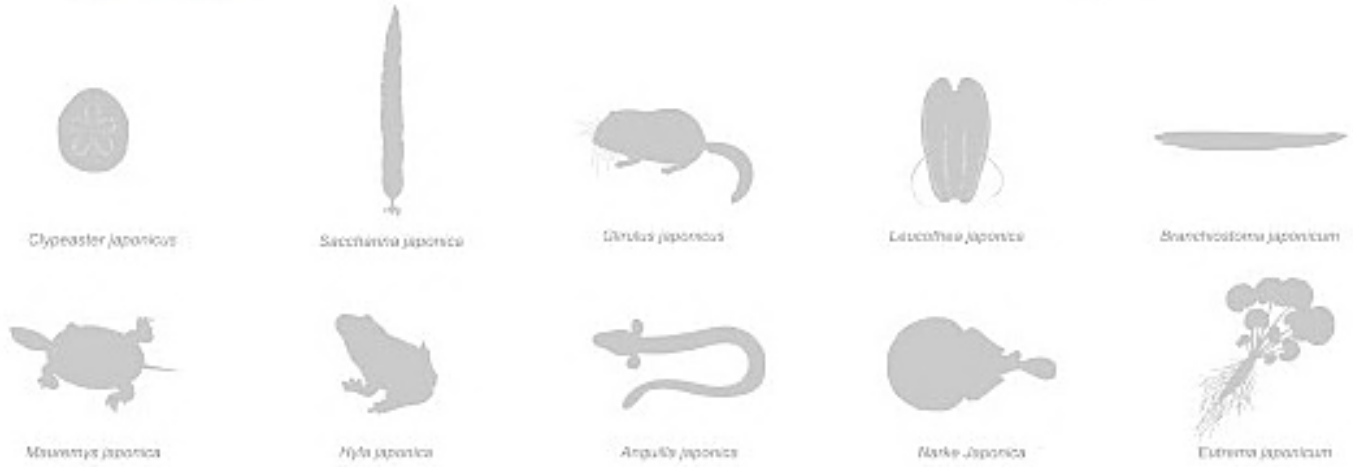
INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

IBO Challenge 2020

A Substitute for The 31st IBO 2020 Nagasaki, JAPAN



Theoretical Exam 2

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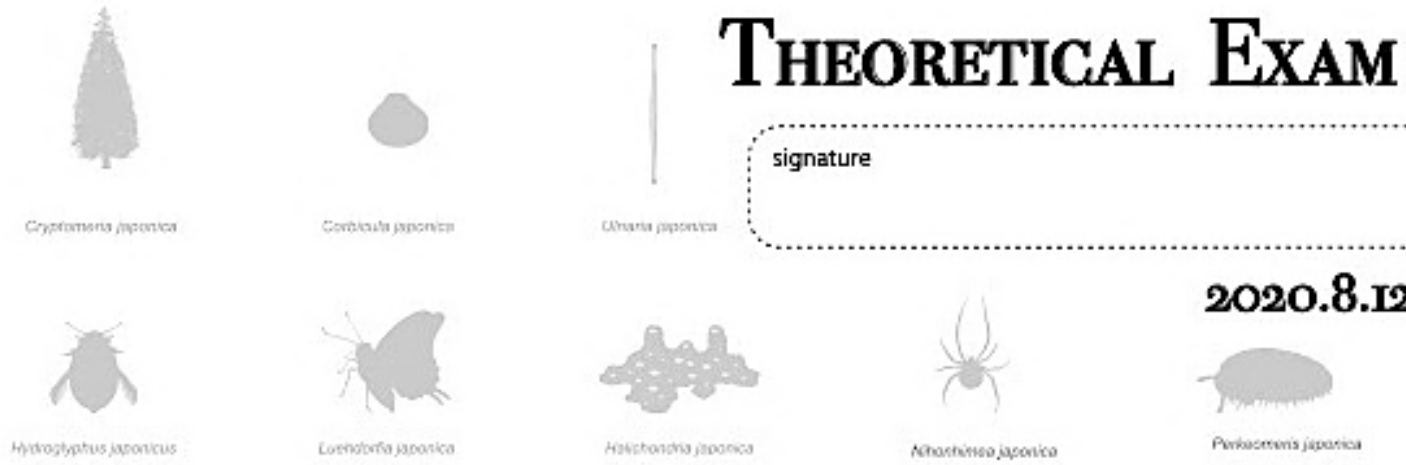


General instructions for theoretical examinations

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.

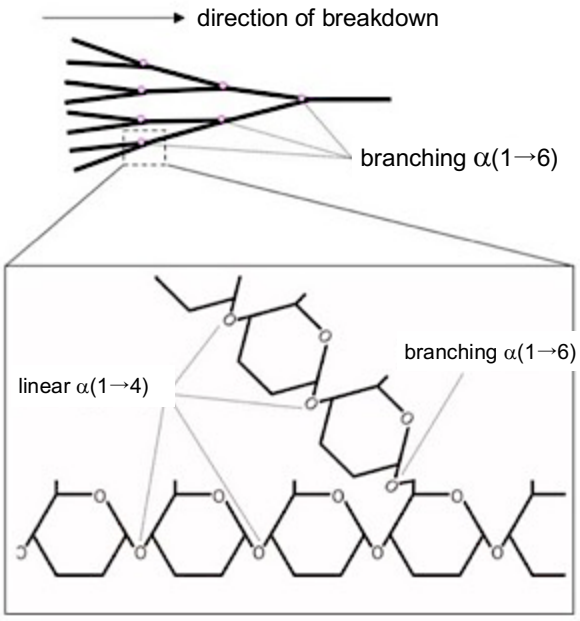


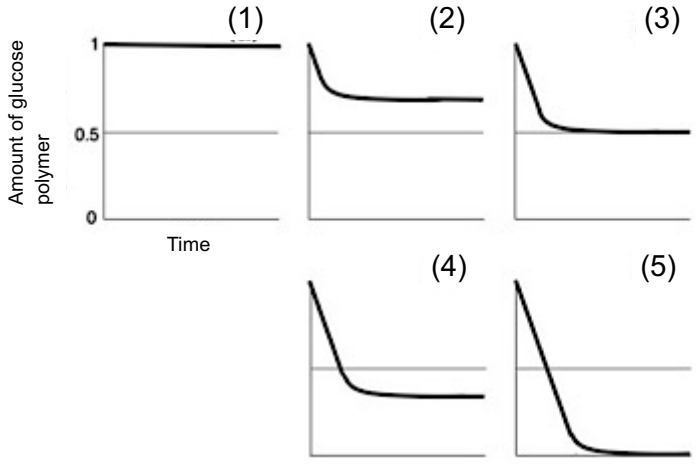
Figure 1 Breakdown of glycogen in cells.

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

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

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

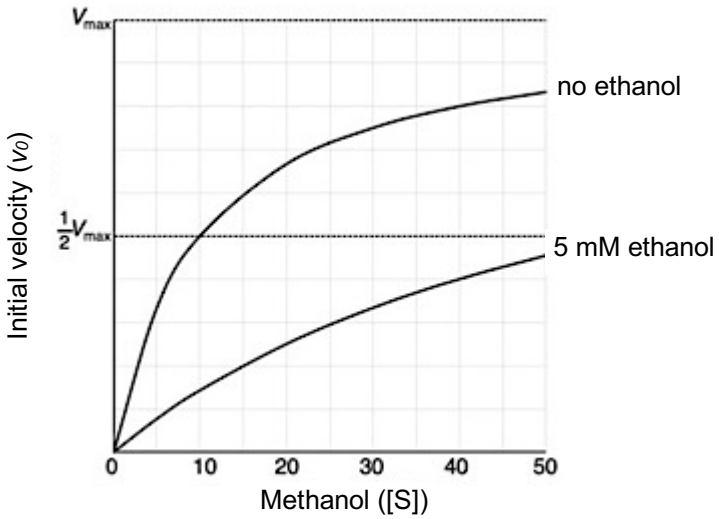


Figure 1 Methanol conversion with or without ethanol

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.

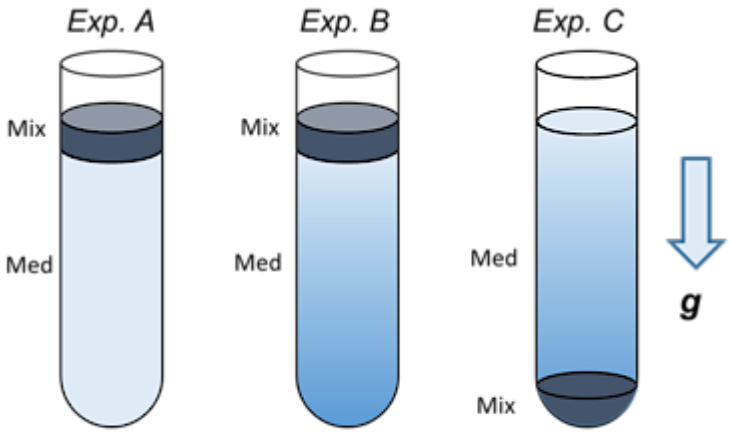


Figure 1

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)

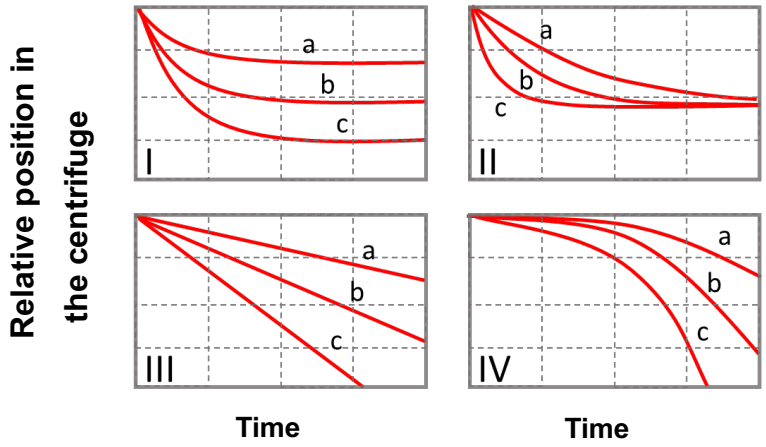


Figure 2

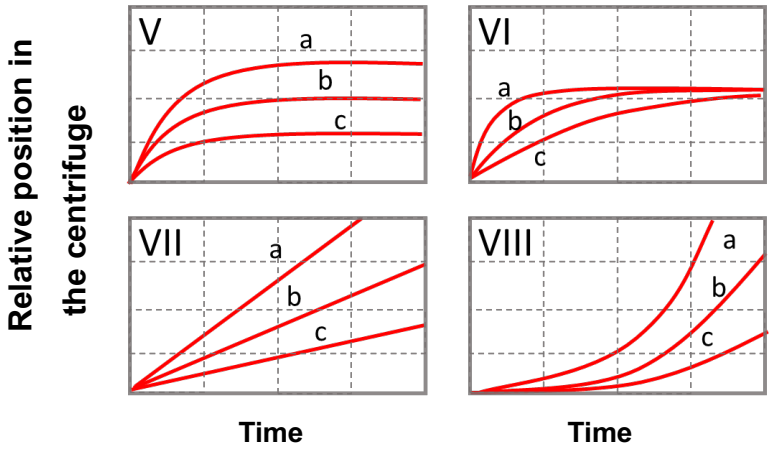


Figure 3

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.

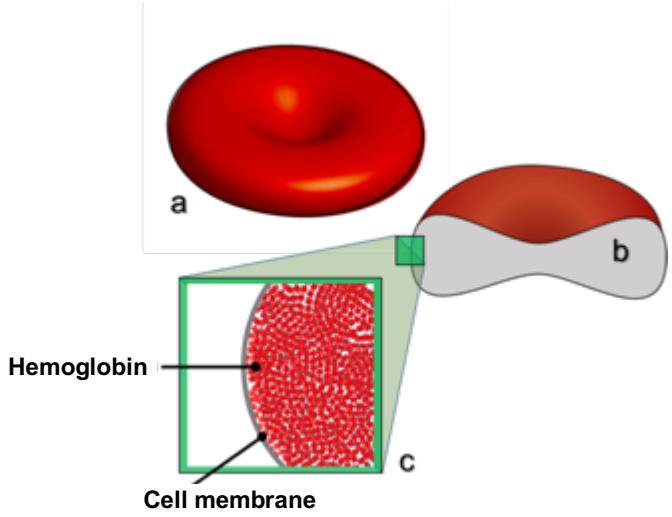


Figure 1 Schematic drawing of red blood cells (RBCs). c, Hemoglobin molecules assumed in a RBC cross section (b).

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.

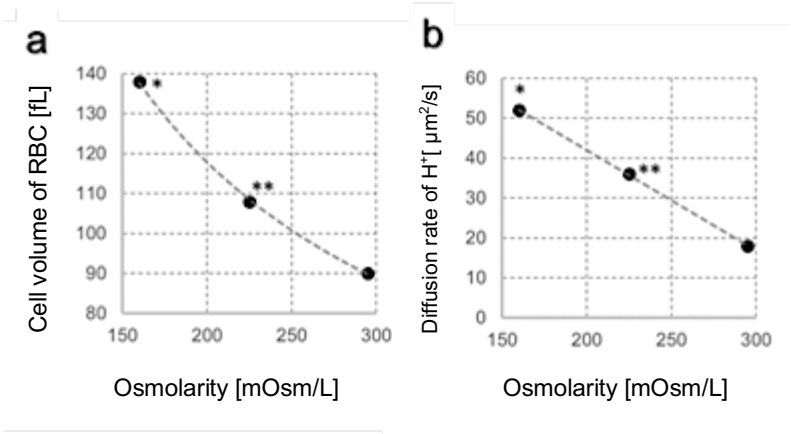


Figure 2 Examination of the relationship between (**a**) the cell volume of RBC (fL = 1×10^{-15} L) and (**b**) the measured diffusion rate of hydrogen ions [$\mu m^2/s$] versus osmolarity [mOsm/L] of saline solutions. 300 mOsm/L corresponds to the osmosis of the body fluid in a healthy human.

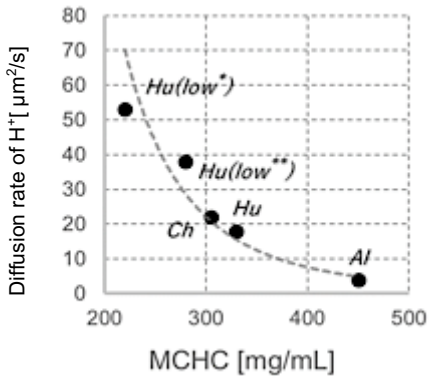


Figure 3 Diffusion rate of intracellular hydrogen ions measured using RBCs from different animal species. *Hu*, *Ch*, and *Al* represent humans, chickens, and alpacas from which RBCs were derived. *Hu (low*)* and *Hu (low**)* indicate the results obtained at 155 and 225 mOsm/L (* and ** in Figure. 2),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

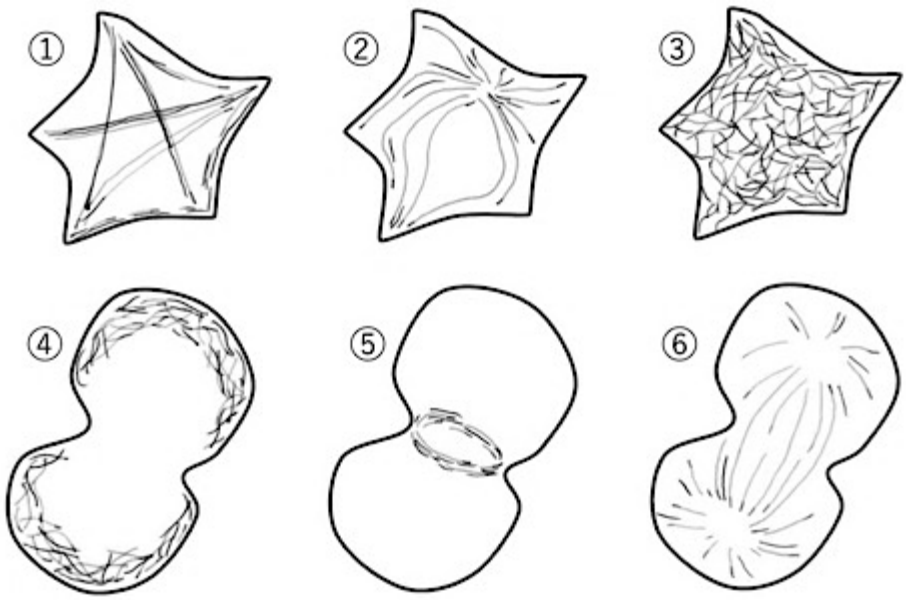


Figure 1

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)

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

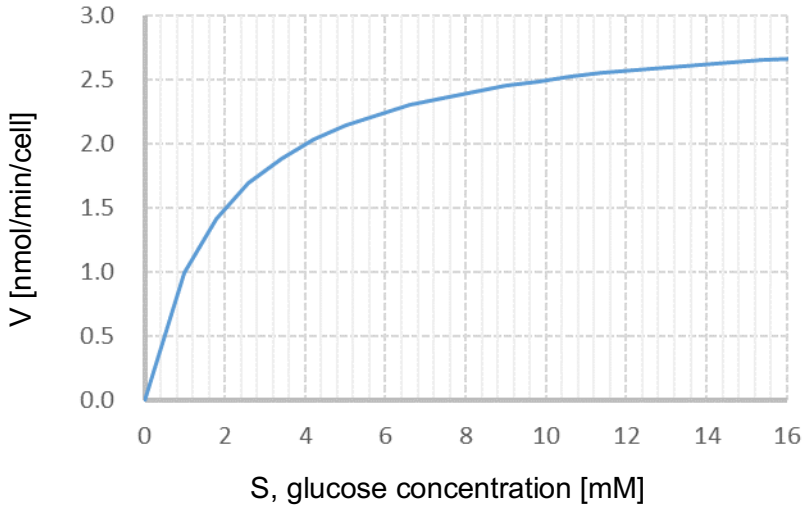


Figure 1 Relationship between S (extracellular glucose concentration, mM) and V (the rate of glucose uptake into red blood cells).

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

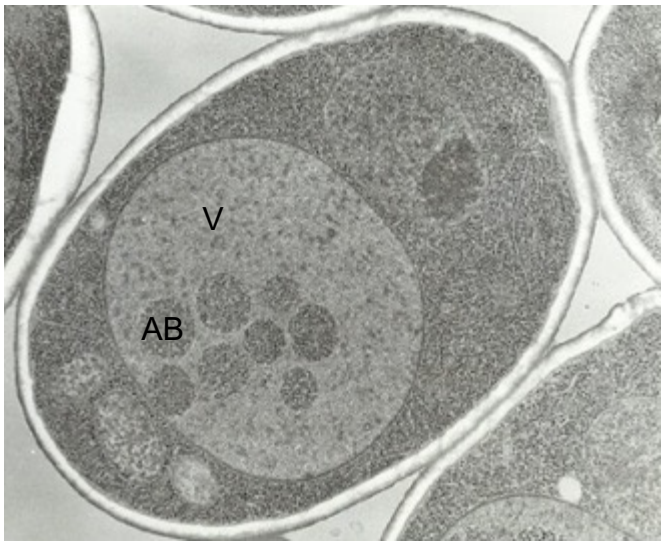
D. Increasing the concentration of CO_2 in the air increases the carboxylase activity of Rubisco. 46

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.

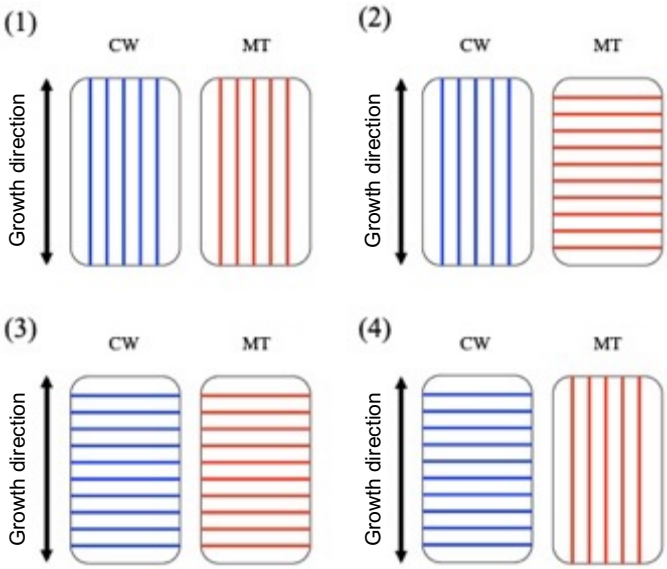
When diffuse growth occurs in plant cells, the cell wall must be loosened, and the growth direction is affected by the orientation of the cellulose microfibrils constituting the cell wall. In cells undergoing diffuse growth, a cellulose synthase complex synthesizes cellulose microfibrils while moving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

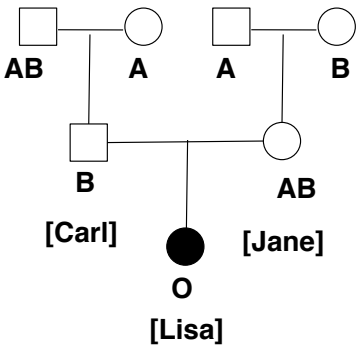


Figure 1

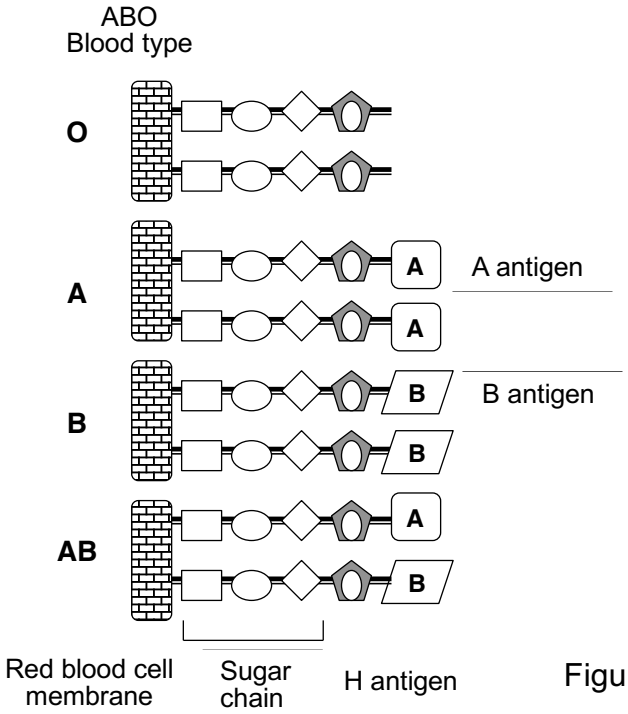


Figure 2

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

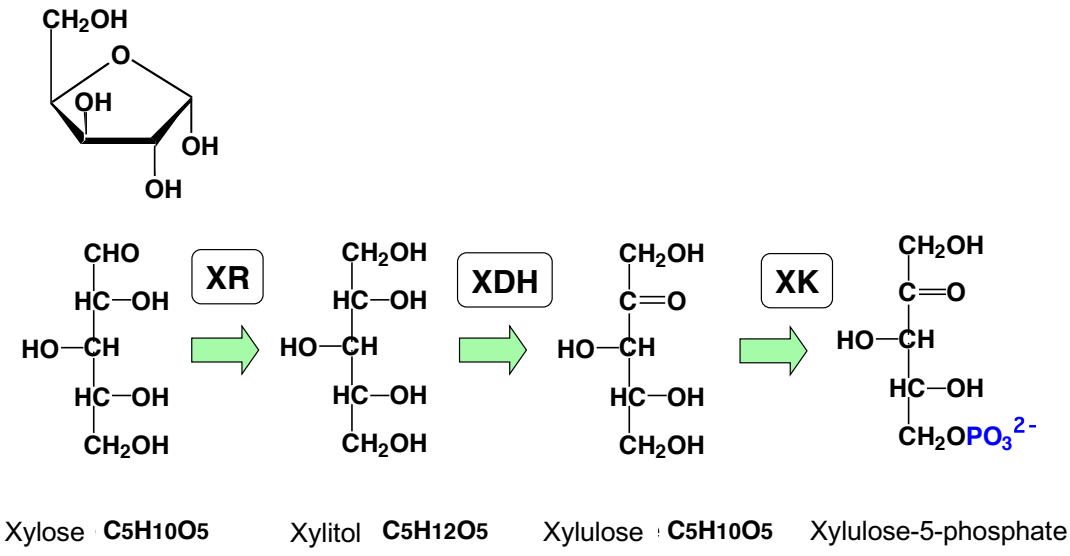
No.	Cycles	Limiting component
(1)	7	Primer pairs
(2)	7	dNTPs
(3)	7	DNA polymerase
(4)	8	Primer pairs
(5)	8	dNTPs
(6)	8	DNA polymerase
(7)	9	Primer pairs
(8)	9	dNTPs
(9)	9	DNA polymerase
(0)	Others	

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.

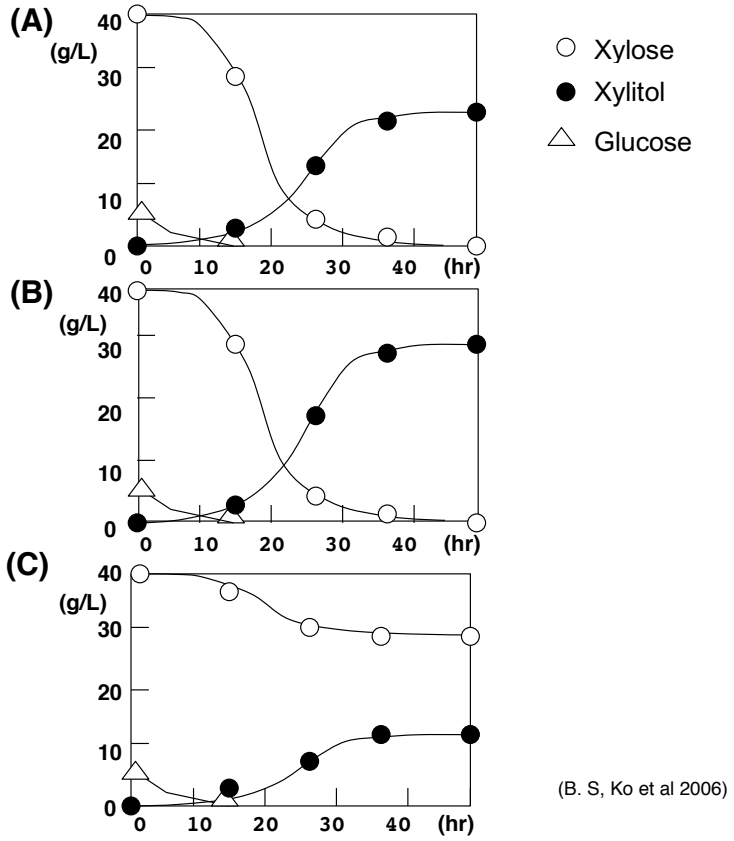


Figure 2

(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.

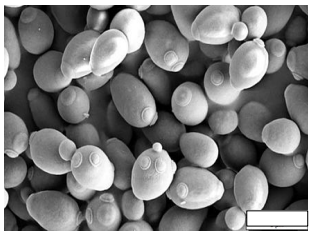
Arrange the genomes of the organisms in the photo in descending order.

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

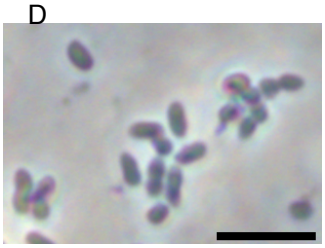
A

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)

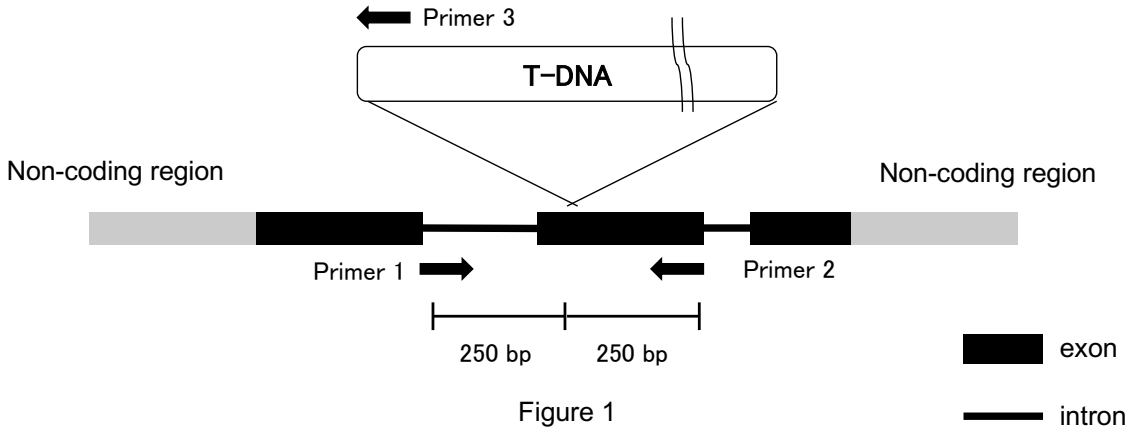
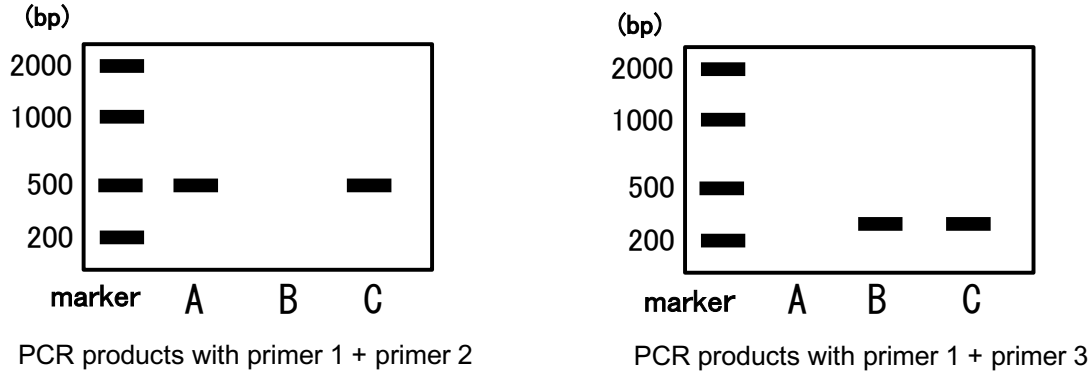


Figure 1



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

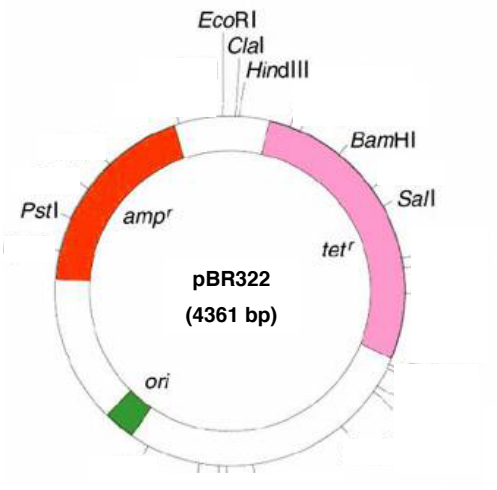
Answer group

(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.

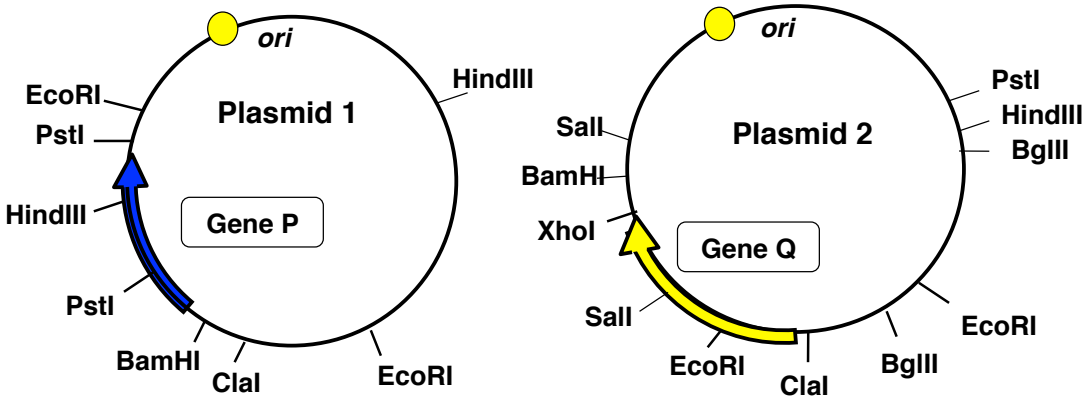


Figure 2 ***ori* : replication origin**

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.

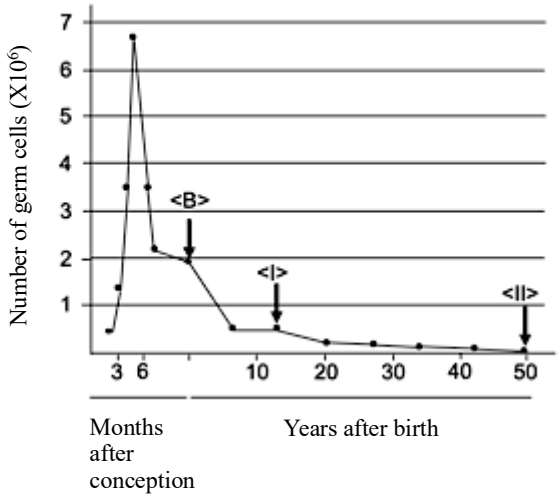
Note: Both native pBR322 and the first recombinant plasmid obtained by Step 1 – 3 are described as pBR322 plasmid.

	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.

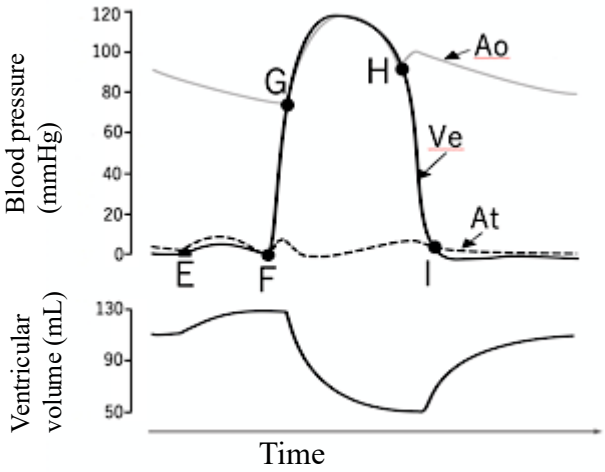


Figure 1

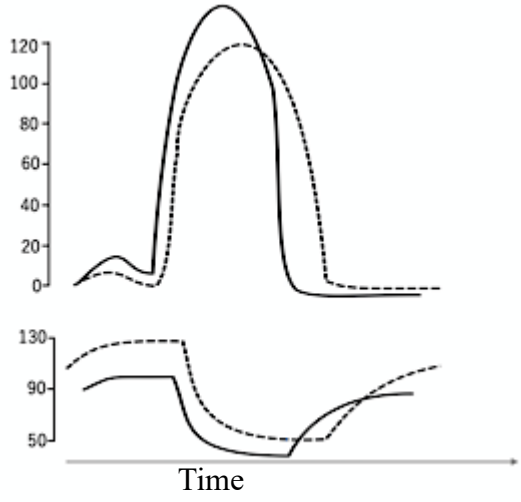


Figure 2

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)

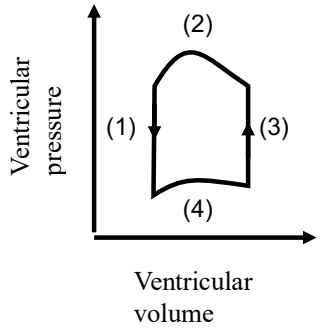
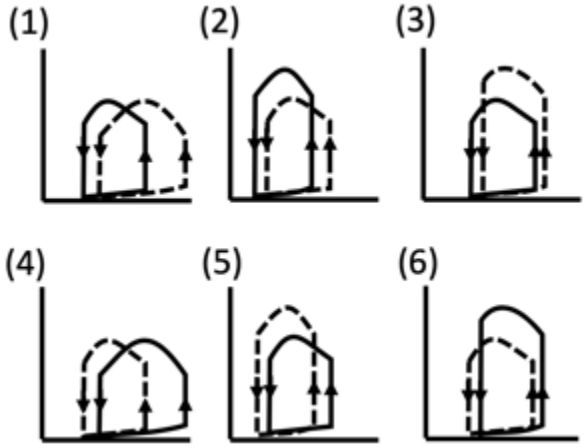


Figure 3

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.

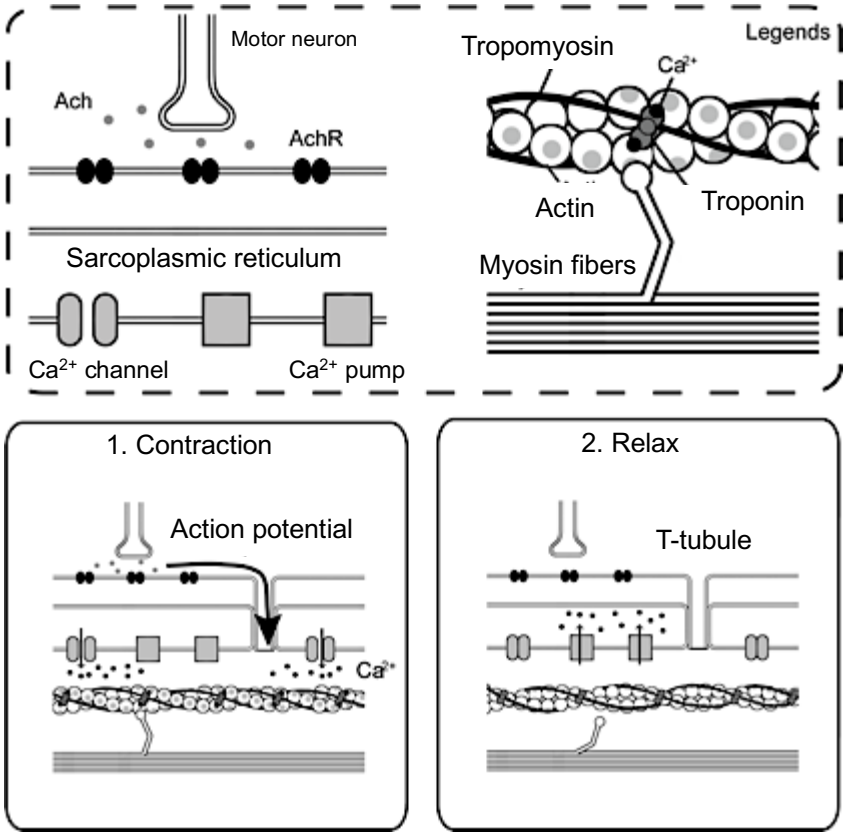
Note that the choices of muscle abnormality are:

(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]

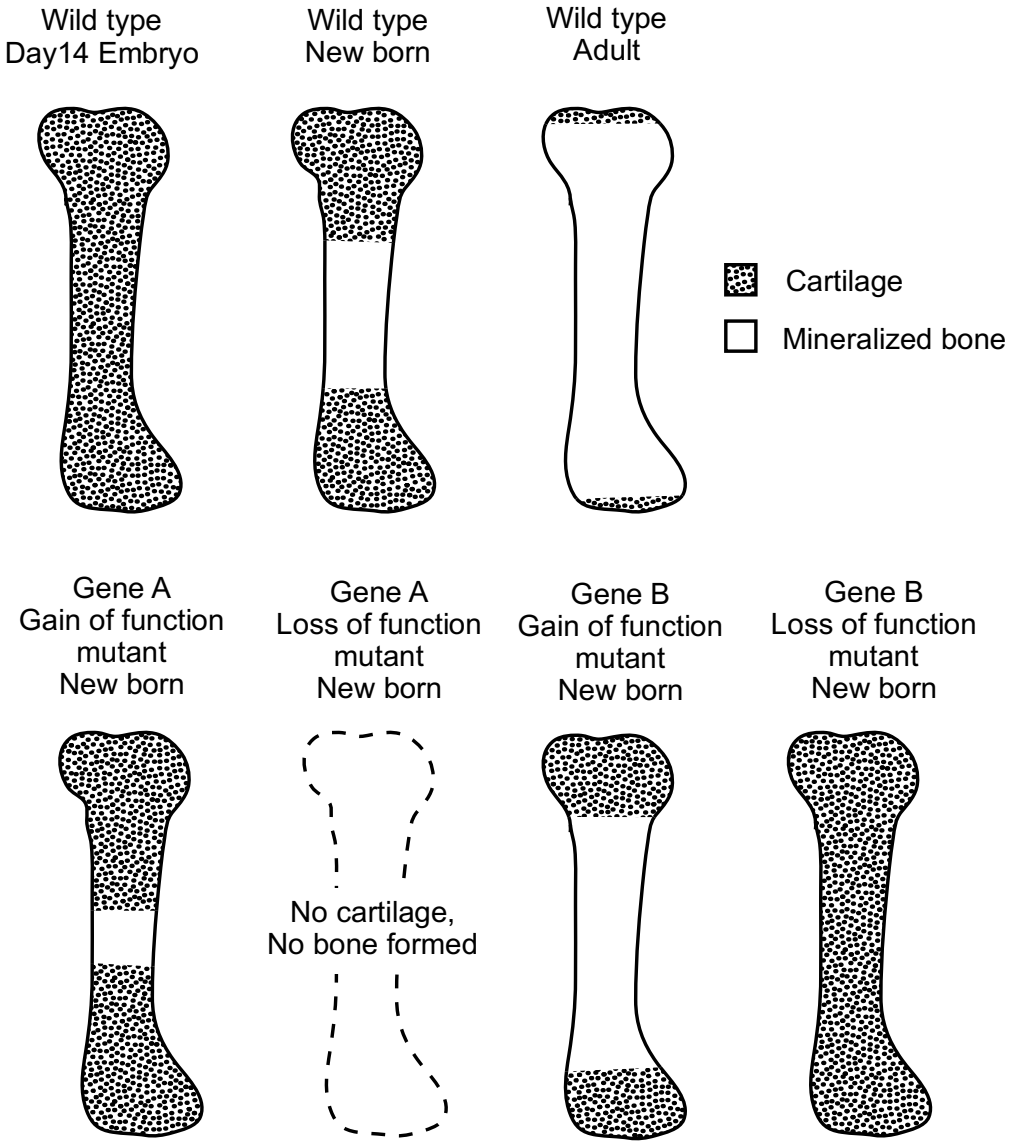
C. Nonsense mutation in Ca^{2+} pump gene, which causes a deficiency in the removal of Ca^{2+} from cytosol. [80]

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.

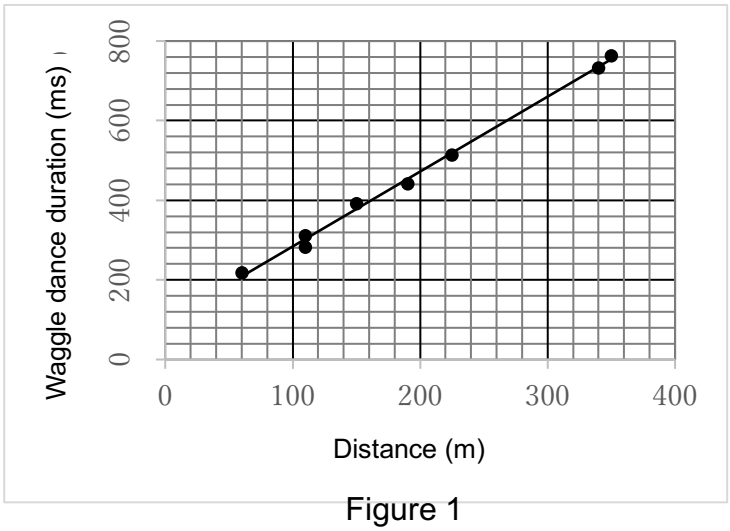


Figure 1

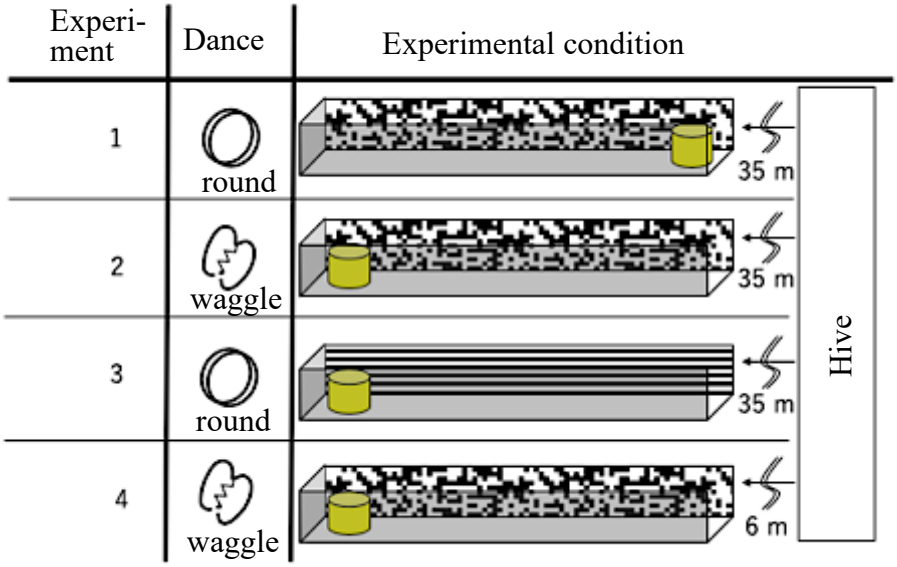


Figure 2

* 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.

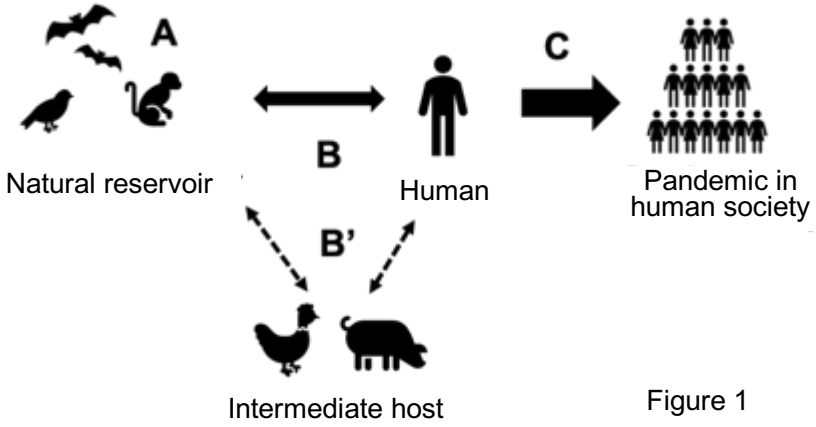


Figure 1

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).

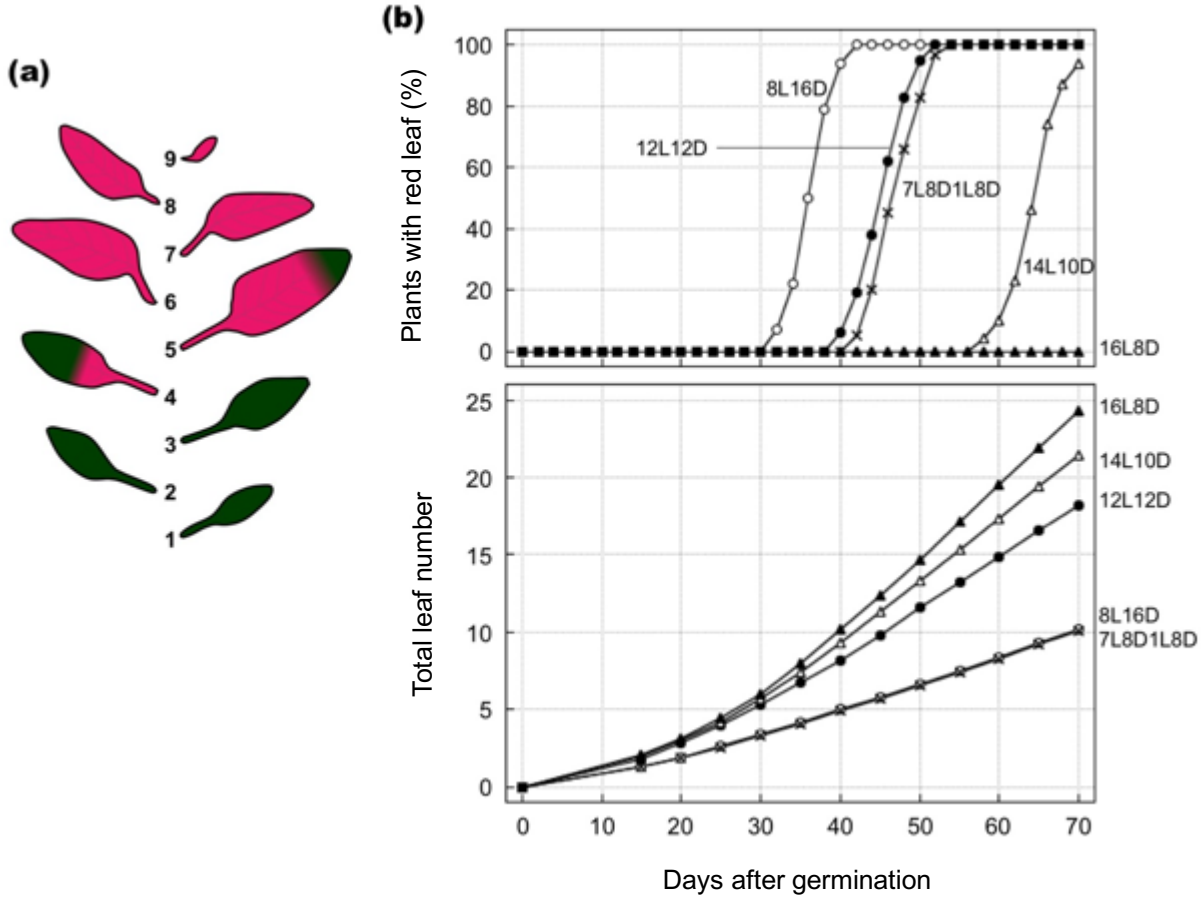


Figure 1

(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).

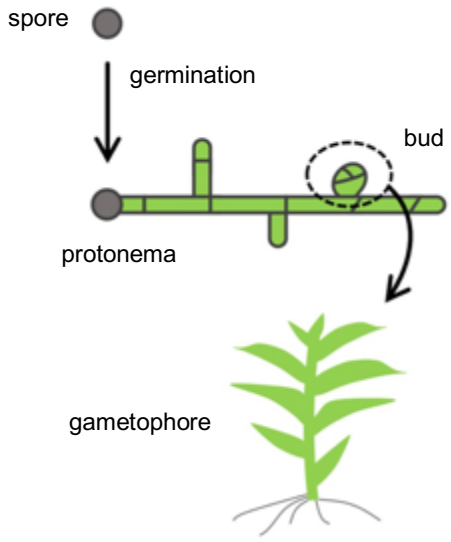


Figure 1

Part of the lifecycle of *Physcomitrella patens*

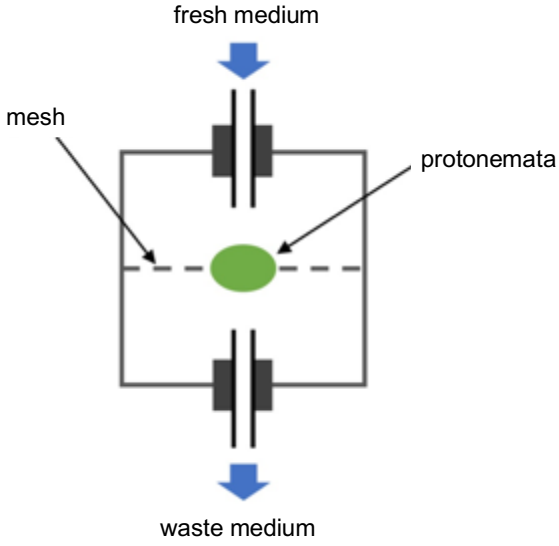


Figure 2

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).

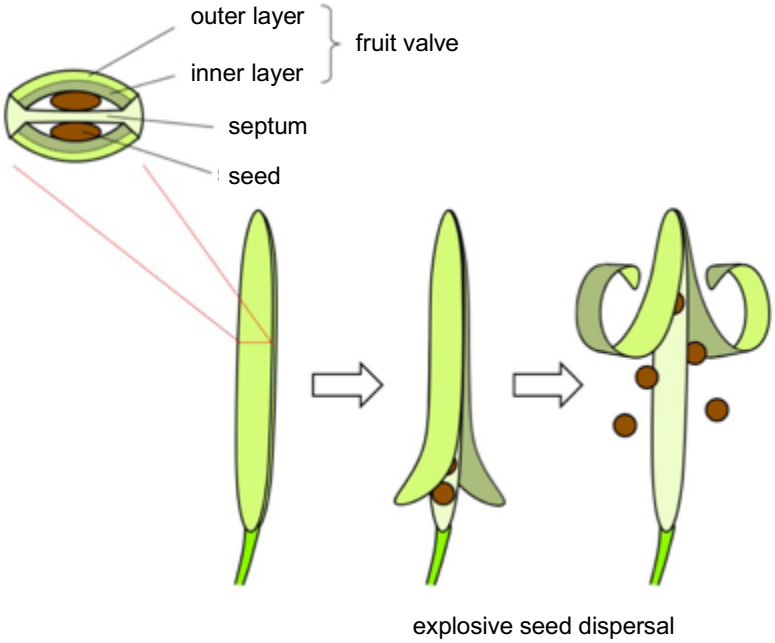


Figure 1. Explosive bending and coiling of a fruit valve of *C. hirsuta*

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).

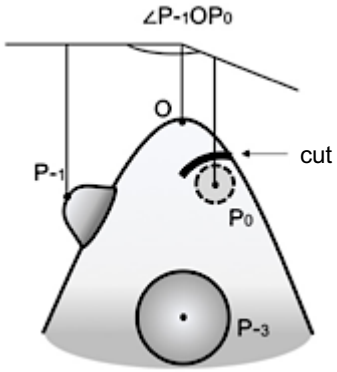


Figure 1. Schematic drawing of the microsurgical experiment
P_{n-1} indicates the leaf immediately preceding to P_n and O represents the center of the stem. P_{-4} and P_{-2} are not shown in this drawing.

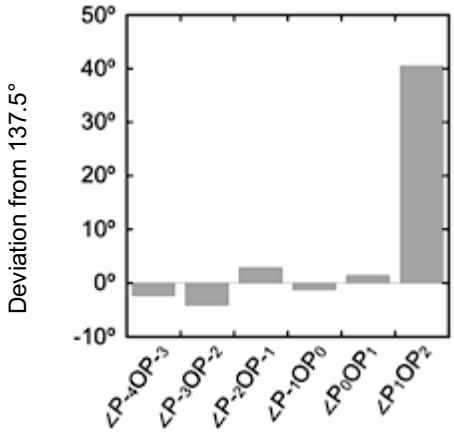


Figure 2. Deviation of the angle between successive leaves from 137.5 degrees

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

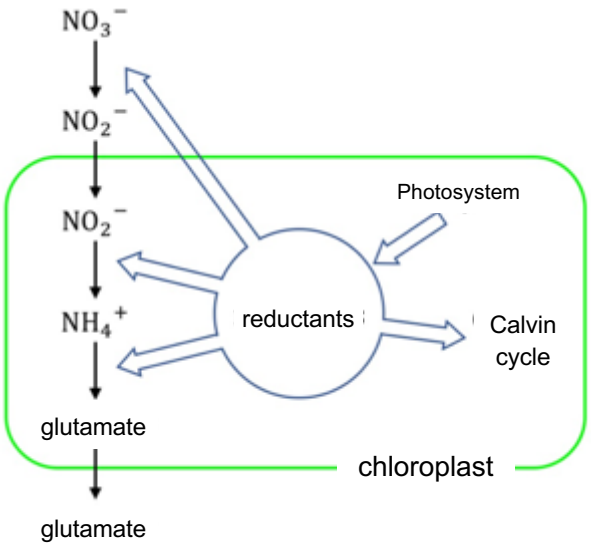


Figure 1

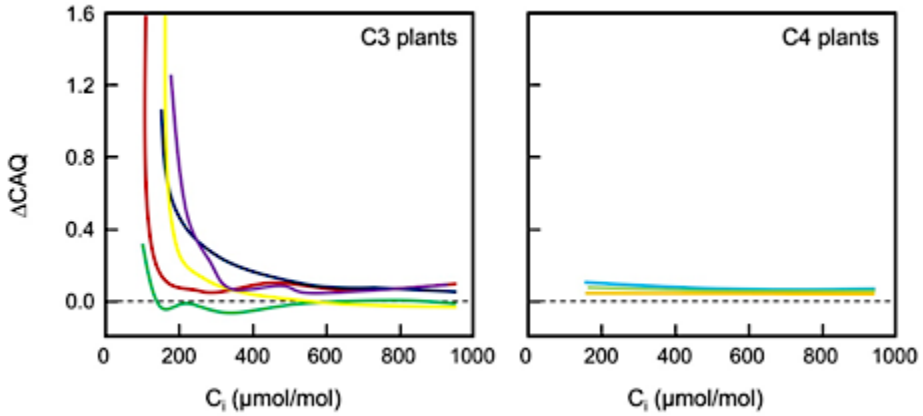


Figure 2

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.

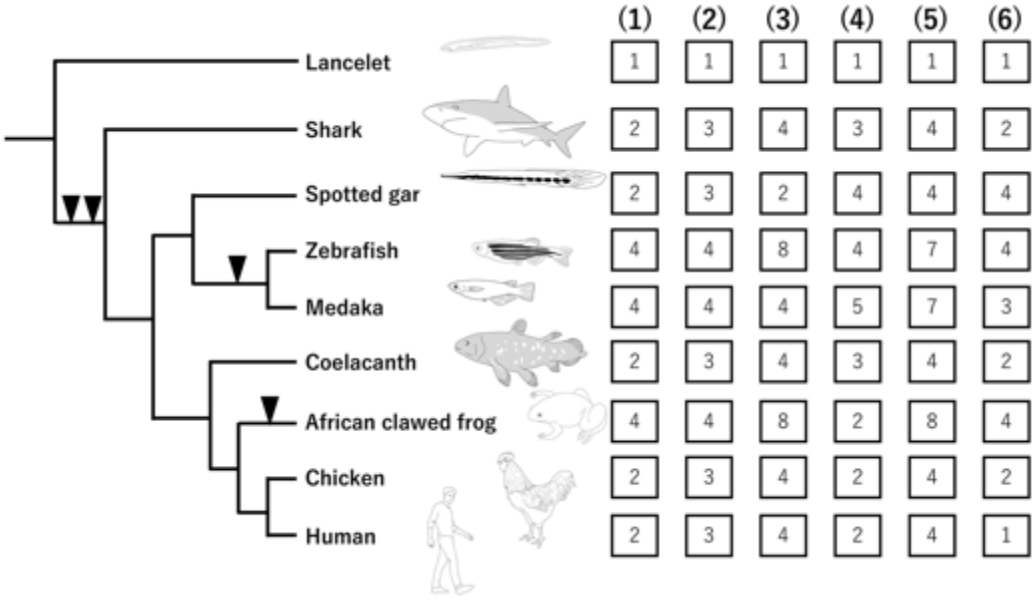


Figure 1. (left) The phylogenetic tree of a broad range of vertebrates and lancelet (an ancestor of vertebrates). (right) The answer options for the number of Hox gene clusters in each species from (1) to (6).

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.

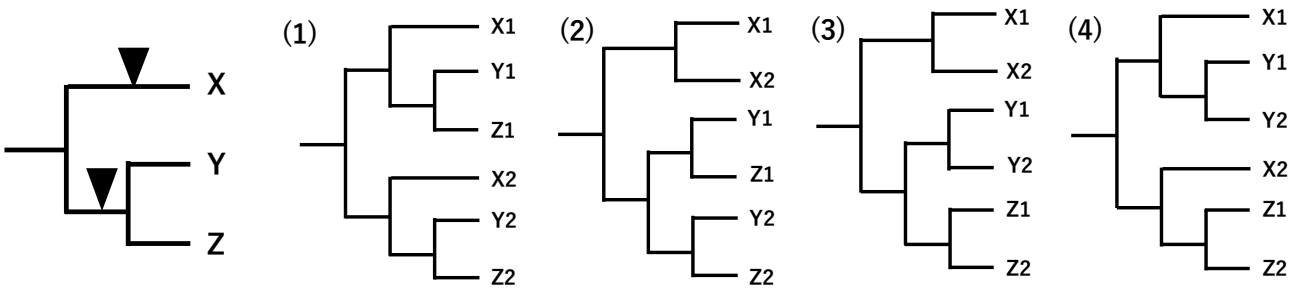


Figure 2 (left) Phylogenetic tree of species X, Y, and Z. (right) The answer options from (1) to (4) for the phylogenetic trees of genes X1 to Z2 of species X, Y, and Z.

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

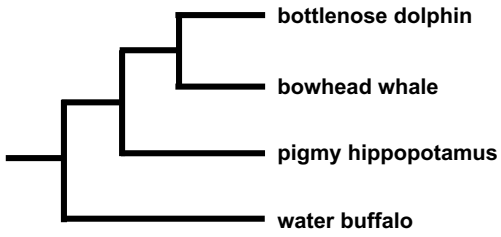
(3) c < b < a

(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?

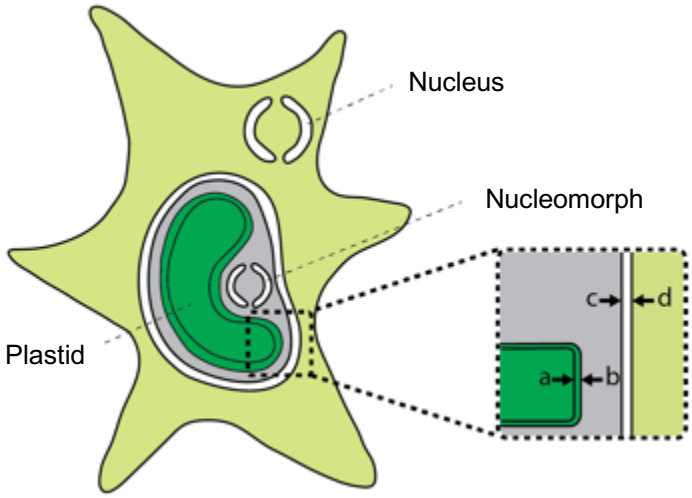


Figure 1. Schematic illustration of the ultrastructure of chlorarachniophytes. The box area is magnified to show that the plastid is bound by four membranes.

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.

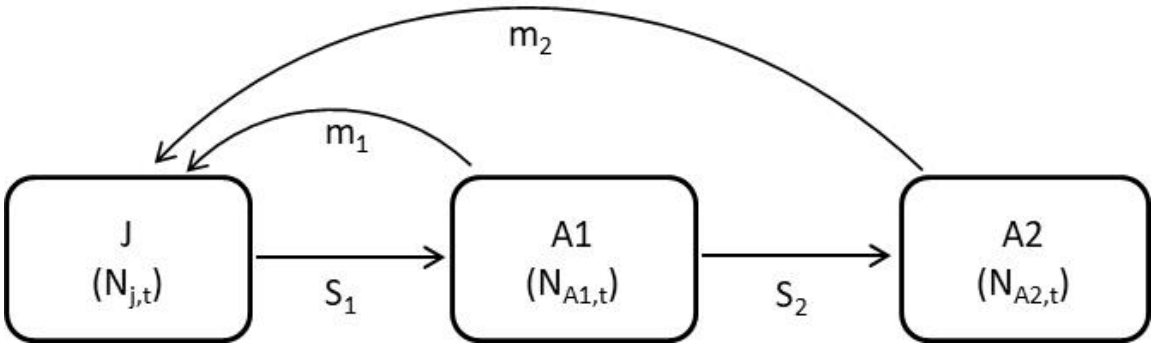
(4) (a) cyanobacterium; (b) cyanobacterium; (c) green alga; (d) green alga.

(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)

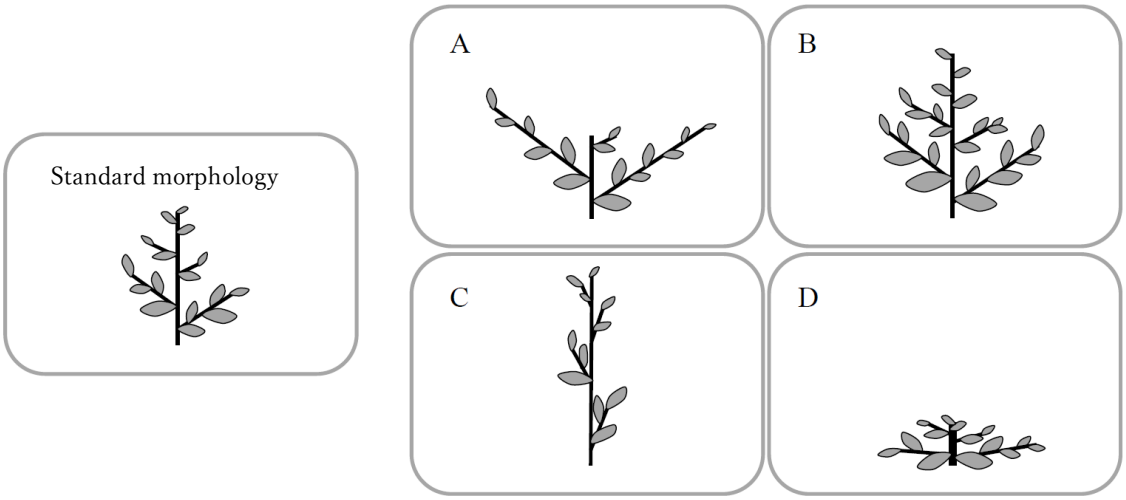
$N_{j,2}$= [114] [115] $N_{A1,2}$= [116] [117] $N_{A2,2}$= [118] [119]

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.

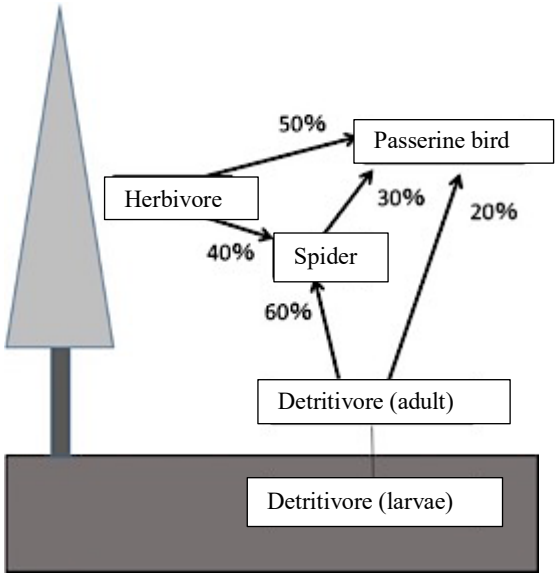


Figure 1

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.

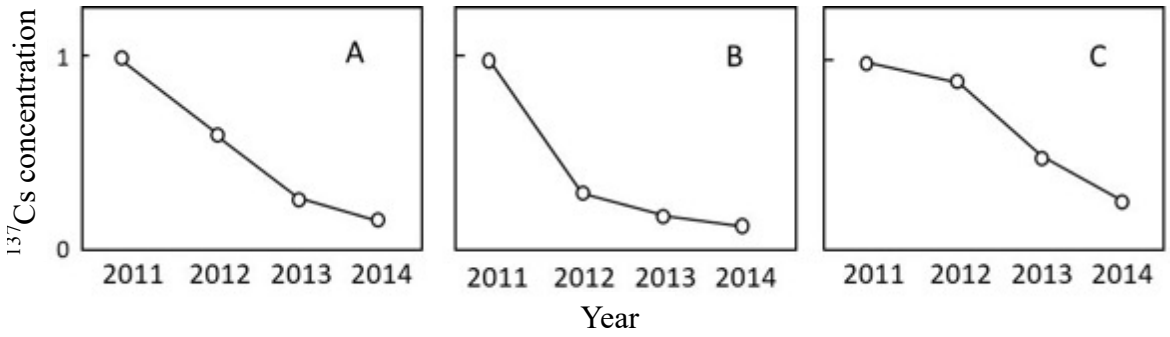


Figure 2 Changes in ^{137}Cs concentration over several years. The concentration represents a relative value, with the value in 2011 being 1.

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)