant C) One of the *XYL2* genes of the AT36 strain was disrupted.

(Disruptant D) Both of the *XYL2* genes of the AT36 strain were disrupted.

Based on the above information, select the number of the most appropriate combination of the culture progress graph (Figure 2) and the disrupted strain. 61 (2 points)

	Graph A	Graph B	Graph C
(1)	AT36	Disruptant A	Disruptant D
(2)	AT36	Disruptant A	Disruptant C
(3)	AT36	Disruptant B	Disruptant D
(4)	AT36	Disruptant C	Disruptant D
(5)	AT36	Disruptant D	Disruptant C
(6)	AT36	Disruptant C	Disruptant B

Genetics

Q16

In order to teach the principles and techniques of DNA replication, professor A instructed graduate students B and C to reproduce the classic experiment of replicating DNA *in vitro* by properly mixing nucleic acids and proteins individually purified from *E. coli* cells.

Professor A was disappointed with the following results of the experiments of student B and C.

Result of student B: Long single-stranded DNA fragments and short single-stranded DNA fragments with attached RNA fragments were replicated, but complete double-stranded DNA was not replicated.

R1: Student B failed to add polymerase I.

R2: Student B failed to add polymerase III.

R3: Student B failed to add DNA ligase.

Result of student C: Long single-stranded DNA fragments and many short single-stranded fragments were replicated, but complete double-stranded DNA was not.

R4: Student C failed to add polymerase I.

R5: Student C failed to add polymerase III.

R6: Student C failed to add DNA ligase.

Choose the combination of the number that most likely caused the failures of students B and C.

62 (2 points)

No.	Student B	Student C
(1)	R1	R5
(2)	R1	R6
(3)	R2	R4
(4)	R2	R6
(5)	R3	R4
(6)	R3	R5

Genetics

Q17

With the progress of genome research, the genomes of many organisms have been analyzed, and it has been revealed that the genomes of organisms vary widely in size.

Faster-growing organisms with a simpler structure tend to have smaller genomes. Most mammalian genomes range from 2.5 to 3.3 billion bases, and human genomes are about 3 billion bases.

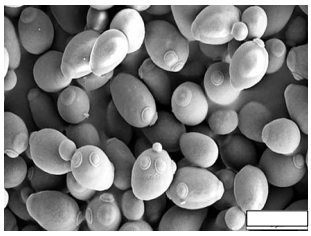
Arrange the genomes of the organisms in the photo in descending order.

Choose the correct one by number. 63 (2 points)

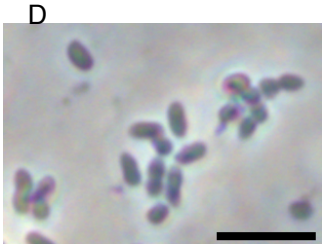
A

B

C



D



E

	Genome size (large - small)
(1)	A – B – C – D – E
(2)	A – B – C – E – D
(3)	A – C – B – D – E
(4)	A – C – B – E – D
(5)	A – B – E – D – C
(6)	A – B – D – E – C
(7)	A – B – E – C – D
(8)	A – C – B – E – D

Genetics

Q18

In its life cycle, baker's yeast *Saccharomyces cerevisiae* has haploid and diploid generations. The haploid has α-type and a-type mating types and grows independently. When α-type cells and a-type cells meet, they undergo sexual conjugation and become diploid (a/α-type) cells. When the nitrogen source is depleted, the diploid cells undergo meiosis and form four spores (two a-type cells and two α-type cells) inside the cell.

Wild type genes of yeast are written in capital letters and mutant genes are written in lower case. For example, the genes encoding leucine biosynthetic enzymes are written as *LEU1, LEU2*..., and the corresponding mutant genes are written as *leu1, leu2*.... Strains that do not have the *LEU2* gene cannot grow in a medium without leucine.

The haploid XY-1A strain (genotype: *a, ura3, leu2*) requires uracil and leucine for growth, and the XY-2B strain (genotype: *α, his3, leu1*) requires histidine and leucine for growth.

A diploid XY-3C strain (*a/α, ura3/URA3, leu2/LEU2, LEU1/leu1, HIS3/his3*) was obtained by sexual mating of the XY-1A strain and the XY-2B strain. Out of 160 spores obtained from the XY-3C strain, approximately [64] spores can grow on a medium containing uracil but not leucine/histidine.

The genes of the mating type, the *URA3*, the *LEU2*, the *LEU1*, and the *HIS3* are all present on different chromosomes.

Choose the appropriate number that is most likely. [64] (2 points)

No.	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
spores	10	20	25	40	50	80	120	150

Genetics

Q19

As part of the functional analysis of the april gene found in a diploid plant, a mutant strain was discovered in which a DNA fragment (T-DNA) of 3 kb or more was inserted into one april gene. Since this strain is considered heterozygous for the april gene, seeds were obtained by self-pollination.

Figure 1 shows the gene map of the april gene and the T-DNA insertion site. The arrow in the figure indicates the region in which the primers used for genotyping PCR were designed.

The obtained seeds were grown, genomic DNA was extracted from three different plants (A, B, C), and PCR was performed using the designed primers.

The results of agarose gel electrophoresis shown in Figure 2 indicate that the genotype of the april gene of each of A, B, and C was determined.

Choose the correct homozygous, heterozygous, and wild-type combination for T-DNA insertions in Strain A, B, and C. 65 (2 points)

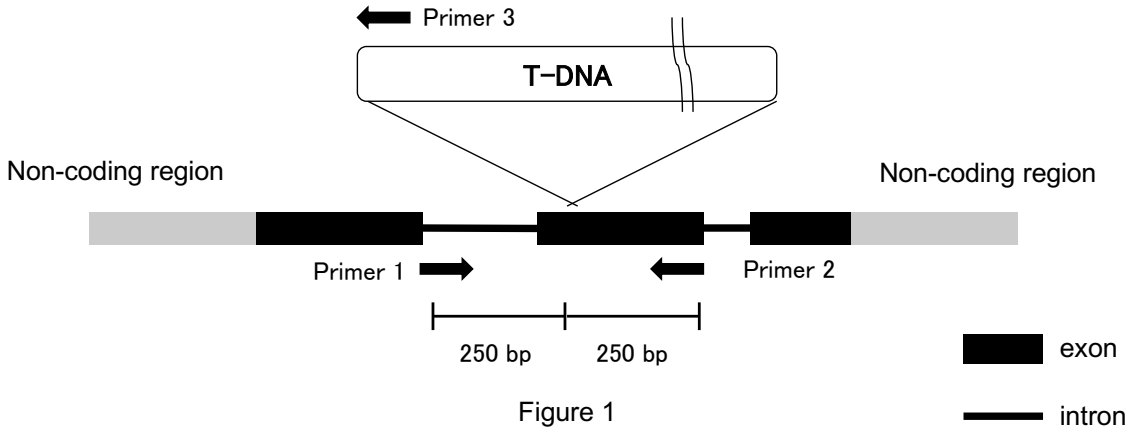
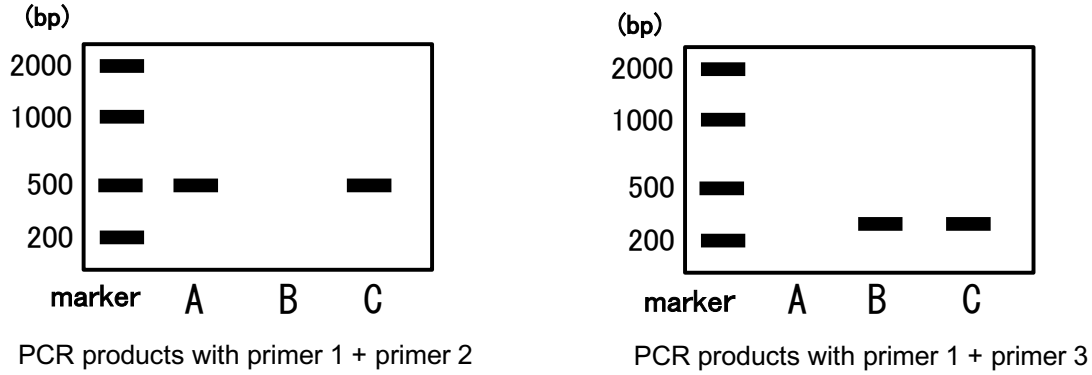


Figure 1



PCR products with primer 1 + primer 2

PCR products with primer 1 + primer 3

Figure 2

	Strain A	Strain B	Strain C
(1)	homo	hetero	wild type
(2)	homo	wild type	hetero
(3)	hetero	homo	wild type
(4)	hetero	wild type	homo
(5)	wild type	homo	hetero
(6)	wild type	hetero	homo

Genetics

Q20

The plants discovered on a remote island have purple, reddish purple, red, blue, light blue, and white flowers. Observations of this plant for several years revealed the following results.

a. This plant is capable of self-pollination and cross-pollination.

b. There was no relationship between the flower color and the seed formation efficiency of this plant.

c. Self-pollination of white-flower individuals revealed that all F1 generation individuals had white flowers. This strain was regarded as a white flower pure strain and was designated as a WW strain.

d. Self-pollination of blue-flower individuals revealed that all F1 generation individuals had blue flowers. This strain was regarded as a blue flower pure strain and was designated as a BB strain.

e. After the self-pollination of light blue flowers, blue, light blue, and white flowers appeared in the F1 generation.

f. After the self-pollination of red-flower individuals, red flower and white-flower individuals appeared in the F1 generation.

g. After the self-pollination of purple flowers, purple and blue flowers appeared in the F1 generation.

h. After the self-pollination of reddish purple flower individuals, flower individuals of all colors appeared in the F1 generation.

i. When blue flowers and white flowers were crossed, light blue flowers appeared in the F1 generation.

j. When a red-flower individual and a white-flower individual were crossed, red and white flower individuals appeared in the F1 generation. Therefore, by repeating the self-pollination of red-flower individuals, a red flower pure strain in which all red-flower individuals appeared was obtained. It was named the RR strain.

k. When a BB strain and an RR strain were crossed, reddish purple-flower individuals all appeared in the F1 generation. This strain was named the BR strain.

The probability that reddish-purple individuals appear in the F2 generation obtained by self-pollination of the BRWW strain is [66] [67] . [68] %. **Mark the appropriate numbers in the Answer boxes.** (3 points)

Note: In this question, descendants resulting from self-pollination are also indicated as “F1”.
The genes related to flower color are not linked in this plant.

Genetics

Q21

Animal viruses are classified by the nucleic acid contained in the capsid. In addition to nucleic acid, some viruses contain enzyme proteins, such as RNA polymerases, inside the virus particles.

From the following animal viruses, select the most appropriate combination of those that must contain an enzyme in the capsid for replication from the answer group, from (1) to (8). [69] (2 points)

Type	Virus	Nucleic acids
A	Smallpox virus	Double-stranded DNA
B	B19 parvovirus	Single-stranded DNA
C	Rotavirus	Double-stranded RNA
D	Rhinovirus	Single-stranded RNA (mRNA)
E	Influenza virus	Single-stranded RNA (template of mRNA)
F	HIV (retrovirus)	Single-stranded RNA

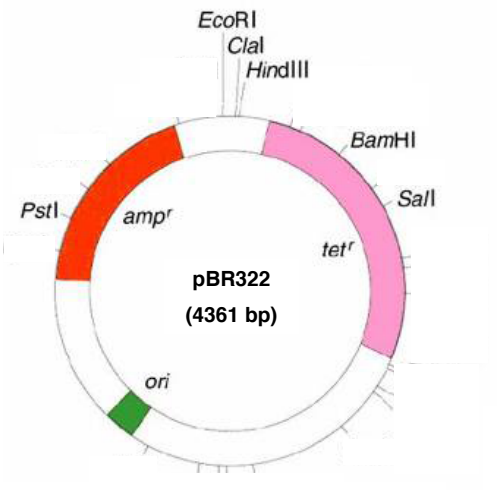
Answer group

(1)	A, C	(5)	B, F
(2)	B, C	(6)	C, E
(3)	B, D	(7)	D, E
(4)	B, E	(8)	E, F

Genetics

Q22

In the 1980s, a plasmid vector called pBR322 was frequently used for DNA recombination experiments. pBR322 is a 4361-base pair plasmid containing ampicillin resistance and tetracycline resistant genes, and has the restriction enzyme sites shown in Figure 1.



EcoRI	5′-G↓AATTC-3′
ClaI	5′-AT↓CGAT-3′
HindIII	5′-A↓AGCTT-3′
BamHI	5′-G↓GATCC-3′
BglII	5′-A↓GATCT-3′
PstI	5′-CTGC↓AG-3′
SalI	5′-G↓TCGAC-3′
XhoI	5′-C↓TCGAG-3′

Restriction enzyme cleavage sites are indicated by arrows

***tet*^{r} : tetracycline resistant gene**
***amp*^{r} : ampicillin resistant gene**
***ori* : replication origin**

Figure 1

In order to learn the technique for the gene recombination experiment, we planned the experiment such that both the gene P (plasmid 1) and the gene Q (plasmid 2) are ligated with pBR322 using only the restriction enzyme and the DNA ligase (Figure 2).

The experimental procedure is as follows.

Step 1: Cleavage of plasmid 1 or plasmid 2 with appropriate restriction enzymes and electrophoresis to obtain DNA fragments containing gene P or Q.

Step 2: Cleavage of pBR322 vector with appropriate restriction enzymes.

Step 3: Ligation of the DNA fragment (containing gene P or Q) with the vector to obtain the first recombinant plasmid.

Step 4: Cleavage of the other plasmid with appropriate restriction enzymes to obtain a DNA fragment containing the second gene (gene Q or P).

Step 5: Cleavage of the first recombinant plasmid with appropriate restriction enzymes.

Step 6: Ligation of the DNA fragment (containing gene Q or P) with the first recombinant plasmid.

Recombinant *E. coli* cells are selected by ampicillin resistant phenotype. The presence of two replication origins in one plasmid results in very low stability and should be avoided. The restriction enzyme reaction should proceed completely.

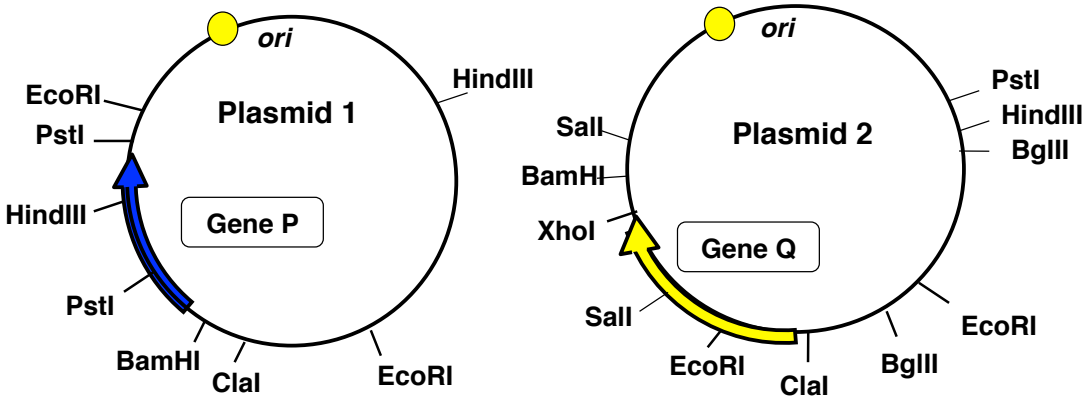


Figure 2 ***ori* : replication origin**

The operations of Step 1 to Step 6 are indicated by A - I, X, and Y. **From the procedures shown in the table, select the number that indicates the appropriate procedure for producing the desired recombinant plasmid.** 70 (3 points)

A: Cleavage of plasmid 1 with EcoRI and BamHI to obtain a DNA fragment containing gene P.

B: Cleavage of plasmid 1 with EcoRI and ClaI to obtain a DNA fragment containing gene P.

C: Cleavage of plasmid 2 with ClaI and BamHI to obtain a DNA fragment containing gene Q.

D: Cleavage of plasmid 2 with ClaI and SalI to obtain a DNA fragment containing gene Q.

E: Cleavage of plasmid 2 with ClaI and XhoI to obtain a DNA fragment containing gene Q.

F: Cleavage of pBR322 plasmid with EcoRI and ClaI

G: Cleavage of pBR322 plasmid with EcoRI and BamHI

H: Cleavage of pBR322 plasmid with ClaI and BamHI

I: Cleavage of pBR322 plasmid with ClaI and SalI

X: Ligation of the DNA fragment containing the gene P with the cleaved pBR322 plasmid.

Y: Ligation of the DNA fragment containing the gene Q with the cleaved pBR322 plasmid.

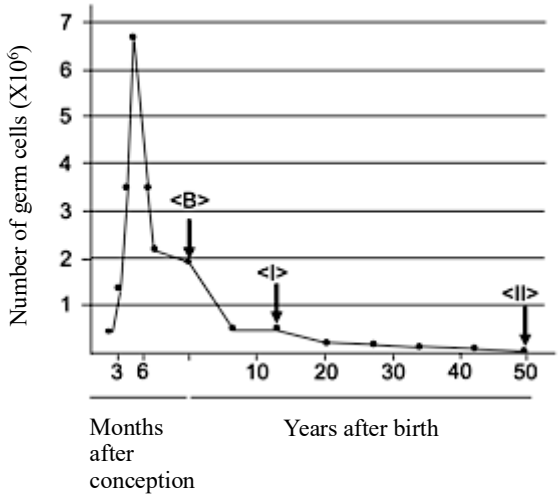
Note: Both native pBR322 and the first recombinant plasmid obtained by Step 1 – 3 are described as pBR322 plasmid.

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6
(1)	A	G	X	C	H	Y
(2)	C	H	Y	A	G	X
(3)	A	G	X	D	I	Y
(4)	D	I	Y	A	G	X
(5)	B	F	X	D	I	Y
(6)	D	I	Y	B	F	X
(7)	B	F	X	E	I	Y
(8)	E	I	Y	B	F	X

Animal biology

Q23

The oocytes in the human ovary is the most numerous in the 5-months-old fetus, in which the number of the oocytes is approximately 7 million. The number of the oocytes then decreases rapidly, reaching about 2 million at birth.



<B> : birth. <I>: menarche <II>: menopause

Q23-1 Indicate whether each of the following statements is True (1) or False (2). (1 point each)

The number of germ cells decreased by 70% at the birth because the oocytes die during the period of meiosis II. [71]

Q23-2 Indicate the most suitable number to fill in the blank from the choices below. (1 point)

The number of oocytes that are ovulated during menstrual periods is less than [72] of the germ cells surviving at menarche.

(1) 0.001% (2) 0.01% (3) 0.1% (4) 1%

Q23-3 The risk of Down syndrome increases with the age of the mother. The ratio of babies with Down syndrome born to a mother in her forties is 10 to 100 times higher than for a mother in her twenties. **Choose the most appropriate sentences below for the reason for this.** [73] (1 point)

(1) accumulation of mutation on oocyte DNA

(2) prolongation of chromosome synapsis in primary oocytes

(3) increase in improperly formed spindle in oocytes

(4) transformation of oocytes

Animal biology

Q24

Figure 1 represents the changes in aortal (Ao), left atrial (At), and left ventricular (Ve) pressures and the left ventricular volume during a human cardiac cycle. During the cycles, the atrioventricular valve opens when the atrial pressure is higher than ventricular pressure, and the aortic valve opens when the ventricular pressure is higher than aortic pressure. Figure 2 shows the changes in ventricular pressure and volume before (dotted line) and after (solid line) a ten-minute period of exercise.

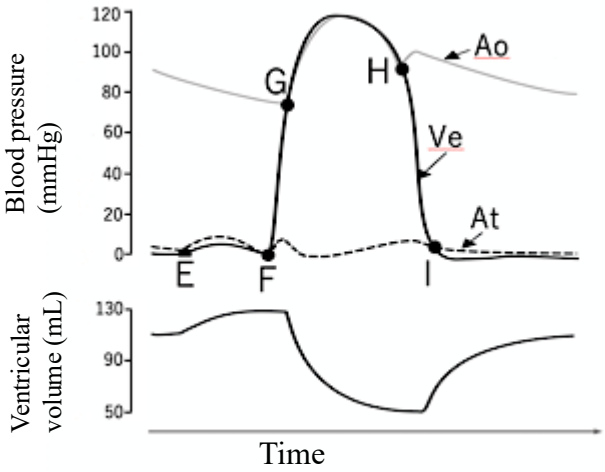


Figure 1

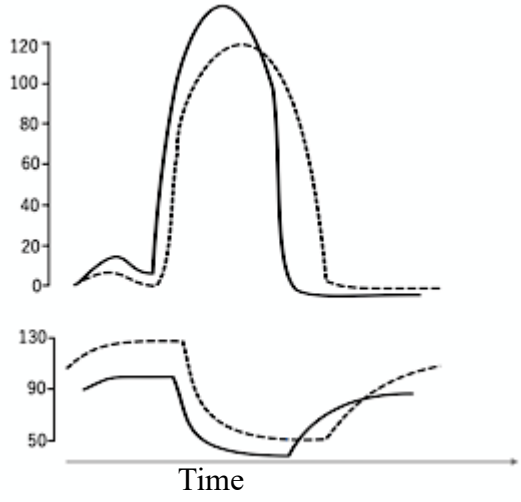


Figure 2

Q24-1 Indicate whether each of the following statement is true (1) or false (2). (1 point)

Elevation of heart rate did not change in duration between the point E and F in Figure 1. [74]

Q24-2 Choose the corresponding period between point H - I in Figure 1 from (1) – (4) of the pressure-volume relationship of the left ventricle (Figure 3). [75] (1 point)

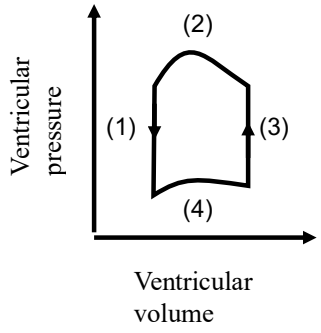
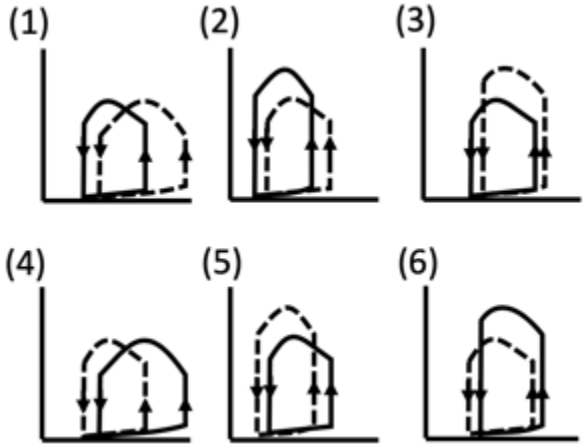


Figure 3

Q24-3 Indicate whether this statements is true (1) or false (2). (1 point)

After point I in Figure 1, blood flows into both atria and ventricles. 76

Q24-4 Choose the most appropriate pressure and volume relationship of left ventricle before (dotted line) and after (solid line) the activation of sympathetic nervous system from (1)-(6). 77 (1 point)



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Animal biology

Q25

The following figures illustrate the molecules in muscle fibers in two states: 1. Contraction, 2. Relaxation. The mutation or insufficient function of those molecules are associated with abnormal muscle functions. For example, a mutation in the Ca^{2+} channel or Acetylcholine receptor (AchR) may cause congenital myopathy.

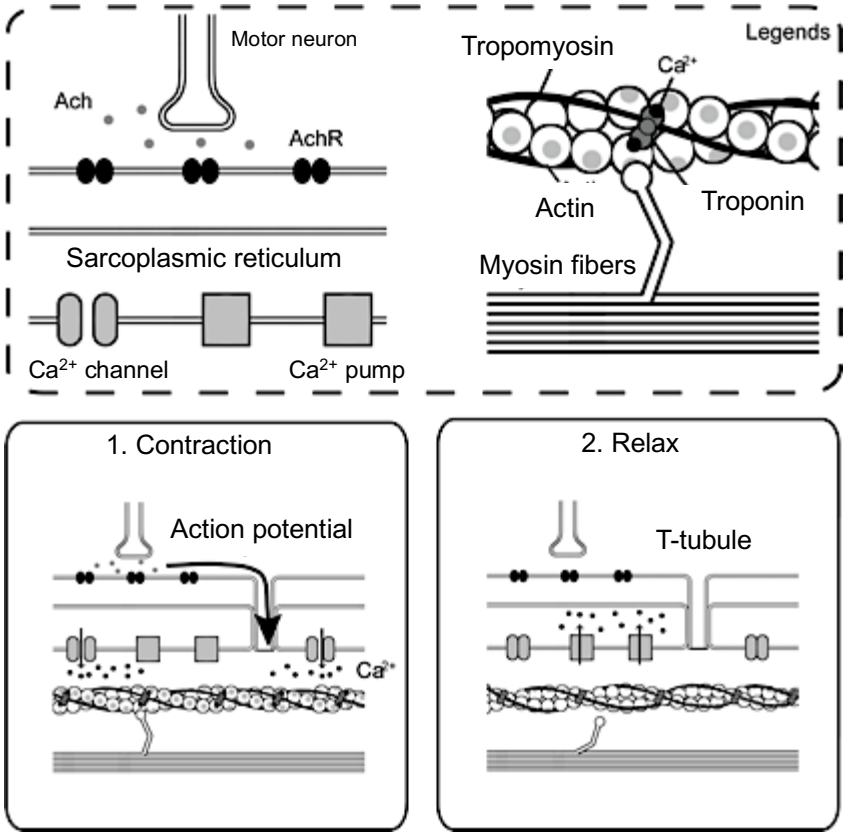
Note that the choices of muscle abnormality are:

(1) Myopathy (muscle weakness)

(2) Difficulties in arm extension

(3) Tetany (involuntary contraction of muscle)

(4) Hypercontractility (contraction occurs quickly, but relaxation occurs slowly)



Indicate the above symptoms (1)-(4) that will occur in each type of muscle abnormality (A-D).

(1 point each)

A. Missense mutation in the Tropomyosin binding site of Actin that causes the muscle to be more sensitive for intracellular Ca^{2+} concentration. [78]

B. Blocking the Ach release by Botulinum toxin treatment. [79]

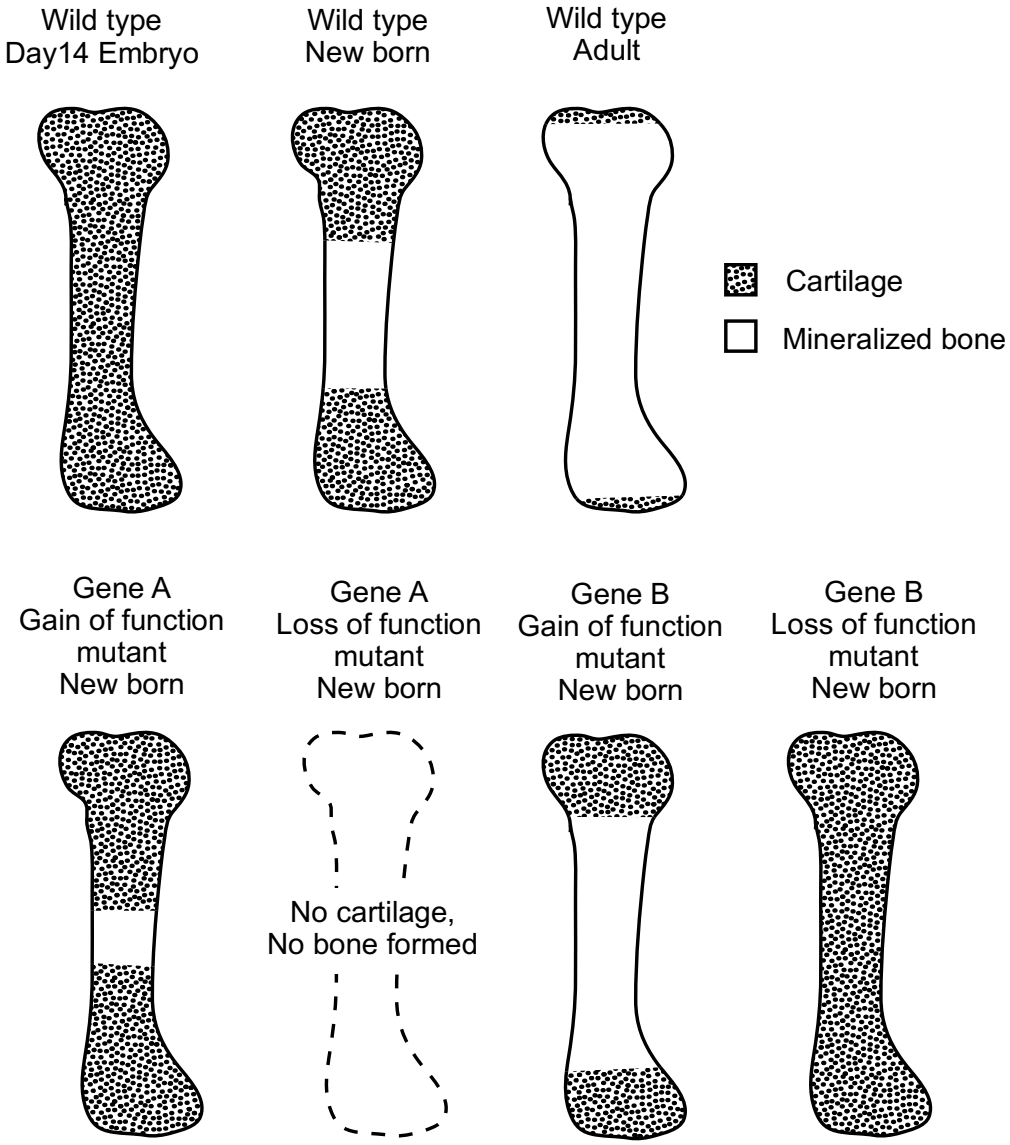
C. Nonsense mutation in Ca^{2+} pump gene, which causes a deficiency in the removal of Ca^{2+} from cytosol. [80]

D. Low blood magnesium level, which results in frequent and uncontrolled depolarization. 81

Animal biology

Q26

Most mammalian bones are formed through a process called "endochondral ossification," in which the cartilages first form the template of skeletal elements and then are mostly replaced by mineralized bones. Below is a schematic drawing of the endochondral ossification of the long bone of mice (upper), and the long bone of the mouse with gain or loss of function in gene A or B (lower). Both gene A and gene B are involved in skeletal development. Choose the correct interpretation of endochondral ossification and the function of genes A and B from (1) – (9) in the table. [82] (2 points)



Choices	Gene A		Gene B	
	Cartilage formation	Cartilage-bone replacement	Cartilage formation	Cartilage-bone replacement
(1)	Not required	Promote	Required	Promote
(2)	Required	Promote	Required	Promote
(3)	Required	Promote	Required	Repress
(4)	Required	Promote	Not required	Promote
(5)	Required	Promote	Not required	Repress
(6)	Required	Repress	Required	Promote
(7)	Required	Repress	Required	Repress
(8)	Required	Repress	Not required	Promote
(9)	Required	Repress	Not required	Repress

Animal biology

Q27

The honeybee (*Apis mellifera*) communicates the distance of a food source to others by dancing. The bee performs “round dances” when the feeder is within 50 m. If the feeder is over 50 m, they perform “waggle dances.” The mean durations (milliseconds, ms) of waggle dances are plotted in Figure 1.

To determine how bees measure this distance, two types of wooden tunnels with a food source were positioned outdoors (Figure 2; the cylinder in the tunnel shows the position of the feeder). The tunnel is 6 m long, 11 cm wide, and 20 cm high. The top of the tunnel was covered with screen cloth, which provided the direction of the sun, and the far end was closed. The walls and floor of the tunnel were randomly patterned (black-and-white pattern of pixel size 1 cm by 1 cm) in experiments 1, 2, and 4, and axially striped in experiment 3. The type of dance that the bees performed in each experiment is shown in Figure 2. In experiments 2 and 4, the mean waggle durations were 529 ms and 441 ms, respectively.

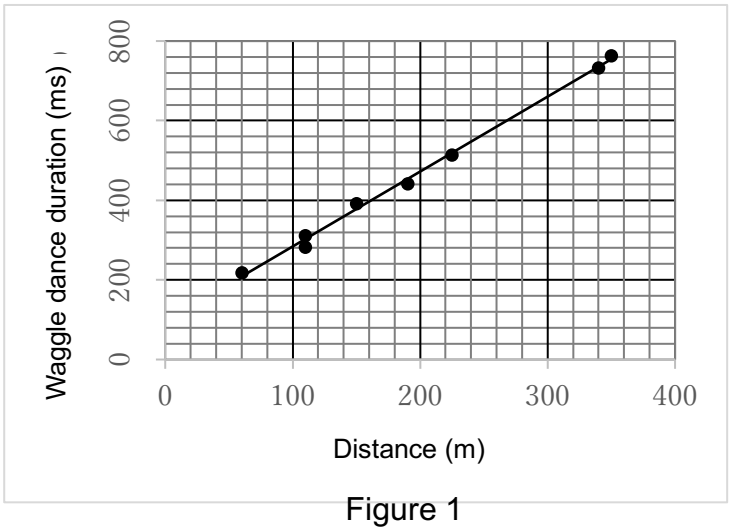


Figure 1

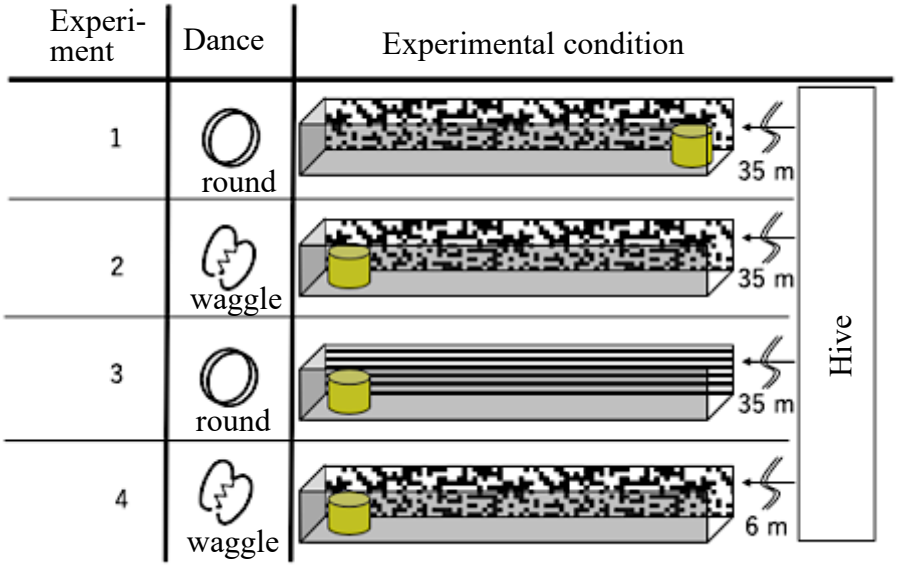


Figure 2

* black-and-white random pattern of pixel size 1 cm by 1 cm

Q27-1 How long is the duration of the waggle dance when the tunnel was extended to be two-times longer in the opposite direction of the hive in experiment 2? **Choose the most appropriate answer from the following.** (3 point)

83

(1) 634 ms (2) 740 ms (3) 846 ms (4) 952 ms

Q27-2 Choose the most appropriate answer from the following statements. 84 (1 point)

1. When the walls and floor are lined with vertically oriented strips in experiment 3, most bees will perform the waggle dance.
2. Bees perform a waggle dance with the same duration for two different feeders. If bees fly to the two feeders with a same speed, the durations from the hive to the feeders are same.
3. The duration of the waggle dance does not change if the bees fly at the different height.

Animal biology

Q28

CYP2C19 belongs to the cytochrome P450 families of enzymes expressed in the liver for detoxification. It is one of the major enzymes that metabolizes and inactivates various drugs. However, there are single nucleotide polymorphisms (SNPs) in the *CYP2C19* gene (Table 1 for combinations of SNPs denoted 1*, 2* and 3*), and the allele frequencies of these SNPs are different among Asian and European populations (Table 2).

Omeprazole is a drug used for the treatment of gastric ulcer and reflux esophagitis. It is metabolized mainly by CYP2C19 for inactivation. Conversely, Clopidogrel is a drug used for the prevention of myocardial and cerebral infarction. Clopidogrel is also metabolized by CYP2C19, and this metabolite inhibits the target molecule on the surface of the platelets, thus showing the drugs' effect.

Table 1

Genotype of *CYP2C19*	Phenotype
**1/*1*	Extensive metabolizer
**1/*2, *1/*3*	Intermediate metabolizer
**2/*2, *2/*3, *3/*3*	Poor metabolizer

Table 2

Country	Allele frequency (%)		
	**1*	**2*	**3*
Sweden	69.4	27.8	2.7
France	56.7	37.2	6.1
China	38.2	47.2	14.6
Japan	27.7	49.9	22.5

A. Orally administered drugs absorbed in the intestinal epithelium are first transported to [85].

(1) Inferior vena cava (2) liver (3) heart (right atrium)

(4) pancreas (5) Large intestine

B. What is the order of genotypes for the effect of omeprazole from the most long-lasting to the shortest lasting effect after oral administration? [86]

(1) **1/*1 > *1/*3 > *2/*2* (2) **1/*1 > *3/*3 > *1/*2*

(3) **3/*3 > *1/*2, > *1/*1* (4) **3/*3 > *1/*1, > *1/*3*

C. Which countries have the highest proportion of patients for whom omeprazole works well? [87]

(1) Sweden (2) France (3) China (4) Japan

D. If clopidogrel does not show sufficient effect due to the *CYP2C19* SNPs in the patient, what is expected by combining omeprazole on the effectiveness of the clopidogrel? [88]

(1) improves the effectiveness (2) no change in the effectiveness (3) worsens the effectiveness

A-D (1 point each)

Animal biology

Q29

Three stages of transmission are considered before a zoonotic disease leads to a pandemic in the human society (Figure 1). Coronaviruses are a group of viruses that can cause such pandemics. The natural host of coronaviruses are generally believed to be bats.

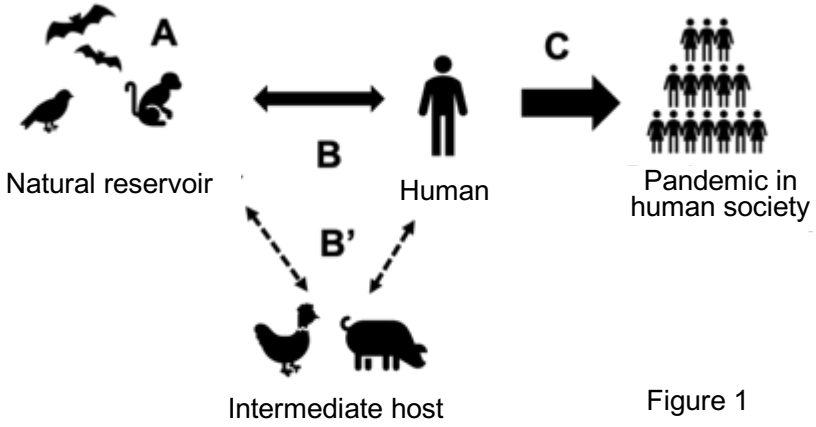


Figure 1

Q29-1 Which factor increases variation of the coronavirus genome in A? **Choose a combination of correct answers.** 89 (1 point)

a) The viral genome is surrounded by capsids.

b) The virus has an envelope.

c) The host DNA replication mechanism has a proofreading function.

d) The genome size is large for a RNA virus.

e) The genome is segmented by some polycistronic genes.

(1) a), b) (2) a), c) (3) a), e) (4) b), c) (5) b), d) (6) c), d) (7) c), e) (8) d), e)

Q29-2 Which of the following facts contribute to the transmission of coronavirus in B and B'? **Choose a combination of correct answers.** 90 (1 point)

a) Disease do not develop in natural host bats.

b) Multiple types of coronaviruses are detected in the fecal masses of bats in one cave by using RT-PCR.

c) Many bat species are nocturnal.

d) Habitat changes that occur due to climate change.

e) Many bat species feed on various insects.

(1) a), b), c) (2) a), b), d) (3) a), b), e) (4) a), c), e)
(5) b), c), d) (6) b), d), e) (7) b), c), d) (8) b), c), e)

Plant biology

Q30

Early splendor, a cultivar of ornamental amaranth (*Amaranthus tricolor*) begins to form red leaves in late summer to early autumn, after producing fully green leaves. The first few red leaves are only partially red, each consisting of distal green and proximal red regions. Finally, fully red leaves form after the formation of partially red leaves (Figure 1a). The color pattern of each leaf remains unchanged after leaf emergence. The timing of red leaf formation is considerably influenced by photoperiodic conditions (Figure 1b).

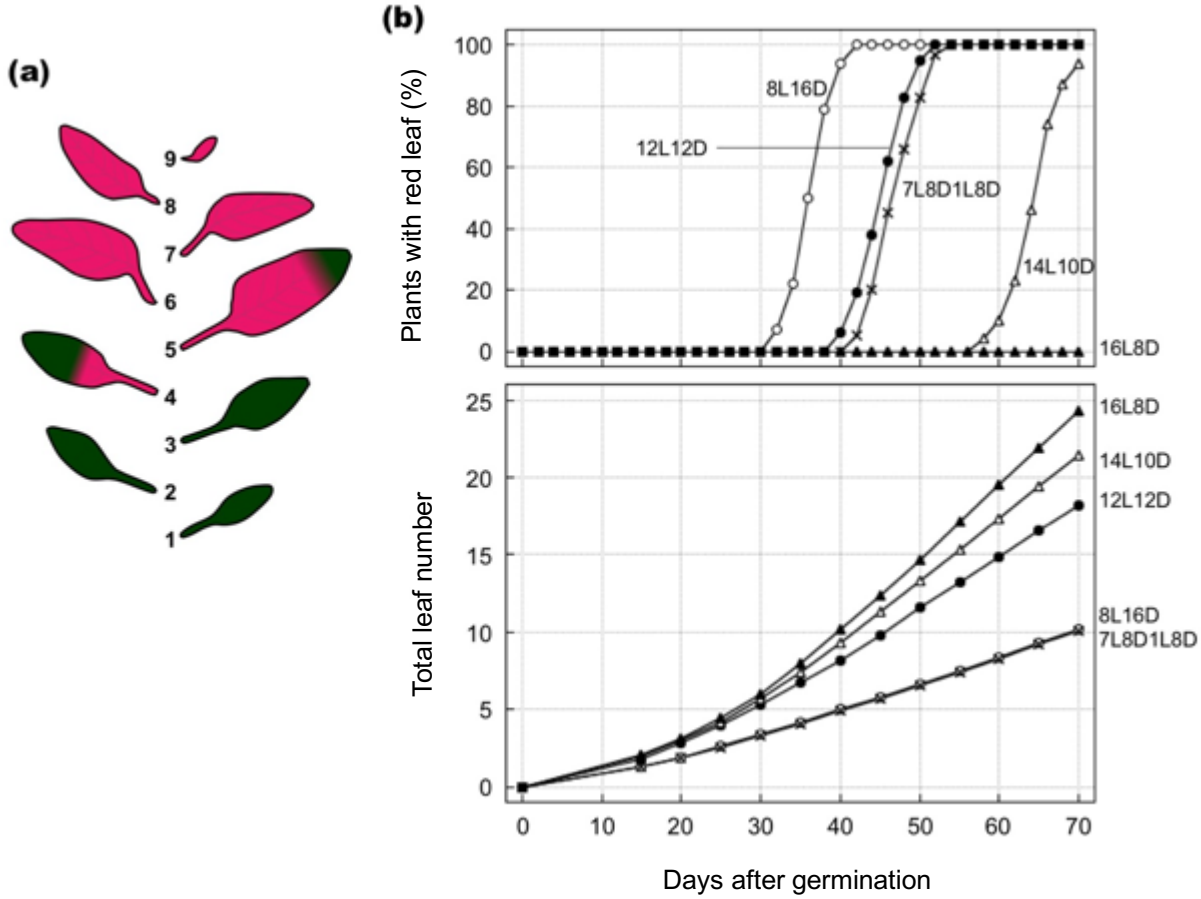


Figure 1

(a) Sketch of leaves excised from a 60-day-old plant cultured under 8-h light/16-h dark conditions. Numbers indicate leaf positions from the base to the apex on the stem.

(b) Plants were cultured in the 16-h light/8-h dark (closed triangles, 16L8D), 14-h light/10-h dark (open triangles, 14L10D), 12-h light/12-h dark (closed circles, 12L12D), 8-h light/16-h dark (open circles, 8L16D), or 7-h light/8-h dark/1-h light/8-h dark (saltires, 7L8D1L8D) conditions. 'Plants with red leaf' indicates plants that form at least one partially red leaf. 'Total leaf number' indicates the average number of total leaves per plant.

Q30-1 The timing of red leaf formation and the growth rate can be related to the number of red leaves. **Rank the 70-day-old plants cultured under different photoperiodic conditions in the order of their average number of red leaves.**

91	>	92	>	93	>	94	>	95	(3 points)

(1) 16L8D
(2) 14L10D
(3) 12L12D
(4) 8L16D
(5) 7L8D1L8D

Q30-2 Assuming that some red leaf-inducing signal X is produced in expanded leaves in response to photoperiodic conditions and transported to the shoot apical region by analogy to the photoperiodic regulation of flowering, the following two hypotheses are considered to explain the color pattern of partially red leaves.

I. The distal part of each leaf primordium requires higher concentrations of the signal X for red coloration than that required by the proximal part.
II. During leaf primordium development, the distal part is determined for coloration earlier than the proximal part.

Which of the following experiments is most informative to distinguish between these hypotheses?

96	(2 points)

(1) Examine color patterns of leaves newly formed on the scion after grafting the scion of the 60-day-old 8L16D plant on the stock of the 60-day-old 16L8D plant.
(2) Examine color patterns of leaves newly formed on the scion after grafting the scion of the 60-day-old 16L8D plant on the stock of the 60-day-old 8L16D plant.
(3) Examine color patterns of leaves newly formed after transferring 60-day-old plants from the 8L16D condition to the 16L8D condition.
(4) Examine color patterns of leaves newly formed after transferring 60-day-old plants from the 16L8D condition to the 8L16D condition.

Plant biology

Q31

When protonemata of the moss *Physcomitrella patens* were cultured without exchanging the culture medium, some of the cells formed buds, which grew into gametophores (Figure 1). The culture medium did not originally contain any plant hormones, but auxin and cytokinin were detected in the medium after the culture of protonemata.

Next, protonemata were cultured while keeping the medium fresh by continuous medium exchange using an apparatus shown in Figure 2, and the effects of addition of auxin and/or cytokinin to the medium on bud formation were examined in this system (Table 1).

Protonemata of mutant ***x***, which do not form buds in natural conditions, were inoculated into hormone-free medium, auxin-containing medium, or cytokinin-containing medium and cultured without medium exchange to examine the effects of the addition of auxin or cytokinin on bud formation (Table 1).

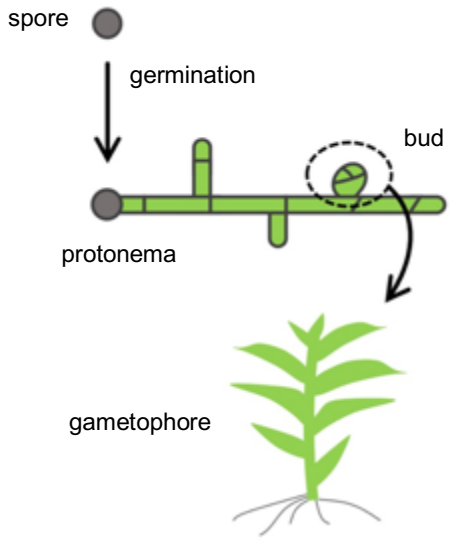


Figure 1

Part of the lifecycle of *Physcomitrella patens*

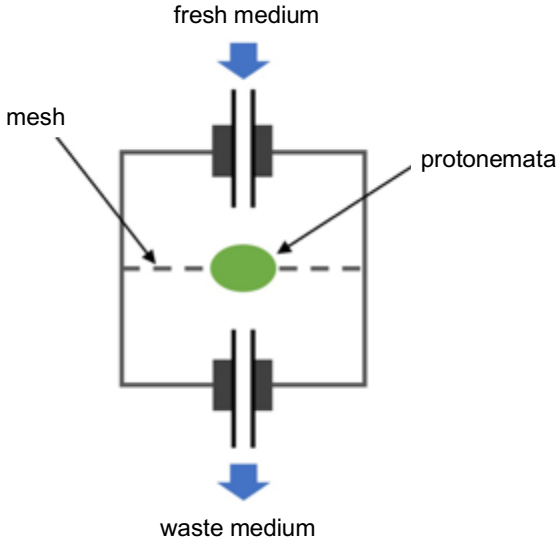


Figure 2

Apparatus for keeping the medium fresh by continuous medium exchange

Table 1

Genotype	Medium exchange	Addition of auxin	Addition of cytokinin	Bud formation
wild type	No	No	No	Occurred
wild type	Yes	No	No	Did not occur
wild type	Yes	Yes	No	Did not occur
wild type	Yes	No	Yes	Did not occur
wild type	Yes	Yes	Yes	Occurred
mutant ***x***	No	No	No	Did not occur
mutant ***x***	No	Yes	No	Did not occur
mutant ***x***	No	No	Yes	Occurred

Choose the most appropriate answer set to fill in the following blanks (A, B and C). 97 (2 points)

- The wild-type protonemata secrete (A).
- Auxin sensitivity is (B) in mutant ***x***.
- Protonemata are more likely to form buds when their growing density is (C).

	A	B	C
(1)	both auxin and cytokinin	lost	higher
(2)	both auxin and cytokinin	lost	lower
(3)	both auxin and cytokinin	normal	higher
(4)	both auxin and cytokinin	normal	lower
(5)	auxin but not cytokinin	lost	higher
(6)	auxin but not cytokinin	lost	lower
(7)	auxin but not cytokinin	normal	higher
(8)	auxin but not cytokinin	normal	lower

Plant biology

Q32

In some plants, fruit valves bend and/or coil explosively to disperse seeds. While, in many cases, dehydration of fruit valves triggers this explosive movement, fruit valves of *Cardamine hirsuta* bend and coil explosively upon mechanical stimulation (e.g., by animals) when the fruits are fresh and turgid (Figure 1).

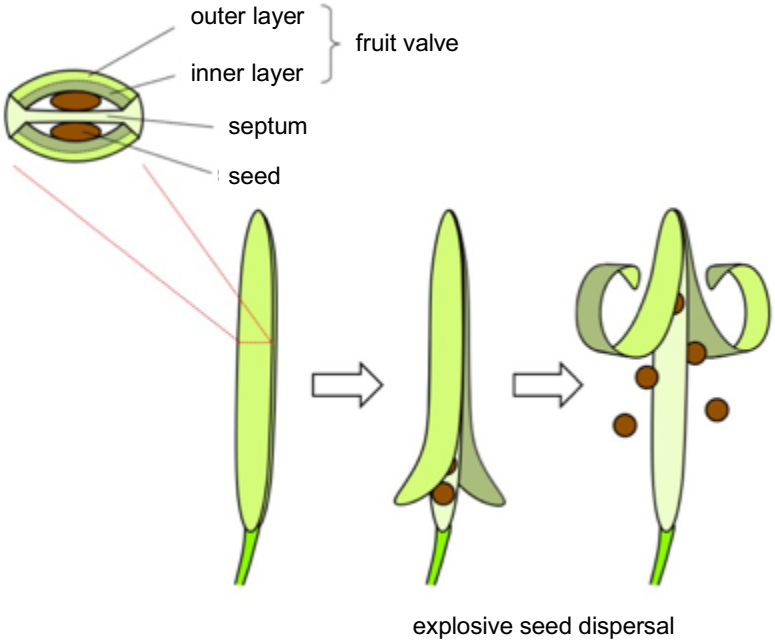


Figure 1. Explosive bending and coiling of a fruit valve of *C. hirsuta*

To study how the fruit valve of *C. hirsuta* builds up energy for bending, researchers examined effects of various treatments and conditions on the bending of fruit valves (Table 1). In this experiment, bending of living intact fruit valves with all layers combined was observed in air, pure water, and 4 M NaCl solution. Fruit valves killed by freeze-thaw disruption of cell membranes and living fruit valves separated into outer and inner layers were also tested for bending in pure water.

Table 1 Bending of fruit valves after various treatments and in various conditions

all layers combined				outer layer only	inner layer only
living	living	living	killed	living	living
air	water	4 M NaCl	water	water	water
+	++	–	–	– (shrank longitudinally)	– (unchanged in length)

++, strong bending; +, bending; –, little or no bending.

Choose the most appropriate answer set to fill in the following blanks (A, B and C). 98 (3 points)

- Higher turgor pressure leads to (A) bending of a fruit valve in *C. hirsuta.*
- A shallow cut made on an intact fruit valve of *C. hirsuta* in the (B) direction would cause the shallow cut to open immediately.
- The outer layer cells of fruit valves of *C. hirsuta* are (C) in water as compared to those in air.

	A	B	C
(1)	increased	longitudinal	narrower and/or thinner
(2)	increased	longitudinal	wider and/or thicker
(3)	increased	transverse	narrower and/or thinner
(4)	increased	transverse	wider and/or thicker
(5)	decreased	longitudinal	narrower and/or thinner
(6)	decreased	longitudinal	wider and/or thicker
(7)	decreased	transverse	narrower and/or thinner
(8)	decreased	transverse	wider and/or thicker

Plant biology

Q33

Many climbing plants have tendrils, a thread-like organ specialized for winding around or clinging to a support. While tendrils are typically modified leaves, some tendrils are modified stems, which can be distinguished by morphological inspection.

Vicia sativa

Cayratia japonica

For a tendril sample, answer which of the following observations is most informative for judging whether it is a modified leaf or a modified stem. 99 (2 points)

(1) Observation of the surface to examine the presence/absence of stomata
(2) Observation of the surface to examine the presence/absence of trichomes
(3) Observation of the surface to examine the thickness of the cuticular wax layer
(4) Observation of the surface to examine the shape of epidermal cells
(5) Observation of the cross section to examine the positional arrangement of the xylem and phloem
(6) Observation of the cross section to examine the number of vascular strands
(7) Observation of the inner tissue to examine the presence/absence of developed chloroplasts
(8) Observation of the inner tissue to examine the presence/absence of the intercellular air space

Plant biology

Q34

Plant leaves are arranged around the stem in regular patterns. A typical example is the Fibonacci spiral, where the angle between successive leaves is near the golden angle of 137.5 degrees. Such regular pattern of leaf arrangement reflects the positional relationship of a new leaf to the existing leaves when it arises at the periphery of the shoot apical meristem. It is considered that the position of new leaf formation is determined under the epidermis-transmitted inhibitory effect from existing leaves. To characterize this effect, the following microsurgical experiments were performed using tomato plants.

Researchers made a shallow cut on the surface at the adaxial side of the incipient leaf P_0 to isolate it from the apical region (Figure 1), and measured the angles between successive leaves after the next leaf P_1 and the second next leaf P_2 were formed (Figure 2).

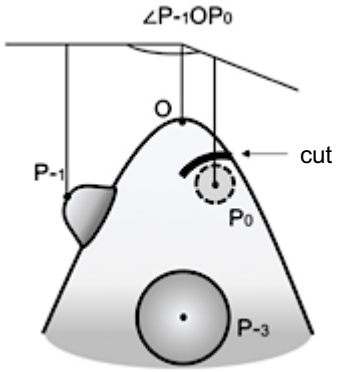


Figure 1. Schematic drawing of the microsurgical experiment
P_{n-1} indicates the leaf immediately preceding to P_n and O represents the center of the stem. P_{-4} and P_{-2} are not shown in this drawing.

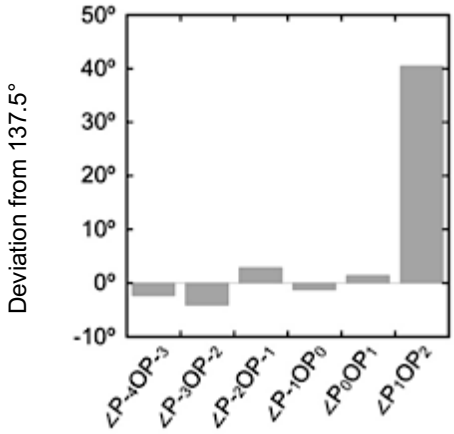


Figure 2. Deviation of the angle between successive leaves from 137.5 degrees

What can be speculated from the above experiment? **Choose the most appropriate set of reasonable speculations.** 100 (3 points)

A. Among existing leaves, only the immediately preceding leaf is critical for the determination of the position of a new leaf formation.

B. Among existing leaves, two preceding leaves are critical for the determination of the position of a new leaf formation.

C. When a leaf is newly arising, the position of the next leaf is not determined yet.

D. When a leaf is newly arising, the position of the next leaf is already determined but the position of the second next leaf is not determined yet.

E. If n is sufficiently large, the deviation of the angle $\angle P_nOP_{n+1}$ from 137.5 degrees is close to 0.

F. If n is sufficiently large, the deviation of the angle $\angle P_nOP_{n+1}$ from 137.5 degrees is close to 42.5 degrees.

(1) A, C, E (2) A, C, F (3) A, D, E (4) A, D, F

(5) B, C, E (6) B, C, F (7) B, D, E (8) B, D, F

Plant biology

Q35

Nitrogen assimilation is a process that requires considerable amounts of reducing power. In leaf cells under moderate light conditions, this process competes with carbon assimilation in the Calvin cycle for reductants supplied by the photosystem, if these reactions are coexistent (Figure 1). Such competition influences the carbon assimilation quotient (CAQ), defined as the ratio of the CO_2 absorption rate to the O_2 evolution rate. Additionally, CAQ is also influenced by the nitrogen source applied to plants. This effect is expressed by ΔCAQ, which is calculated as the difference of CAQ between plants grown with ammonium and plants grown with nitrate.

$$CAQ = CO_2 \text{ absorption rate} / O_2 \text{ evolution rate}$$

$$\Delta CAQ = CAQ_{\text{ammonium}} - CAQ_{\text{nitrate}}$$

* CAQ_{ammonium} : CAQ of plants grown with ammonium as the only source of nitrogen
* CAQ_{nitrate} : CAQ of plants grown with nitrate as the only source of nitrogen

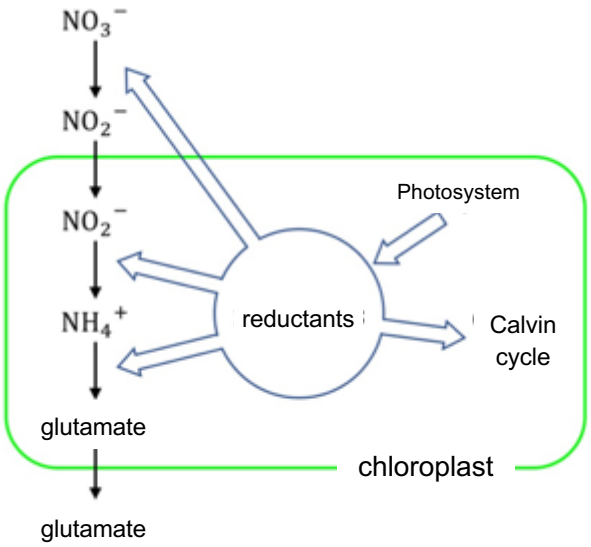


Figure 1

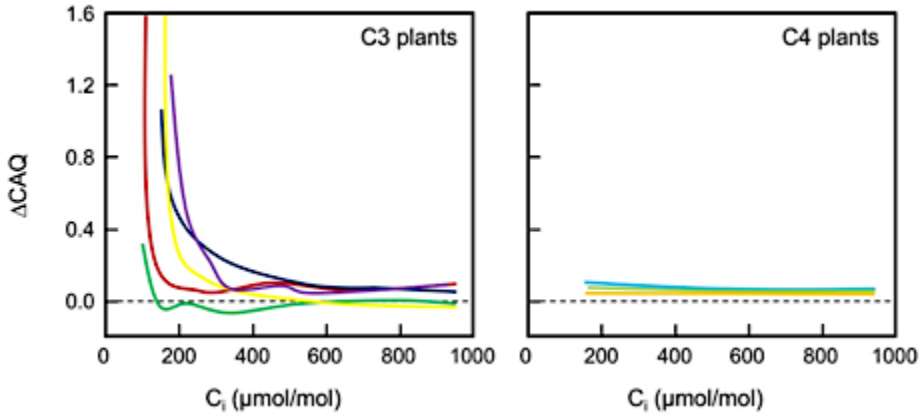


Figure 2

Figure 2 shows ΔCAQ values as a function of the leaf internal CO_2 concentration (Ci) measured in various C3 and C4 plant species. Different colors indicate different species.

Q35-1 Choose the appropriate answer set to fill in the following blanks (A and B). 101 (2 point)

Competition for reductants between nitrogen assimilation and carbon assimilation (A) CAQ. As application of ammonium skips its upstream steps of nitrogen assimilation, ΔCAQ correlates (B) with the activity of the nitrate-to-ammonium conversion process of nitrogen assimilation.

	A	B
(1)	raises	positively
(2)	raises	negatively
(3)	lowers	positively
(4)	lowers	negatively

Q35-2 In C4 plants, which cell is likely to be responsible for the nitrate-to-ammonium and ammonium-to-glutamate processes of nitrogen assimilation? 102 (2 point)

	nitrate-to-ammonium	ammonium-to-glutamate
(1)	mesophyll cell	mesophyll cell
(2)	mesophyll cell	bundle sheath cell
(3)	mesophyll cell	cannot determine from the data provided
(4)	bundle sheath cell	mesophyll cell
(5)	bundle sheath cell	bundle sheath cell
(6)	bundle sheath cell	cannot determine from the data provided
(7)	cannot determine from the data provided	mesophyll cell
(8)	cannot determine from the data provided	bundle sheath cell
(9)	cannot determine from the data provided	cannot determine from the data provided

Evolution

Q36

In a hypothetical organism, a male is known to transmit about 40 *de novo* mutations to his offspring when he mates with a female at the age of 20. In addition, the number of mutations in the germline cells of a male for each year is known to be about two. In this condition, **what is the expected number of deleterious mutations an offspring receives from a 20-year-old and a 40-year-old father, respectively?** Note that the genome size, the number of genes, the average length of a gene, and the probability that a mutation in a gene is deleterious are 1 Gbp, 10,000, 1 kbp, and 70%, respectively. Also note that all deleterious mutations are assumed to remain in the offspring genomes.

Age 20: 0. 103 104 (2 points)

Age 40: 0. 105 106 (2 points)

Evolution

Q37

During the evolutionary history of vertebrates, they experienced several instances of whole-genome duplications that are believed to facilitate genome diversification and dynamic evolution.

A. Figure 1 shows the phylogenetic tree of broad range of vertebrates and lancelet (ancestor of vertebrates) with the timing of whole-genome duplication (black arrowheads). The lancelet possesses one Hox gene cluster in the genome.

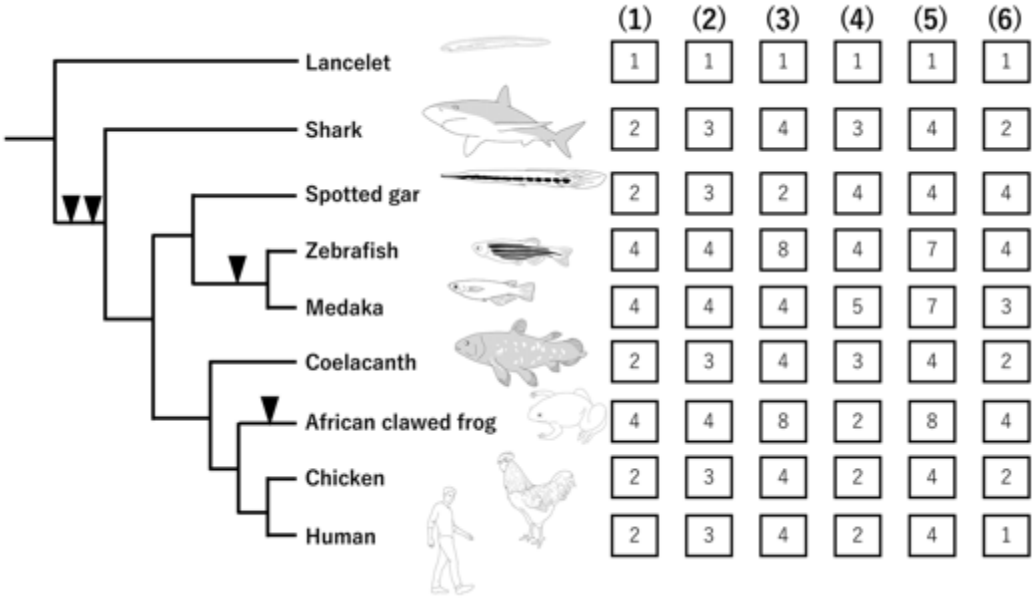


Figure 1. (left) The phylogenetic tree of a broad range of vertebrates and lancelet (an ancestor of vertebrates). (right) The answer options for the number of Hox gene clusters in each species from (1) to (6).

Choose the appropriate combination of the numbers of Hox gene clusters (observed) in vertebrates from (1) to (6). It is noteworthy that Hox gene clusters are rarely lost during evolution. [107] (1 point)

B. In the phylogenetic tree of species X, Y, and Z, whole-genome duplication occurred two times, which is indicated by black arrowheads (Figure 2 left). As a result, each of the species X, Y, and Z possess X1, X2, Y1, Y2, Z1, and Z2 genes.

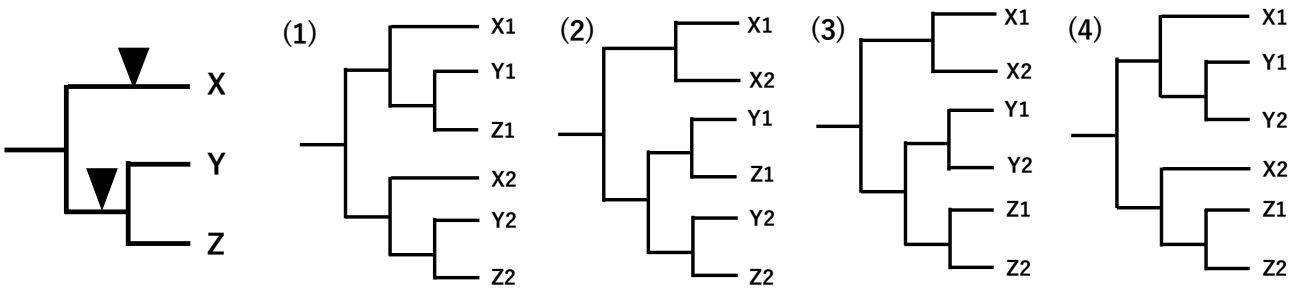


Figure 2 (left) Phylogenetic tree of species X, Y, and Z. (right) The answer options from (1) to (4) for the phylogenetic trees of genes X1 to Z2 of species X, Y, and Z.

Choose the appropriate phylogenetic tree of these genes (X1 to Z2) from (1) to (4). Enough time has passed between gene duplication and the subsequent speciation. 108 (1 point)

Evolution

Q38

Zuckerkandl and Pauling proposed the molecular clock hypothesis, in which amino acid differences in a protein accumulate at a uniform rate among species. The concept was applied to estimate the divergence time between species of various organisms. In addition, rate of the amino acid substitution was revealed to vary among proteins because of the difference in functional importance. Here, we focus on two proteins X and Y. Their lengths (X: 400 a.a., Y: 600 a.a.) and the substitution rates (X: 0.625×10^{-9}, Y: 1.25×10^{-9} substitution/site/year) are both different.

A. In each of the proteins X and Y, how many substitutions were expected to be accumulated at a maximum between human and mouse, which was diverged 80 million years ago (MYA).

Choose the right choice. 109 (1 point)

(1) X: 25, Y: 40 (2) X: 40, Y: 100 (3) X: 40, Y: 120 (4) X: 10, Y: 40

B. In Protein X, 6 amino acid substitutions were observed between human and a mammal species. **Choose the right choice for the divergence time between them.** 110 (1 point)

(1) 3 MYA (2) 6 MYA (3) 9 MYA (4) 12 MYA

C. The rate of amino-acid substitution also varies in the different domains of particular proteins in case that the functional importance is different among domains. Human insulin is a peptide hormone, which is first synthesized as a single polypeptide called preproinsulin (110 a.a.). Preproinsulin subsequently undergoes maturation into active insulin composed of A-B domain (51 a.a.), by releasing predomain (24 a.a.) and C domain (31 a.a.). We can expect that the functional importance varies among these domains.

Choose the most appropriate choice for the relative values of the substitution rates of a. pre-domain, b. A-B domain, and c. C-domain. 111 (1 point)

(1) a < b < c

(2) b < c < a

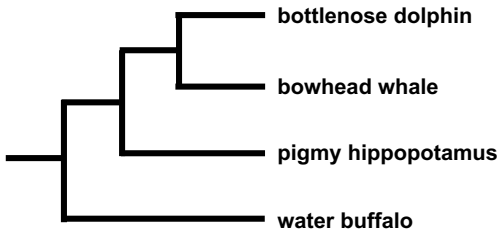
(3) c < b < a

(4) a < c < b

Evolution

Q39

There is a laboratory storing extracted DNA of diverse mammal species. One day, a laboratory staff investigated stored DNA tubes and found three tubes lacking labels. He also found three labels removed from tubes in the same shelf where the three label-less tubes were stored. These three labels are: “bowhead whale”, “pigmy hippopotamus” and “water buffalo”. There was one more tube stored in the same shelf with a label “bottlenose dolphin”. Here, he sequenced a specific genomic region from the DNA of these three label-less tubes (#2, #3, #4) and the bottlenose dolphin (#1). The phylogenetic relationship of the four species and sequence alignment are shown below. It has been assumed that this genomic region has been evolved under a constant evolutionary rate among cetartiodactyls.



Tube #1 bottlenose dolphin	T	A	A	A	T	A	T	C	G	C	A	T	T	T	A	G	T	T	G	C	C
Tube #2	A	T	A	A	T	T	T	G	G	C	A	A	A	T	T	C	A	T	G	T	G
Tube #3	T	A	A	A	T	A	T	C	C	C	C	A	T	A	T	A	G	T	A	G	C	C
Tube #4	T	A	T	A	T	T	T	C	G	C	A	T	A	A	T	G	T	T	G	G	C

	Tube #1	Tube #2	Tube #3	Tube #4
(1)	bottlenose dolphin	bowhead whale	pigmy hippopotamus	water buffalo
(2)	bottlenose dolphin	bowhead whale	water buffalo	pigmy hippopotamus
(3)	bottlenose dolphin	pigmy hippopotamus	bowhead whale	water buffalo
(4)	bottlenose dolphin	pigmy hippopotamus	water buffalo	bowhead whale
(5)	bottlenose dolphin	water buffalo	bowhead whale	pigmy hippopotamus
(6)	bottlenose dolphin	water buffalo	pigmy hippopotamus	bowhead whale

Based on the result above, indicate in the answer sheet which is the most likely combination of tubes and labels. 112 (2 points)

Evolution

Q40

It is well-established that photosynthetic eukaryotes (i.e., algae and plants) acquired plastids through the primary symbiotic uptake of a cyanobacterium. Plastids possess two membranes. Furthermore, it is believed that the ancestors of chlorarachniophytes, which had no plastids, acquired their plastids through the secondary symbiotic uptake of a green alga. The plastids of chlorarachniophytes are bound by four membranes (Figure 1). From which organism is each of the membranes derived?

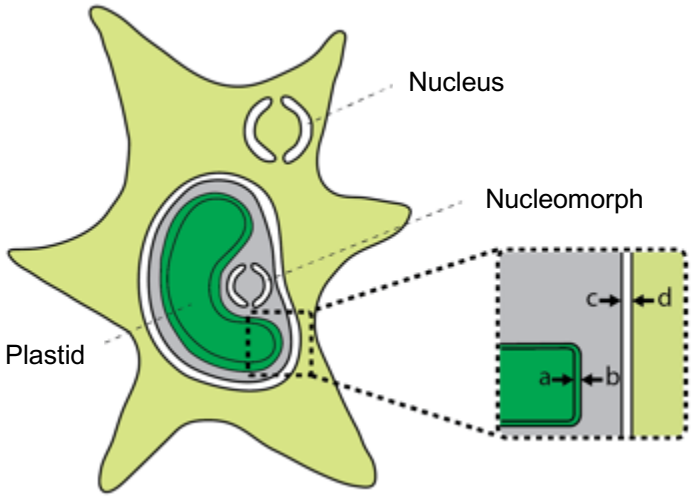


Figure 1. Schematic illustration of the ultrastructure of chlorarachniophytes. The box area is magnified to show that the plastid is bound by four membranes.

Choose the most appropriate combination in the following. 113 (2 points)

(1) (a) cyanobacterium; (b) green alga; (c) ancestor of chlorarachniophytes; (d) ancestor of chlorarachniophytes.

(2) (a) cyanobacterium; (b) green alga; (c) green alga; (d) ancestor of chlorarachniophytes.

(3) (a) cyanobacterium; (b) cyanobacterium; (c) green alga; (d) ancestor of chlorarachniophytes.

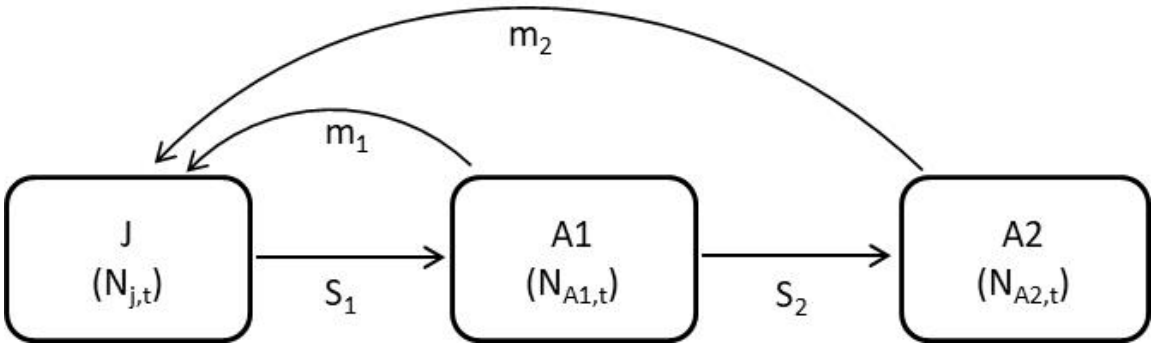
(4) (a) cyanobacterium; (b) cyanobacterium; (c) green alga; (d) green alga.

(5) (a) cyanobacterium; (b) cyanobacterium; (c) ancestor of chlorarachniophytes; (d) ancestor of chlorarachniophytes.

Ecology

Q41

The population dynamics are principally determined by birth and death rates. The diagram below shows the life cycle of an animal species with three age structures, namely juvenile (J), adult 1 (A1), and adult 2 (A2). The population size (females only) at each stage in a given year (t) is shown in parentheses. S_1 and S_2 denote the survival rates between two successive stages, and m_1 and m_2 show the numbers of juveniles produced by an adult individual at the two stages. All individuals that have survived enter the next stage the following year, and mothers give birth to juveniles immediately after entering the next stage. For instance, the number of juveniles at year 1 ($N_{J,1}$) is the total number of offspring produced by adult 1 ($m_1 \times N_{A1,1}$) and adult 2 ($m_2 \times N_{A2,1}$). All individuals in the adult 2 group die the following year.



Q41-1 Given the following demographic parameters and initial population size (year = 0), what will be the number of individuals at each stage class two years later? Note that population size here represents females only.

S_1=0.2, S_2=0.5, m_1=3, m_2=2, $N_{J,0}$=100, $N_{A1,0}$=20, $N_{A2,0}$=20

Choose the appropriate value for each of the following boxes. (2 points if 2 digits are correct)

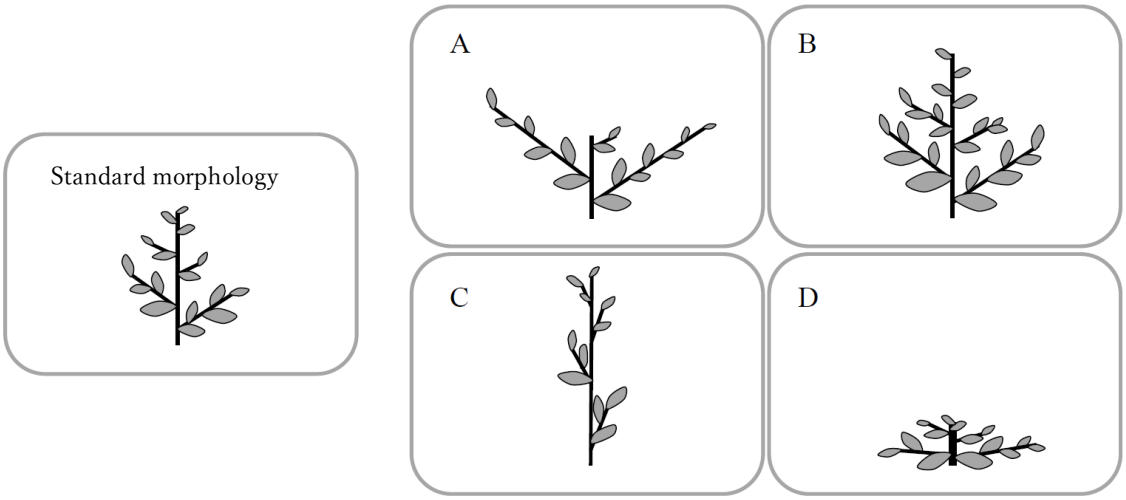
$N_{j,2}$= [114] [115] $N_{A1,2}$= [116] [117] $N_{A2,2}$= [118] [119]

Q41-2 The population with the above parameters will go extinct in the near future. To prevent extinction, at least one parameter value must be increased. When other parameters are held constant, m_1 should be increased, such that $m_1 \geq$ [120]. **Choose the appropriate value for the box.** (1 point)

Ecology

Q42

Plants show morphological plasticity and can change their morphology in response to different environmental conditions. The four figures below (A to D) show simplified diagrams of a plant's typical response to environmental conditions.



Match the following statements (a to d) with the corresponding diagrams (A to D) shown above and choose the appropriate number from the table below. 121 (2 points)

a. Response to soil fertilization

b. Response to apical damage

c. Response to shade condition

d. Response to trampling pressure

	A	B	C	D
(1)	a	d	c	b
(2)	a	c	d	b
(3)	b	a	c	d
(4)	b	d	c	a
(5)	c	a	d	b
(6)	c	a	b	d

Ecology

Q43

The table below presents data on the reproductive success of four different genotypes, A to D in a Hymenopteran insect. The sex determination of hymenopteran insects (bees and wasps) is haplodiploidy: males develop from unfertilized eggs and are therefore haploid, and females develop from normally fertilized eggs and are diploid. If a female mates with only one male, any two of her daughters will share, on average, 3/4 of their genes.

Females	Number of their own offspring	Number of siblings	Average number of offspring produced by each sibling
Genotype A	12	3	7
Genotype B	2	8	12
Genotype C	8	4	6
Genotype D	9	6	5

Q43-1 Provide the direct fitness of genotype A, assuming that all offspring are females and females with different genotypes do not compete. [122] (1 point)

Q43-2 Rank genotypes A to D in descending order of inclusive fitness, assuming that all offspring are females. Choose the number from the table below. [123] (1 point)

(1)	A>B>D>C
(2)	A>D>B>C
(3)	B>A>D>C
(4)	B>D>A>C
(5)	C>A>D>C
(6)	C>D>A>C

Ecology

Q44

The Figure 1 below shows a simplified food web in temperate forests. Spiders living on shrubs and trees are generalist predators that consume herbivores and detritivores, which belong, respectively, to grazing and detrital food webs. Detrital insects spend their larval period in soil, but move to aboveground as winged adults, becoming potential prey for spiders. Passerine birds are also generalist predators that consume herbivores, detritivores, and spiders. Spiders and passerine birds therefore integrate two pathways from grazing and detrital food webs aboveground.

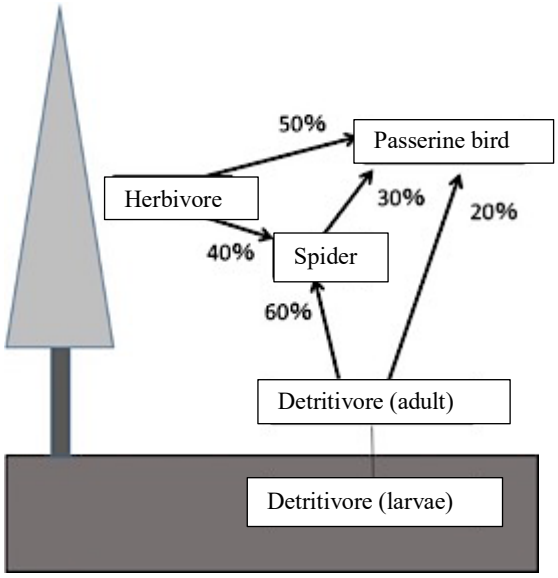


Figure 1

Q44-1 The prey biomass of spiders consists of 40% herbivores and 60% detritivores, while that of passerine birds consists of 50% herbivores, 30% spiders, and 20% detritivores. What is the contribution of the pathway from detrital food web to passerine birds, as expressed by % biomass of detritivores relative to the biomass of herbivores and detritivores combined? Note that the conversion efficiency is assumed to be 10% for any pairs of adjacent trophic levels. **Answer with a 2-digit integer by cutting off numbers after the decimal points.** [124] [125] (2 points if 2 digits are correct)

Q44-2 A huge amount of radionuclides were released into the environment from the Fukushima Daiichi Nuclear Power Plant accident after the earthquake and subsequent tsunami of March 2011. Cesium 137 (^{137}Cs) is the most worrying radionuclide, which spread from the atmosphere to forests. ^{137}Cs was initially retained on plant surfaces and then entered into soil through rain and defoliation. ^{137}Cs is bound to the organic materials of soil by ion-exchange adsorption, or bound strongly to mica minerals in soil, which makes it

difficult for vascular plants to absorb cesium from roots several years later. However, fungi absorb and accumulate a large amount of ^{137}Cs, which is consumed by detritivores. The Figure 2 below presents a schematic representation of how ^{137}Cs concentration changes over the years for three types of organisms after the initial cesium fallout.

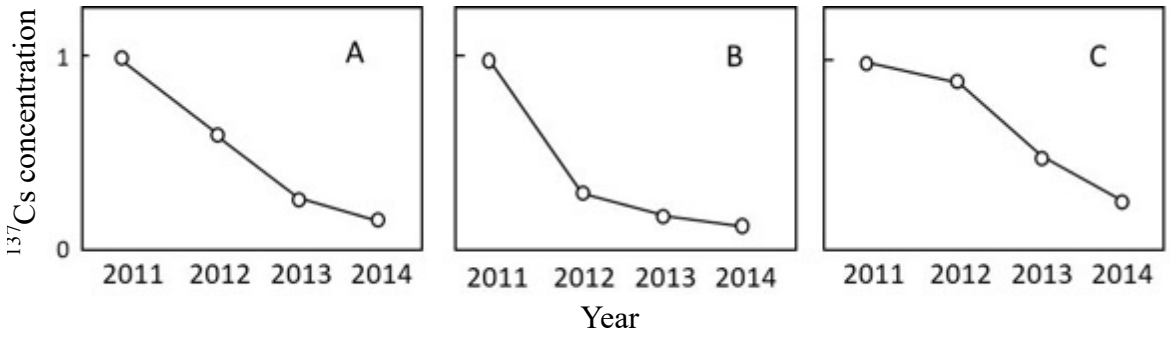


Figure 2 Changes in ^{137}Cs concentration over several years. The concentration represents a relative value, with the value in 2011 being 1.

Given the above information, organisms in different trophic positions in forest food webs are expected to show different temporal changes in cesium concentrations. The above graphs (A,B,C) represent the responses of three organisms. **Choose the most appropriate combination of organisms from below.** Note that the data were gathered in autumn, and bioaccumulation through trophic levels does not occur. 126 (1 point)

	A	B	C
(1)	Grasshopper	Spider	Earthworm
(2)	Grasshopper	Earthworm	Spider
(3)	Spider	Earthworm	Grasshopper
(4)	Spider	Grasshopper	Earthworm
(5)	Earthworm	Grasshopper	Spider
(6)	Earthworm	Spider	Grasshopper

Ecology

Q45

Primary succession can begin in a virtually lifeless area, characterized by early-seral plant species followed by the replacement of these species by other late-seral plant species. An example of this process can be seen in Alaska, where glaciers have retreated as a result of climatic warming during the Holocene. Through this succession, key soil properties such as nitrogen (N) and phosphorus (P) content also change. Nitrogen enters into the soil through the biological pathway of nitrogen fixation, the conversion of N_2 to forms that can be used to synthesize organic nitrogen compounds. Phosphorus is added into the soil through the weathering of rocks. Plants in each successional stage use these nutrients for growth and survival. After the death of plants, the elements stored in the plants can reenter into the soil through the activities of microorganisms, which decompose and mineralize detritus. Soil nutrients can be absorbed and utilized by plants again over time, but some are lost through leaching out from ecosystems.

Choose a panel from (1) to (4) that represents temporal changes in nutrient accumulation in soil through primary succession after glacial retreat in Alaska. The climax stage is boreal forests. In the panels, N and P represent the total amount. 127 (2 points)