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.

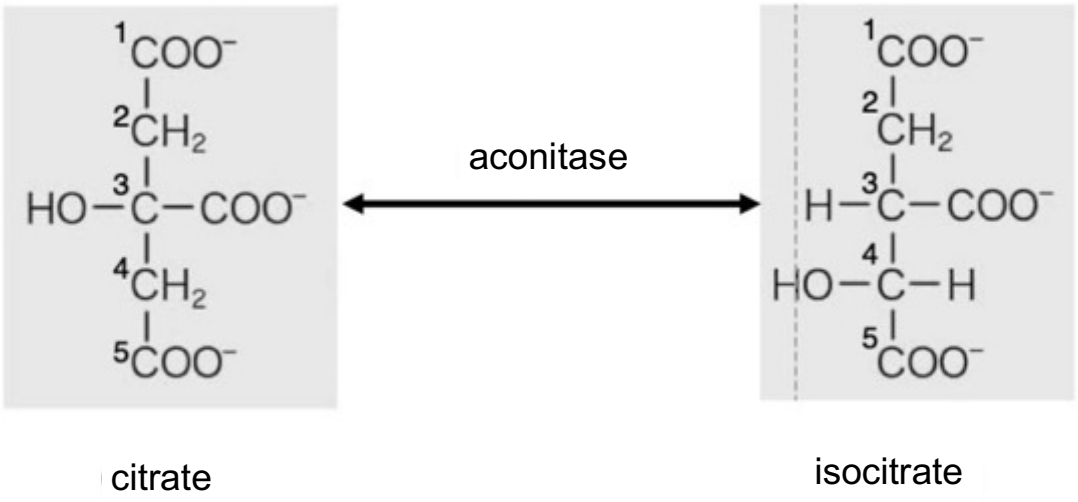


Figure 1

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.

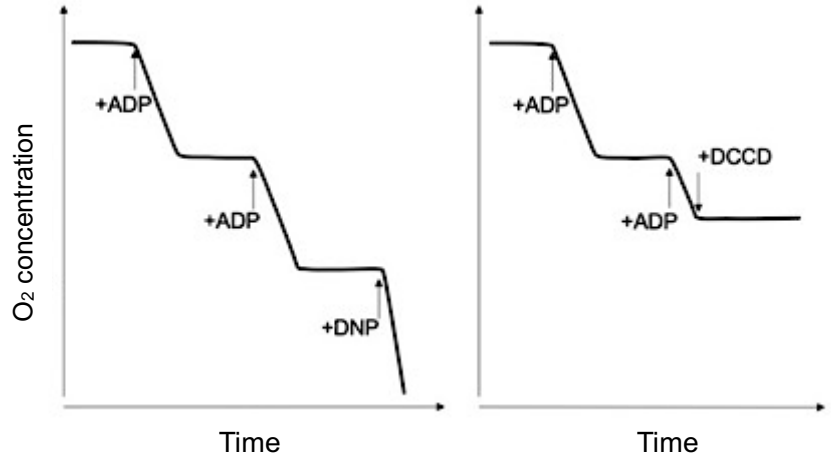


Figure 1. Oxygen consumption of mitochondria in suspension. Identical aliquots of ADP were added in both experiments.

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

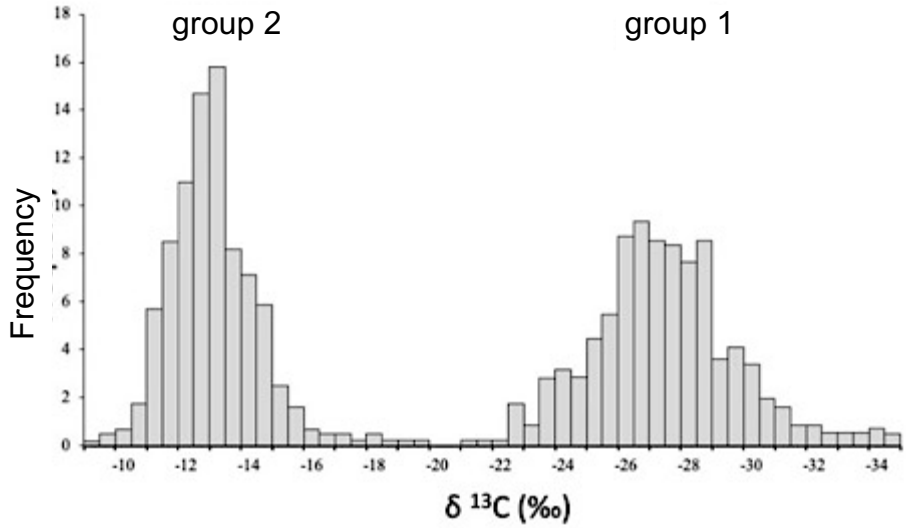


Figure 1 Distribution of the carbon isotope fractionation ($\delta^{13}C$ value) of various plants.

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 $^{24}Na^+$.

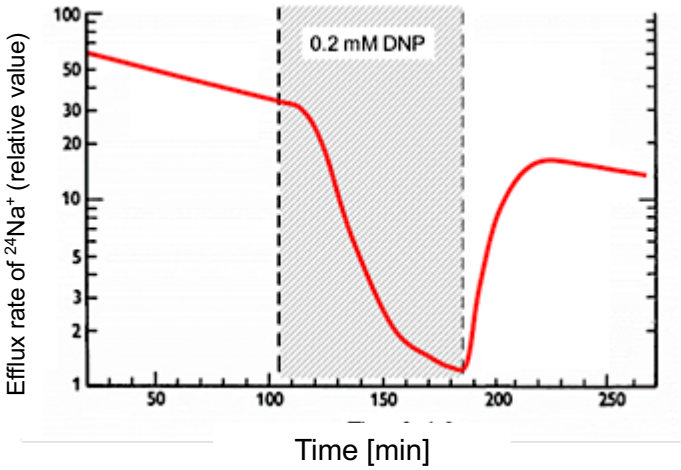


Figure 1 Investigation of the efflux rate of radioisotope ^{24}Na from a squid giant axon to the external solution (seawater). At 0 min, a buffer solution containing $^{24}Na^+$ was injected into the giant axon. For 100-190 min, the external seawater was replaced with a solution (seawater) containing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

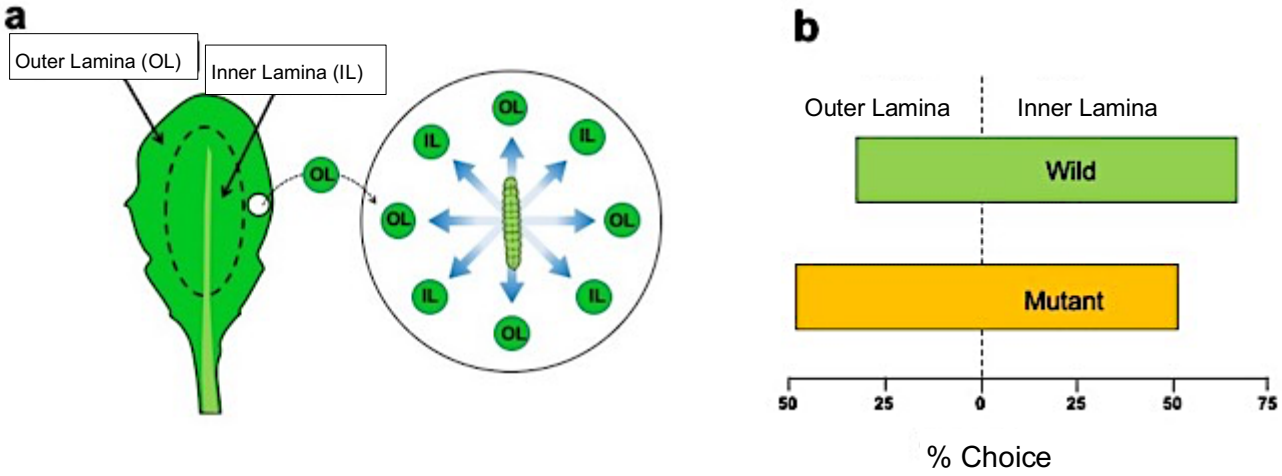


Figure 1 (**a**) Experimental design, (**b**) Result of choice by insect larvae.

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.

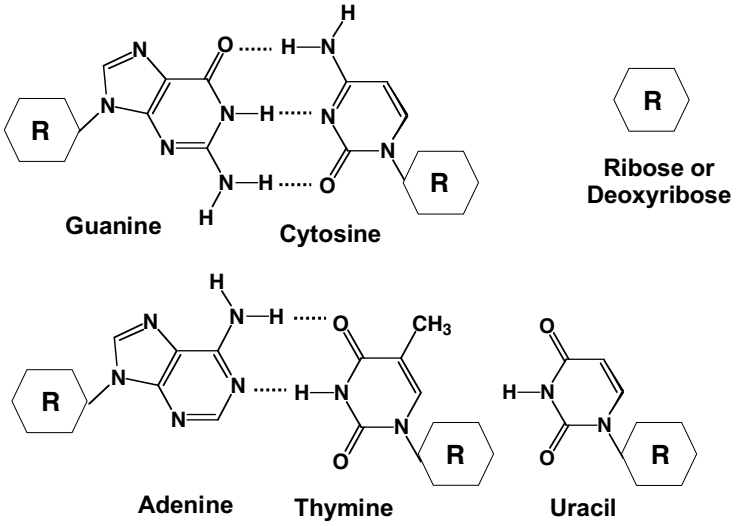


Figure 1

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.

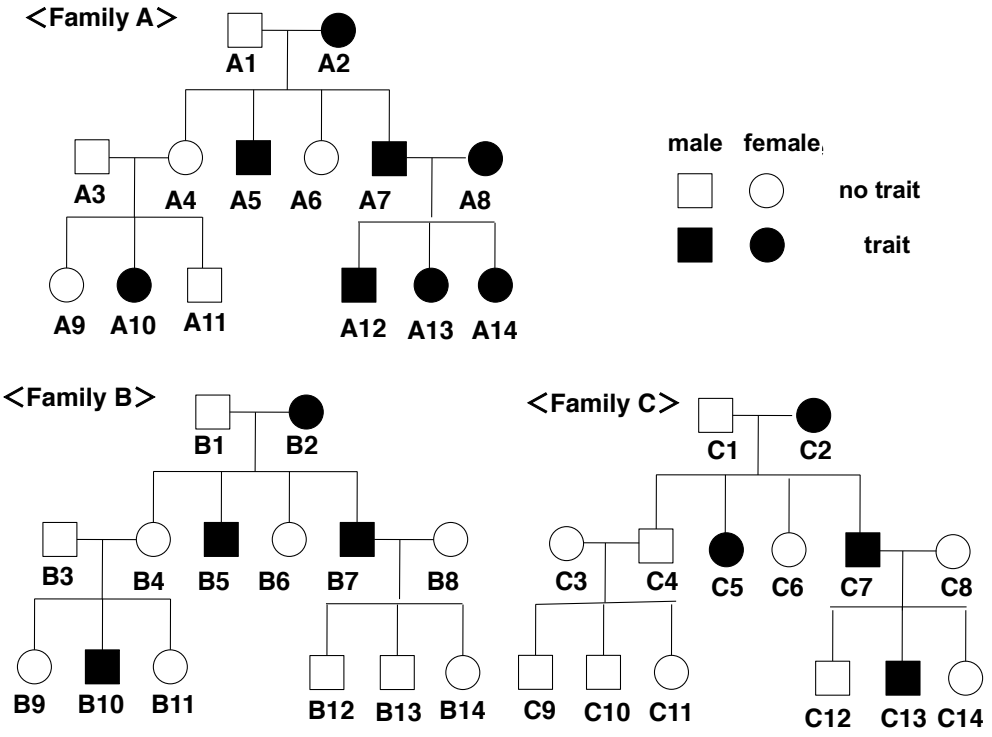


Figure 1

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.

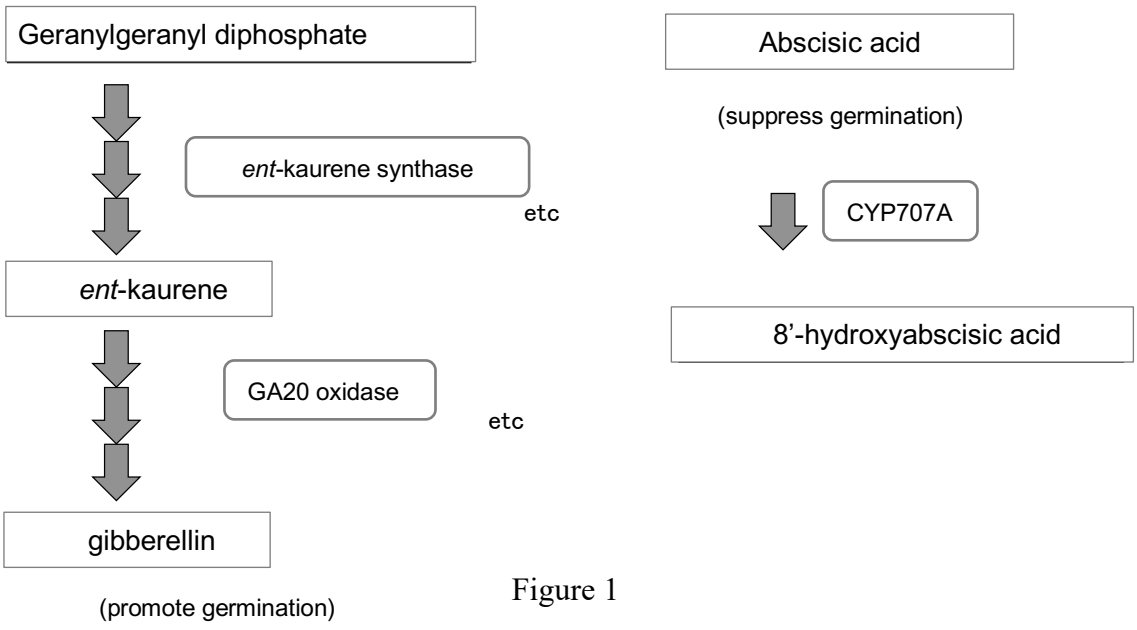


Figure 1

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----,

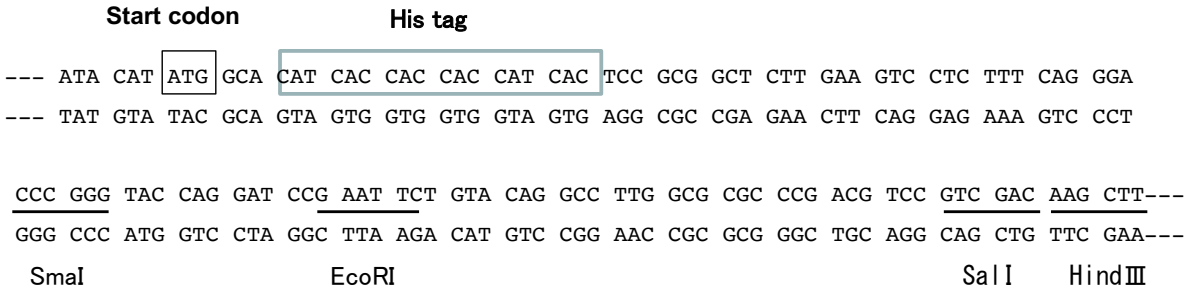


Figure 1. DNA sequence of the cloning region of vector A (double strands).

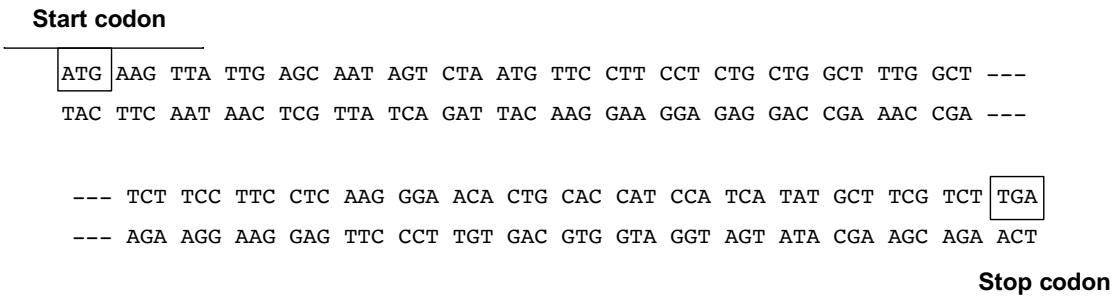


Figure 2. DNA sequence of the gene X showing 5' region and 3' region: 1566 base pair

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

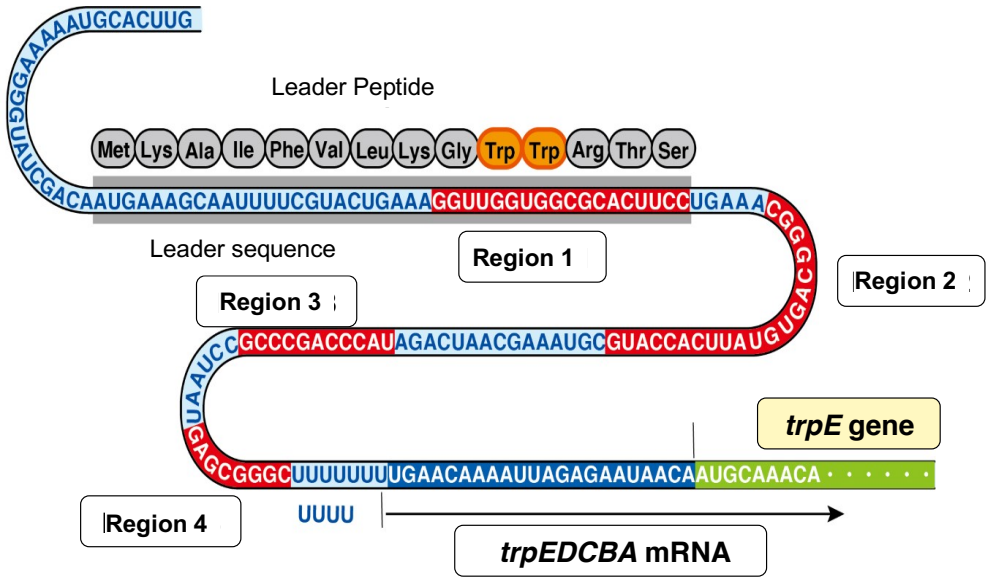
D. It is highly likely that the activity of enzyme A is lost in mutant 4. 67

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）.

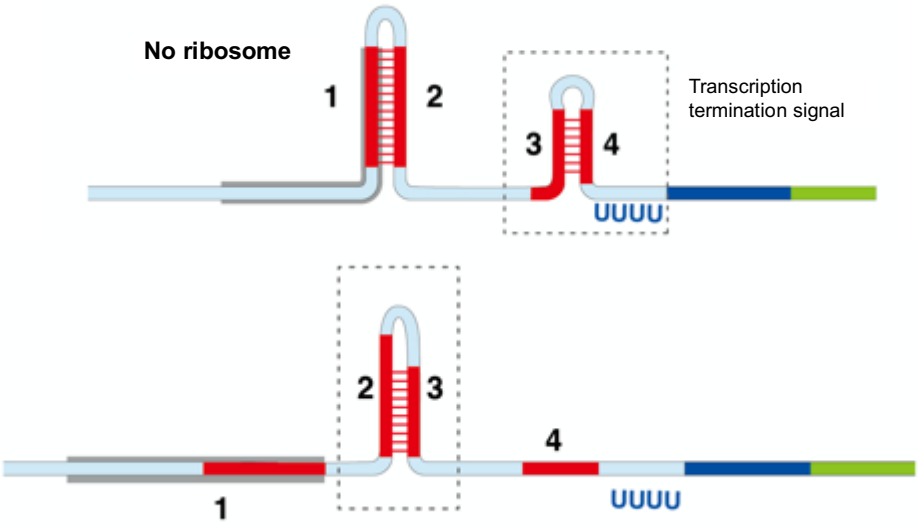


Figure 2

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.

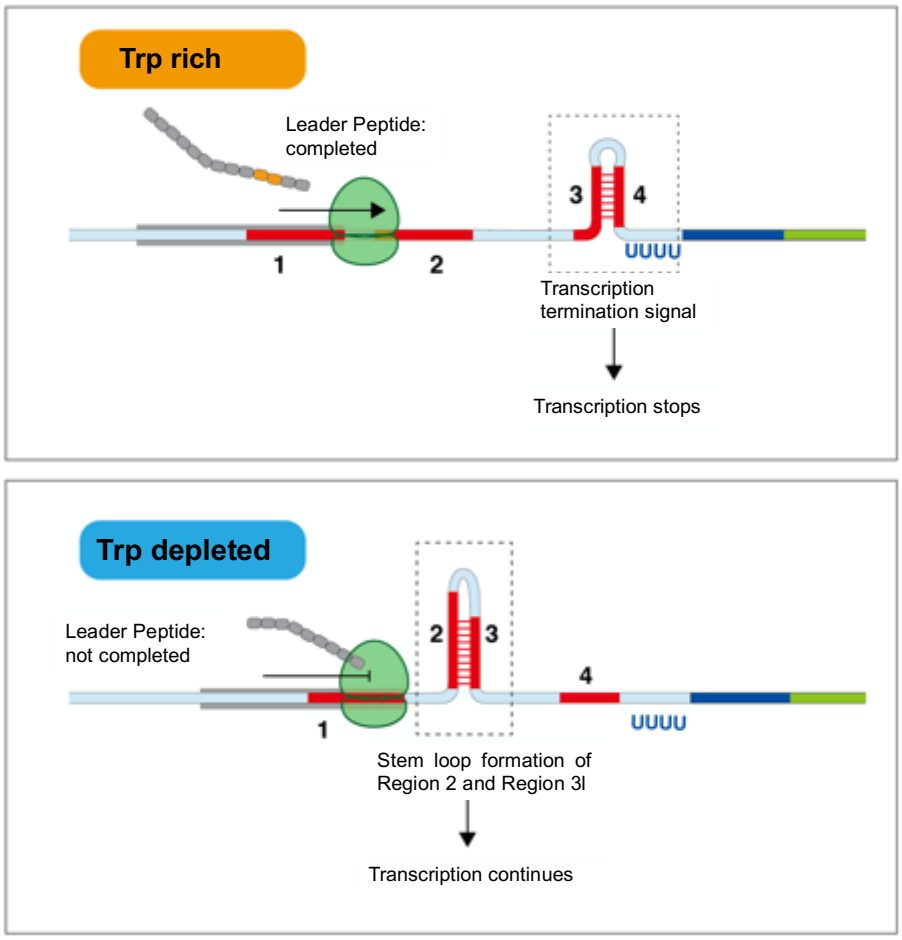


Figure 3

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.

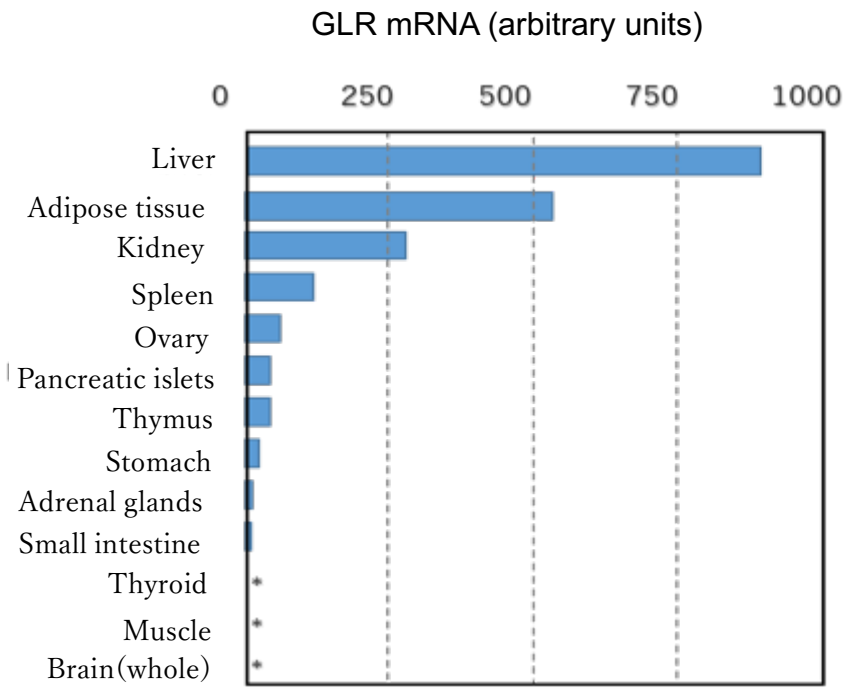


Figure 1 Relative abundance of GLR (glucagon receptor) mRNA in rat tissue. * indicates less than detectable level.

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.

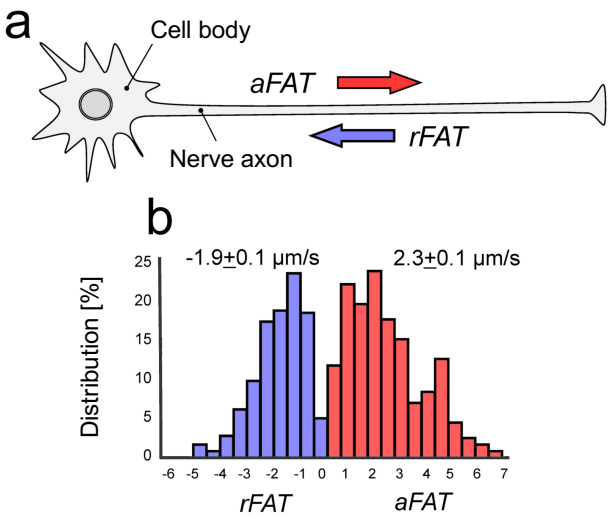


Figure 1 Fast axonal transport (FAT) in nerve cells. **a**, active transportation of synaptic vesicles and mitochondria outward to the nerve ends is called anterograde FAT (*aFAT*). Transportation in the opposite inward direction is called retrograde FAT (*rFAT*). Measured velocity and its distribution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

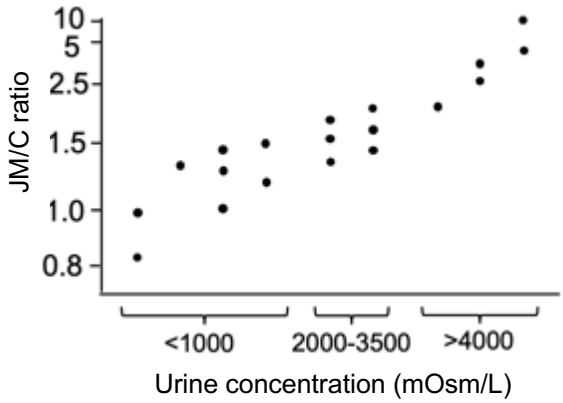
D. ATP produced by glycolysis is crucial for the FAT of mitochondria. 83

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.

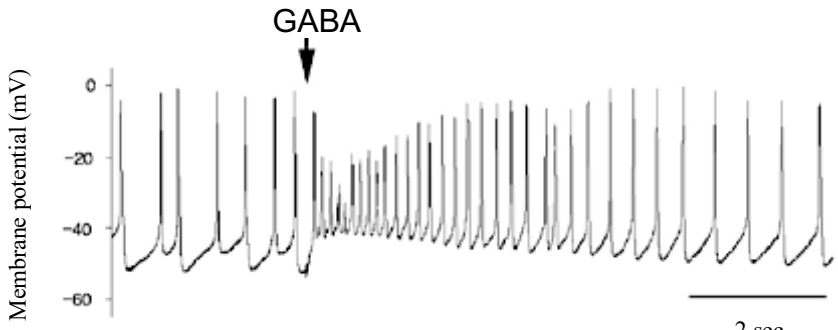


Figure 1

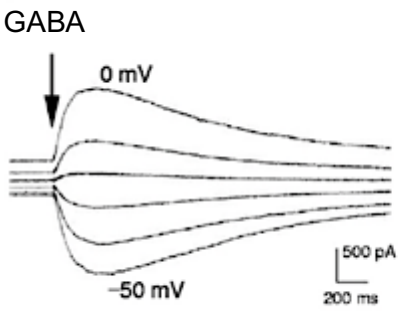


Figure 2

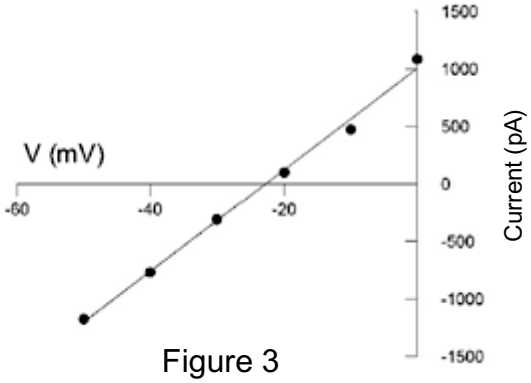


Figure 3

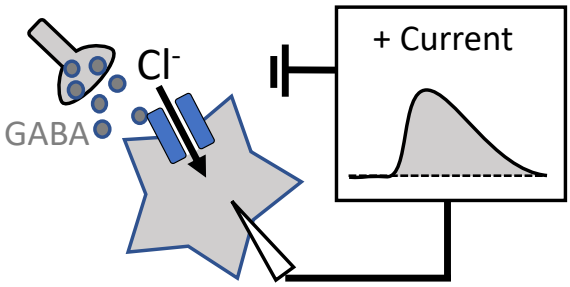


Figure 4

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.

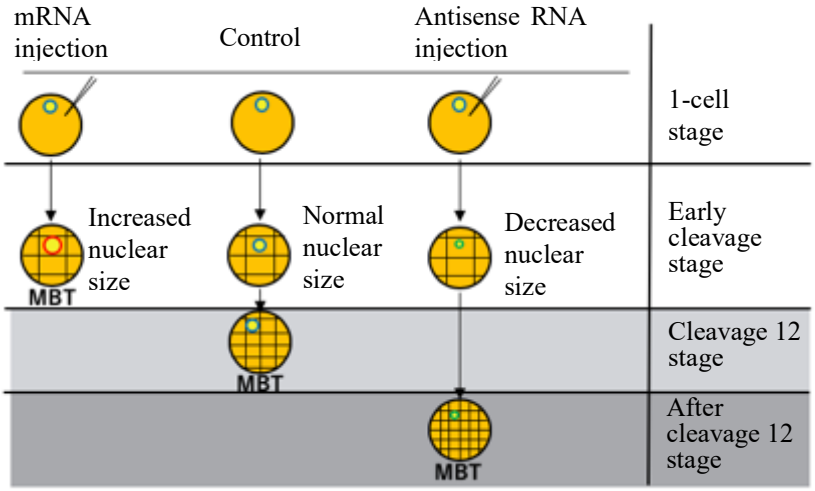


Figure 1

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

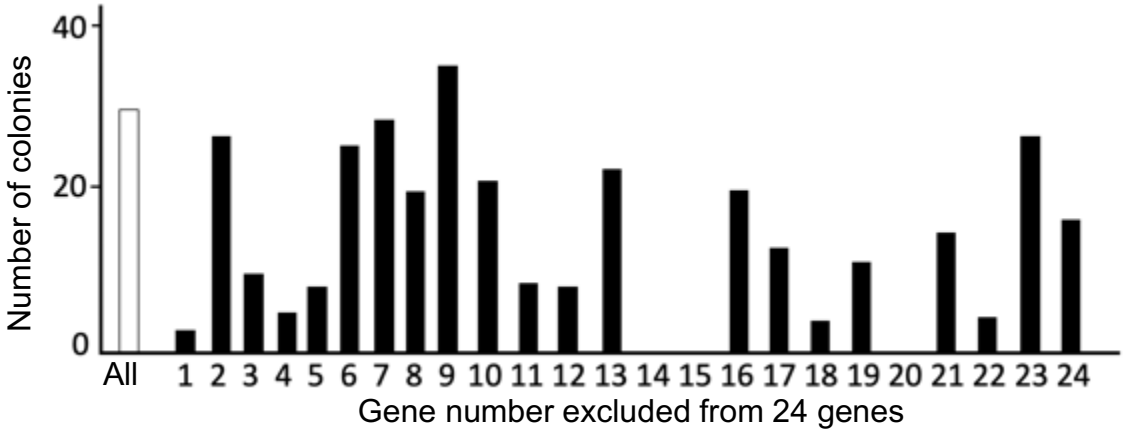
C. When a female Burmese python incubates eggs, her oxygen consumption in a cold room is less than that in warm room. 102

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.

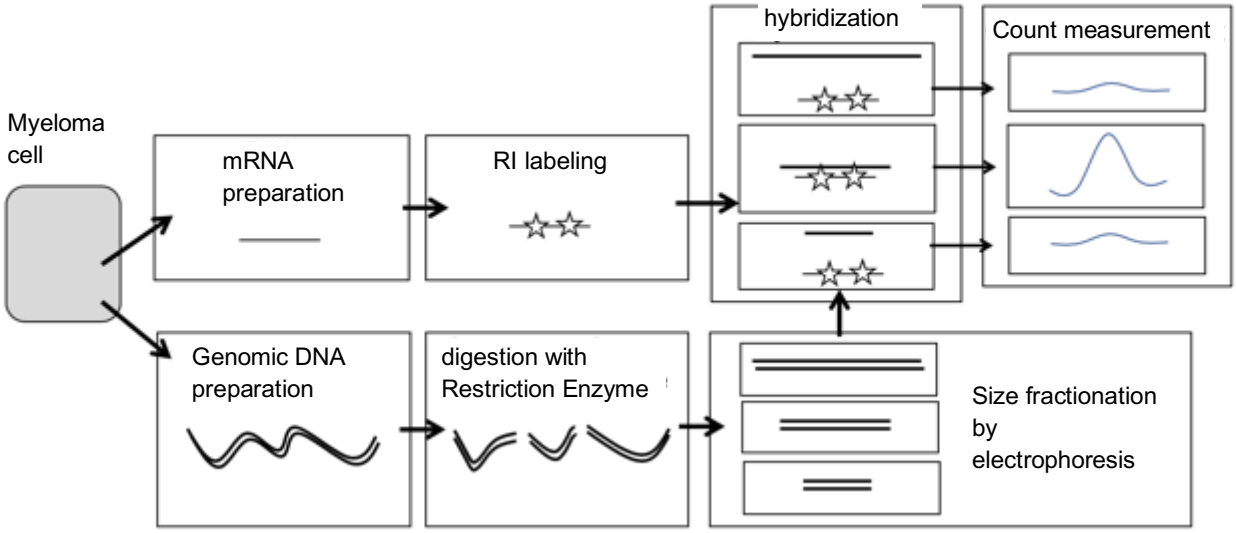


Figure 1

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

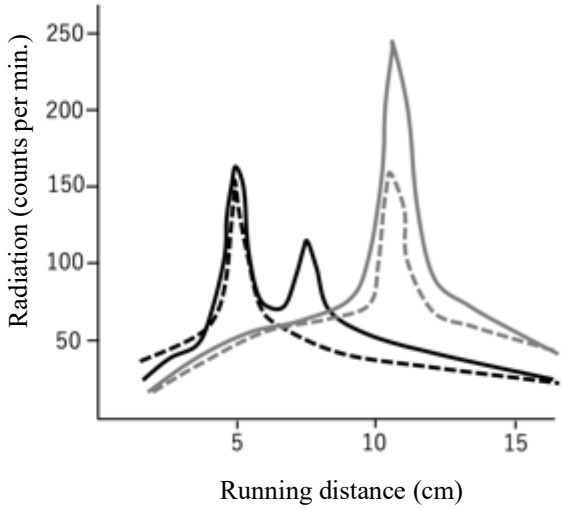


Figure 2

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.

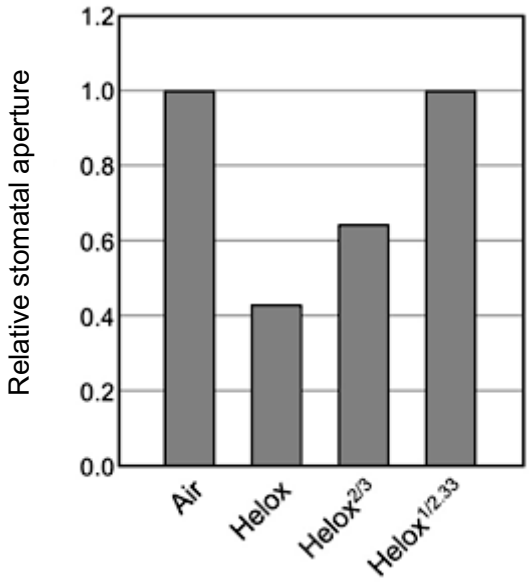


Figure 1 Relative stomatal apertures in various conditions

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.

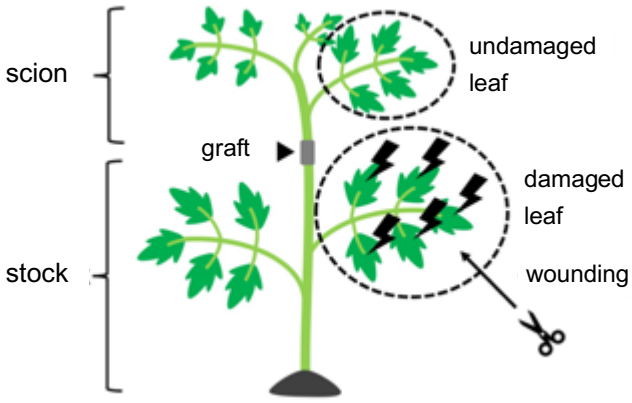


Figure 1. Schematic illustration of graft experiments

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.

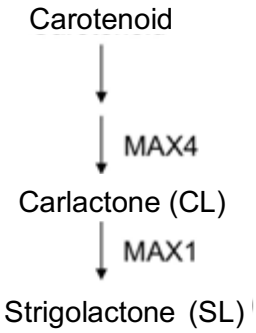


Figure 1.
Biosynthetic pathway of strigolactone

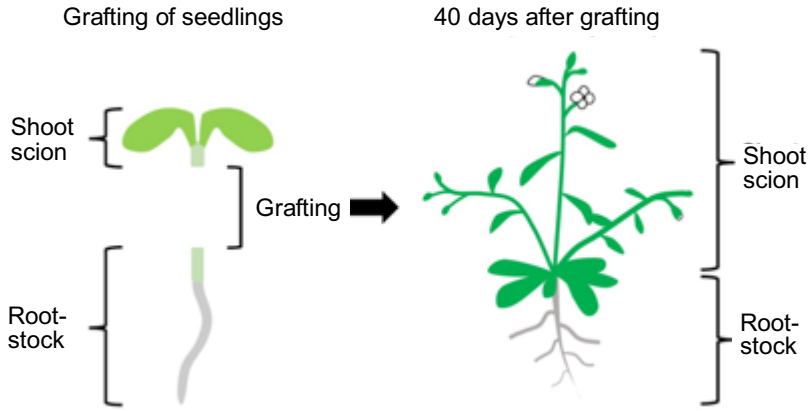


Figure 2.
Schematic illustration of grafting experiments

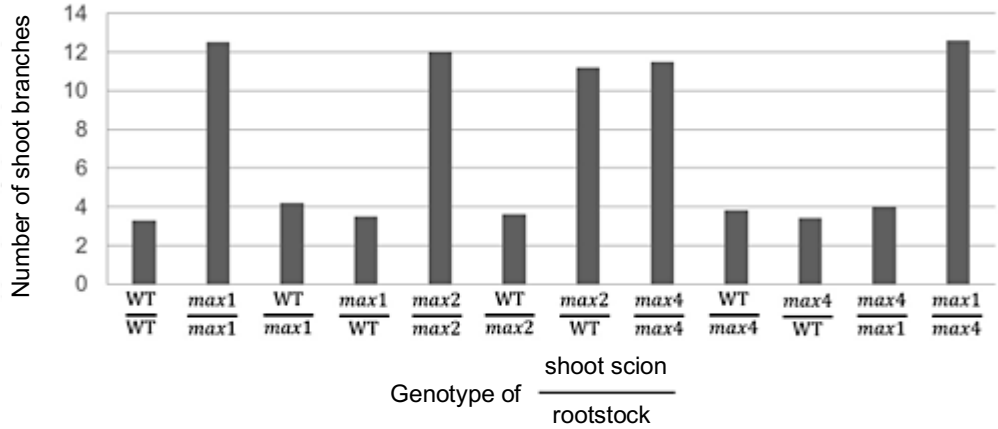


Figure 3. Number of shoot branches in the grafted plants

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+}.

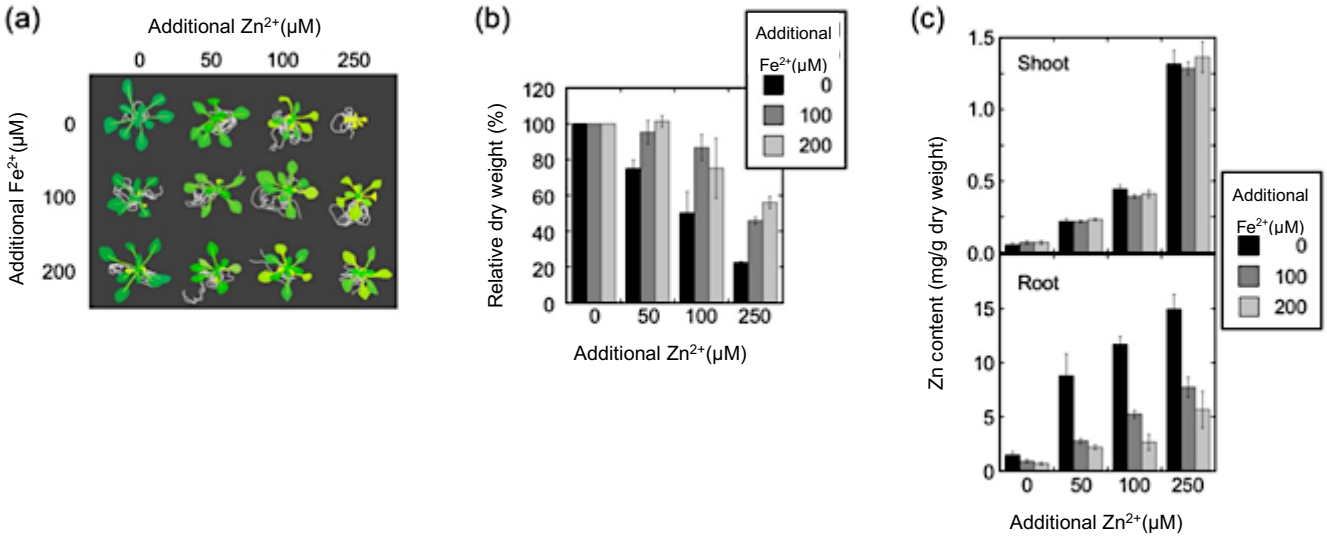


Figure 1. Effects of additional Zn and Fe ions on plant growth

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.

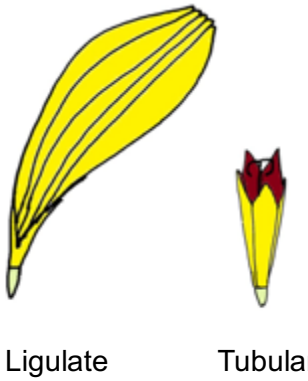


Figure 1 Ligulate and tubular florets of sunflower

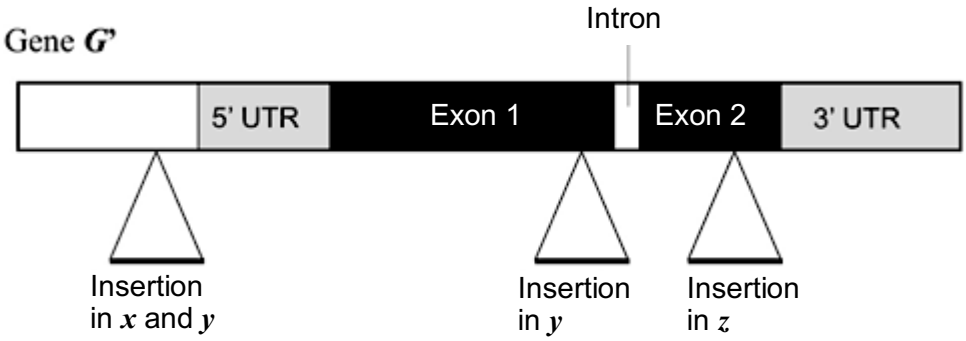


Figure 2 DNA insertions in gene ***G'*** in the sunflower variants

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.

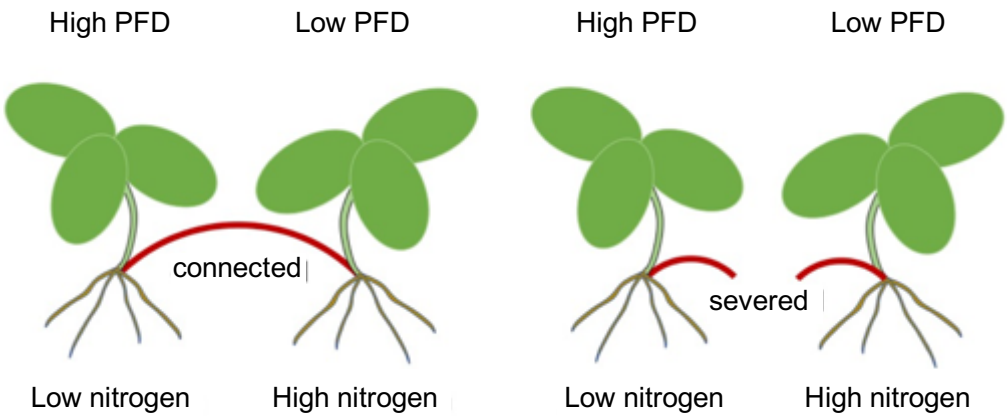


Figure 1 Schematic cartoon of experimental setup

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*.

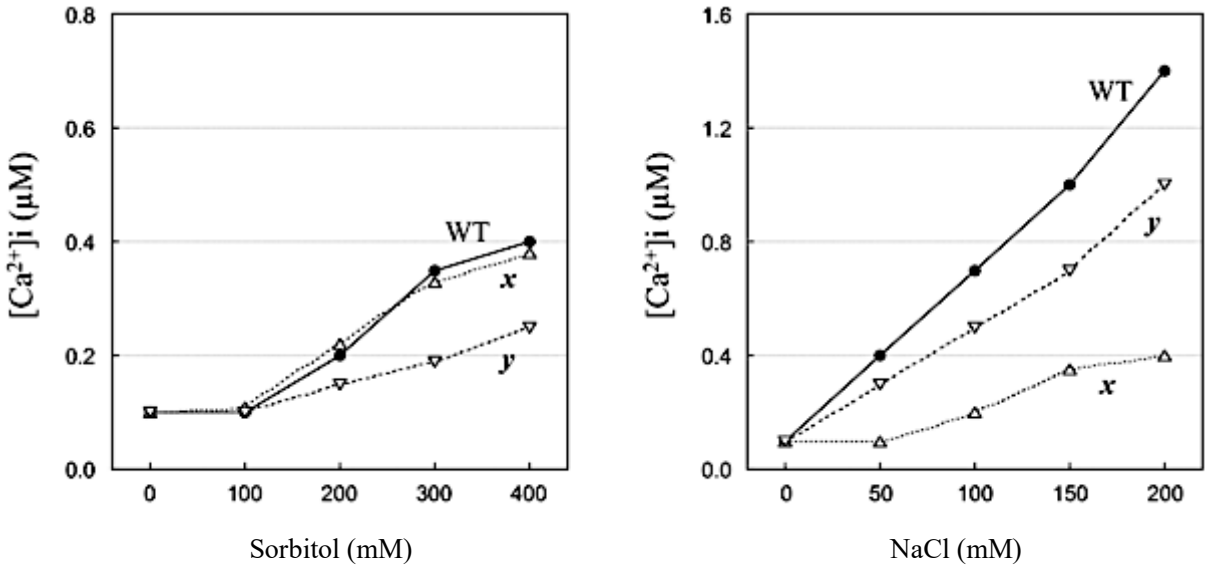


Figure 1

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.

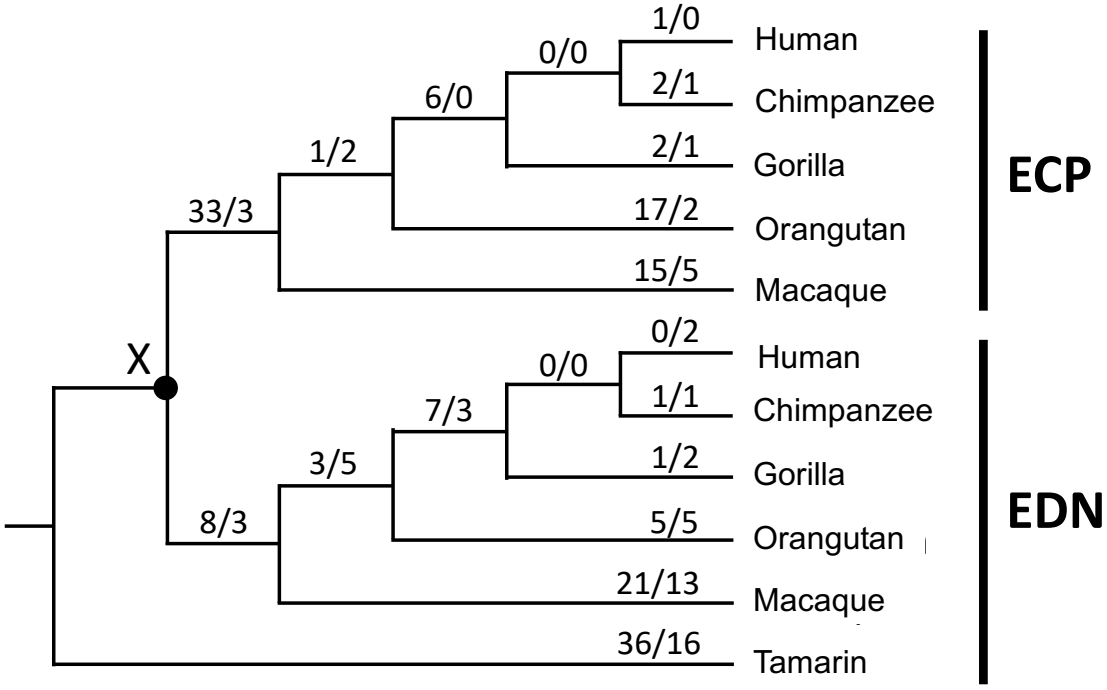


Figure 1. A molecular phylogenetic tree of *ECP* and *EDN* genes in primates based on amino-acid sequences. The numerator and denominator along each branch show the numbers of nonsynonymous and synonymous nucleotide substitutions (substitutions that cause and do not cause amino-acid changes), respectively. 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.

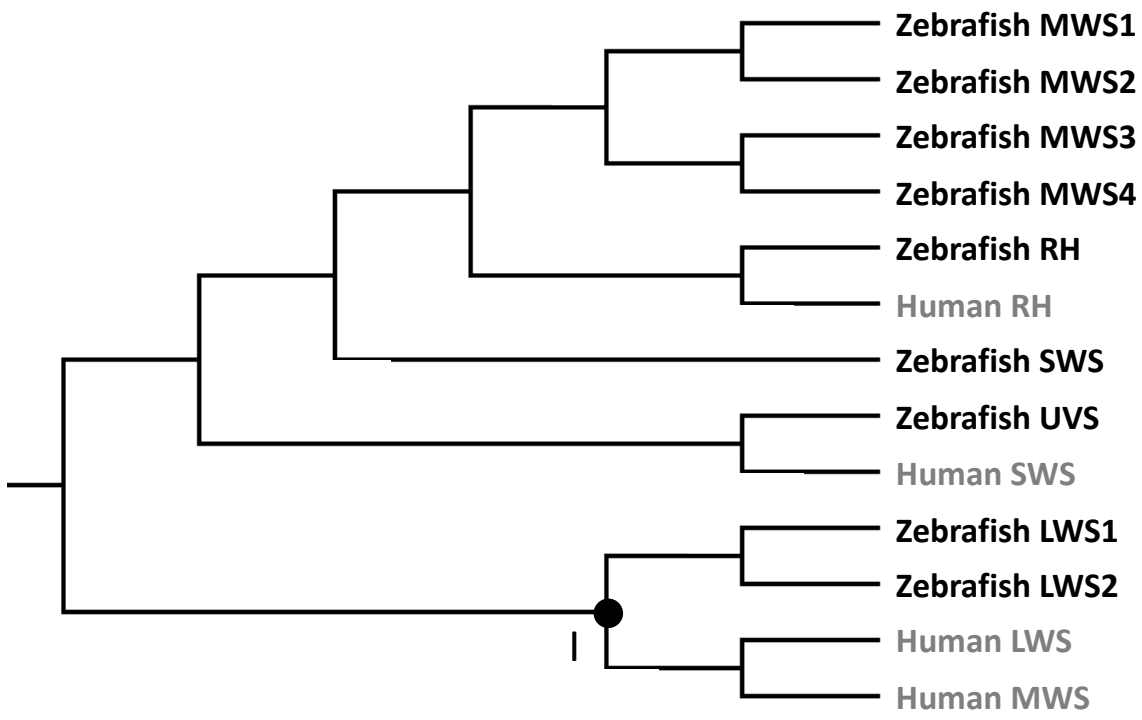


Figure 1. A phylogenetic tree based on the amino-acid sequences of all opsin genes in the human and zebrafish genomes. LWS: Long Wavelength Sensitive opsin, MWS: Middle Wavelength Sensitive opsin, SWS: Short Wavelength Sensitive opsin, UVS: Ultra Violet Sensitive opsin, RH: Rhodopsin type opsin. Branch 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.

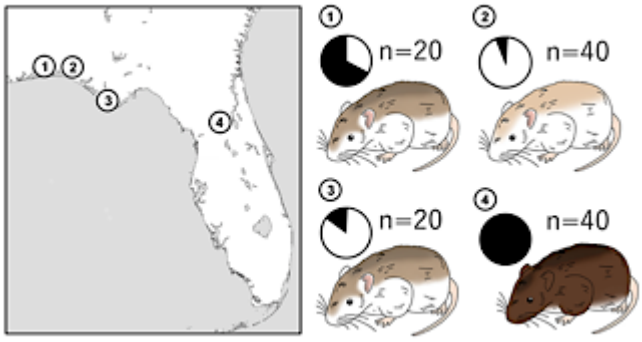


Figure 1

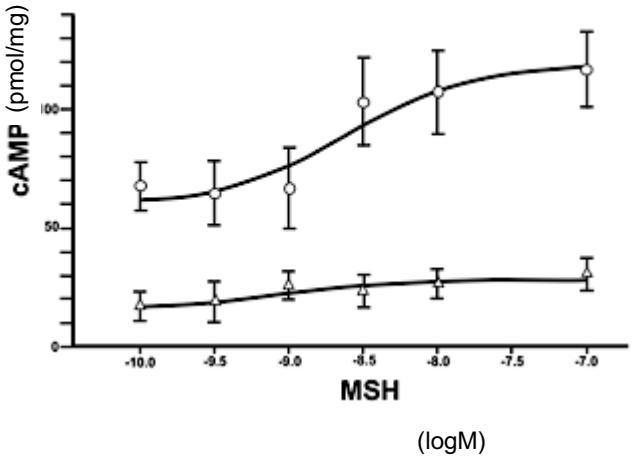


Figure 2

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.

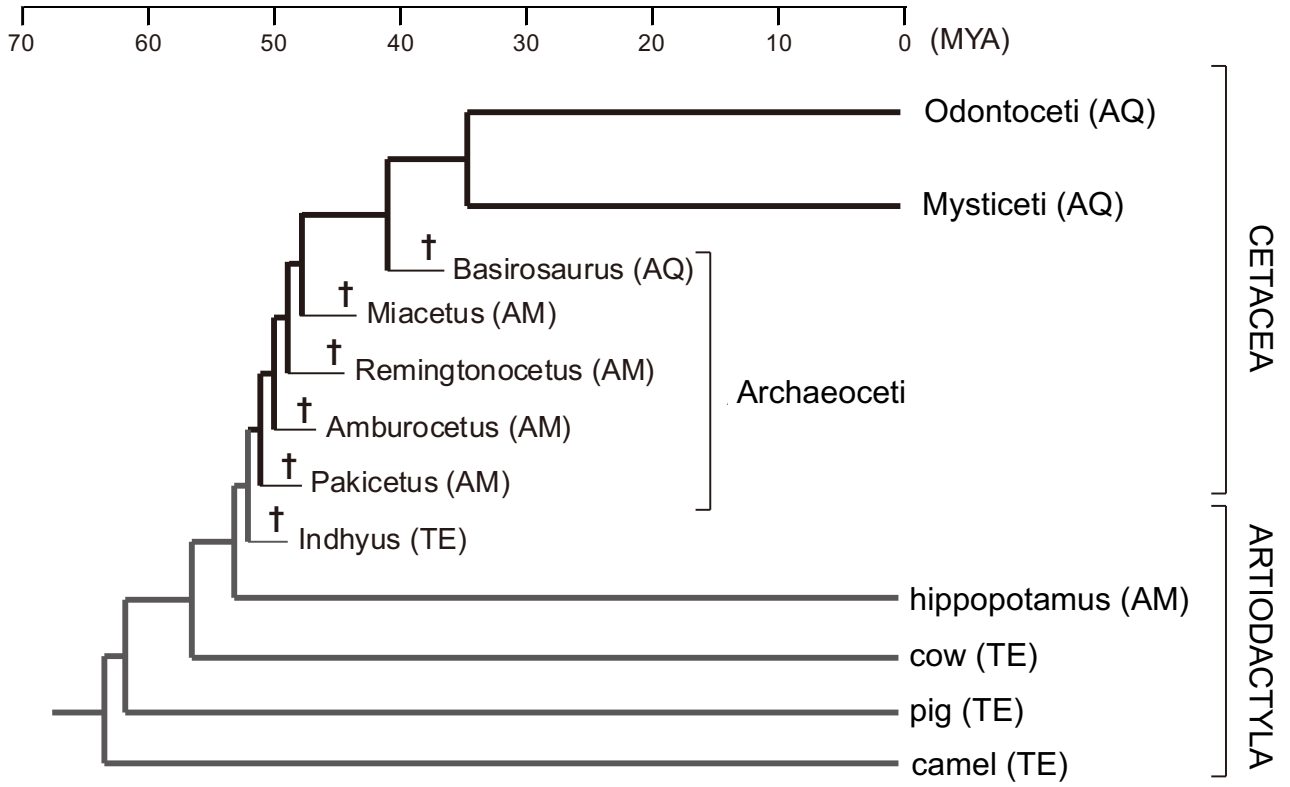


Figure 1. A phylogenetic tree of cetaceans and artiodactyls. Extinct fossil species are shown with †. All extant cetaceans are classified into two subfamilies: Mysticeti (baleen whales, which do not possess enamel-based teeth but possess baleen plates) and Odontoceti (toothed whales, which possess teeth). 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).

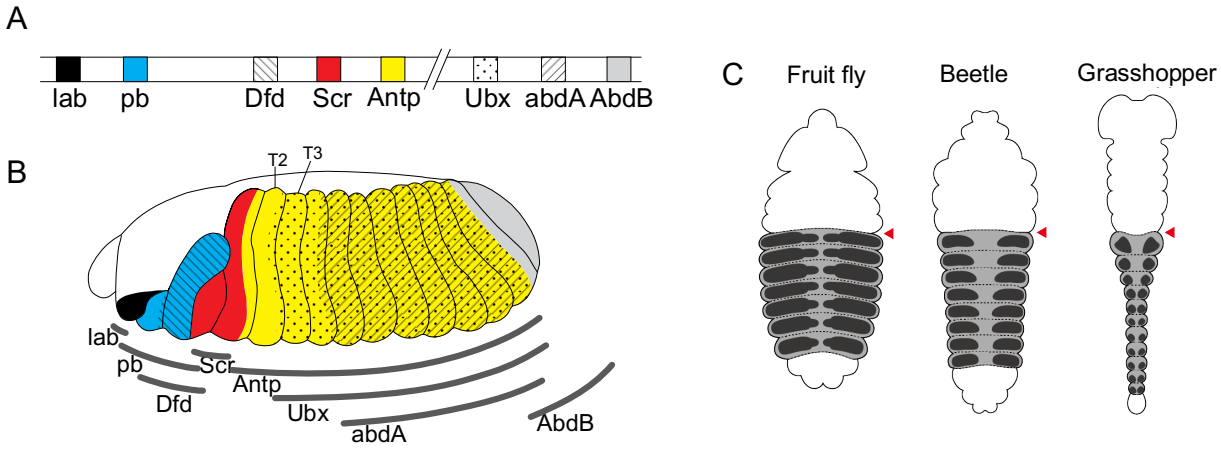


Figure 1. (A) Eight *Hox* genes on the fruit fly genome: *lab*, *pb*, *Dfd*, *Scr*, *Antp*, *Ubx*, *abd-A*, and *Abd-B*. (B) Segmental expression pattern of the *Hox* genes in the fruit fly embryo. Anterior is the left. The expression patterns for each gene are illustrated by labels that correspond to (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).

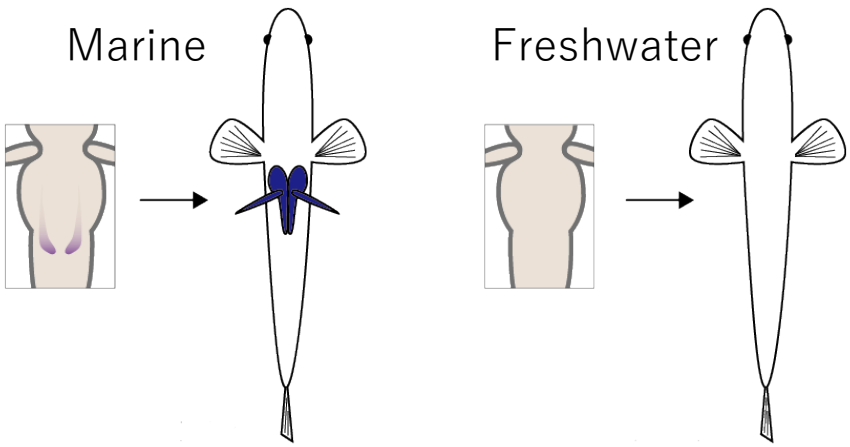


Figure 1. Ventral view of marine (left) and freshwater (right) sticklebacks, showing the presence/absence of pelvic spines (shown by dark blue). Anterior is to up. (Boxes) Magnified ventral view of stickleback embryos showing *Pitx1* expressions in the pelvic fin buds.

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

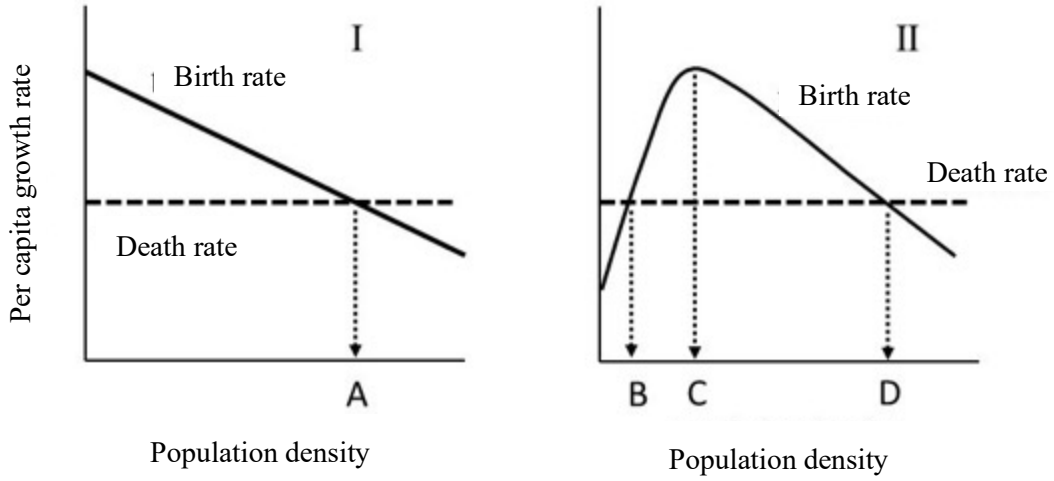
C. The *Pitx1*-knockout individual of the marine population are likely to show similar phenotypes to those of the freshwater population. 177

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

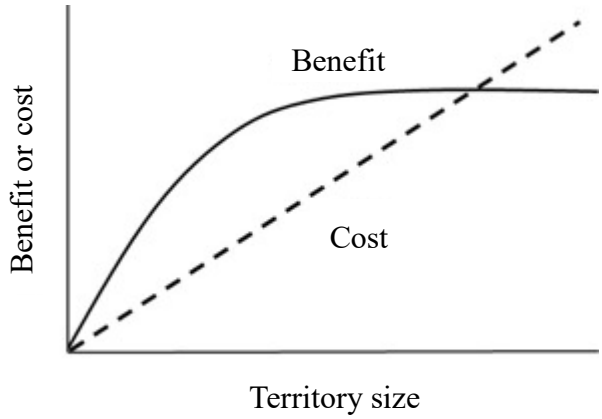
3. The aggregation of individuals is advantageous, rather than detrimental, below the density threshold of C. 181

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

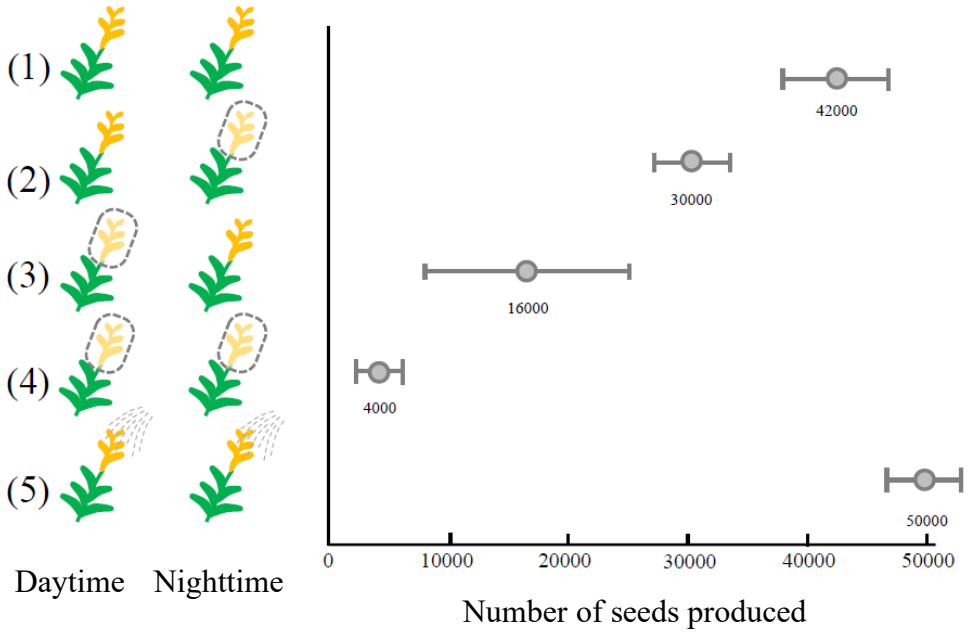
C. When resources become scarce while the cost line is unchanged, the optimal territory size becomes larger. 185

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

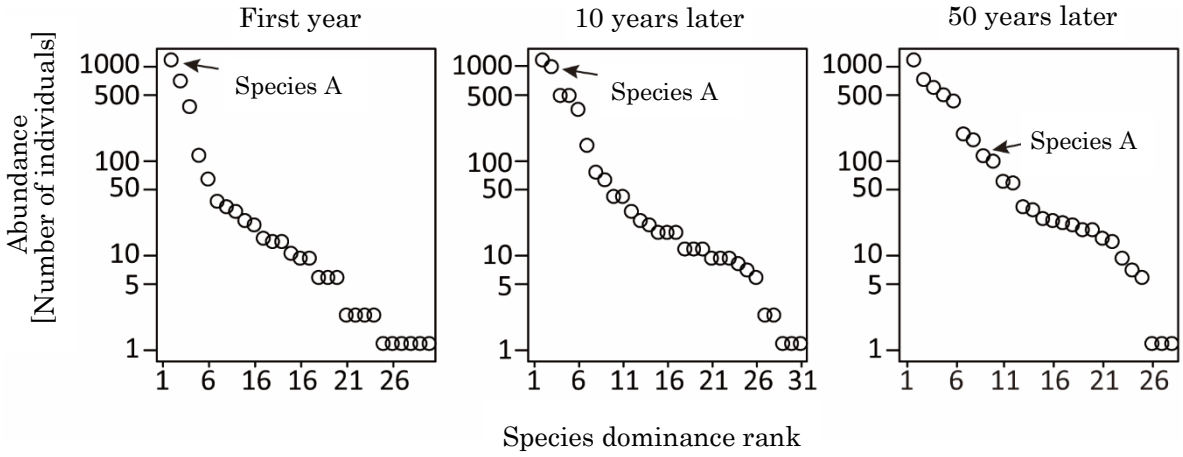
C. Resistant individuals (r/r) are likely to have lower fitness than others (s/r, s/s) in the absence of pesticide application. 193

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

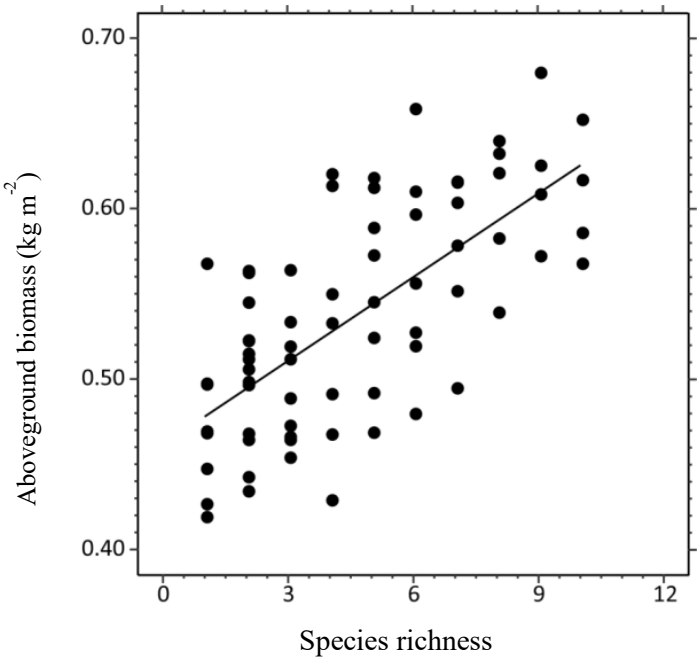
C. The top three species account for more than 75% of the total number of individuals in the first year. 197

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