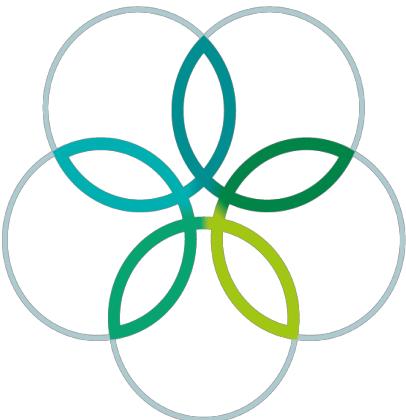
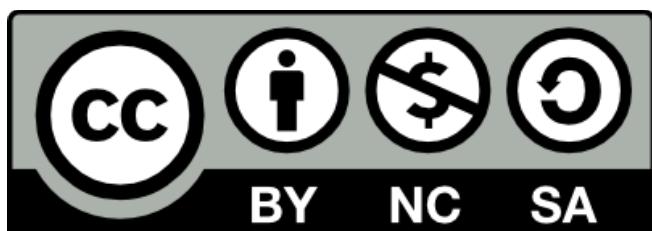


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ANSWER KEYS FOR THEORETICAL TEST

PART A

(The final version)

Mark “✓” for True or “✗” for False statements.

PART A										
Q.	A	B	C	D	Q.	A	B	C	D	
1	✗	✗	✗	✓	26	✓	✗	✗	✓	
2	✗	✗	✓	✓	27	✓	✓	✓	✗	
3	✓	✗	✓	✓	28	✓	✗	✓	✓	
4	✓	✓	✓	✗	29	✗	✓	✓	✗	
5	✗	✓	✗	✗	30	✓	✓	✗	✗	
6	✓	✗	✗	✓	31	✗	✓	✓	✓	
7	✓	✗	✗	✗	32	✗	✗	✗	✓	
8	✗	✗	✓	✓	33	✓	✓	✓	✗	
9	✗	✓	✗	✗	34	✗	✓	✓	✓	
10	✗	✓	✗	✓	35	✗	✗	✓	✗	
11	✗	✗	✗	✓	36	✗	✓	✓	✓	
12	✓	✗	✓	✗	37	✗	✗	✗	✓	
13	✓	✓	✗	✗	38	✗	✓	✓	✓	
14	✓	✗	✓	✗	39	✓	✓	✗	✓	
15	✗	✓	✗	✓	40	✓	✓	✓	✗	
16	✗	✗	✗	✗	41	✓	✓	✗	✓	
17	✓	✓	✗	✓	42	✓	✓	✗	✓	
18	✗	✓	✓	✓	43	✗	✓	✓	✗	
19	✓	✗	✓	✓	44	✓	✓	✗	✗	
20	✓	✗	✓	✓	45	✗	✗	✗	✓	
21	✗	✓	✓	✗	46	✗	✓	✓	✓	
22	✓	✓	✗	✗	47	✓	✗	✓	✓	
23	✓	✗	✓	✗	48	✗	✓	✗	✓	
24	✓	✗	✓	✓	49	✓	✓	✓	✗	
25	✓	✓	✗	✓	50	✗	✓	✓	✗	

Country:

Student Code:

27th International Biology Olympiad

17th-23rd July, 2016
Hanoi, Vietnam



THEORETICAL TEST

PART A

Total points: 50 points

Duration: 180 minutes

Dear Participants,

- Please write your **student code** in the given box.
- Write down your answers using a pen in the **Answer Sheet**. Only answers given in the **Answer Sheet will be evaluated**.
- Part A consists of 50 questions:
 - Q1-Q10: Cell Biology
 - Q11-Q17: Plant Anatomy and Physiology
 - Q18-Q30: Animal Anatomy and Physiology
 - Q31-Q32: Ethology
 - Q33-Q42: Genetics and Evolution
 - Q43-Q47: Ecology
 - Q48-Q50: Biosystematics
- There are two types of questions: True/False multiple choice questions and gap filling questions.
 - For each True/False multiple choice question, there are four statements. Mark “√” for True statements and “×” for False statements in the Answer Sheet. If you need to change an answer, you should strikethrough the wrong answer and write in the new one. See the example below:

	Statement	True	False
A		✗	×
B			✗
C			✗
D		✓	

- For each gap filling question, there are four designated spaces to fill in numbers or codes.
- Scoring for one question:
 - If all four answers are correct, you will receive 1 point.
 - If only three answers are correct, you will receive 0.6 point.
 - If only two answers are correct, you will receive 0.2 point.
 - If only one answer is correct, you will not receive any points (0).
- You can use the ruler and the calculator provided.
- Stop answering and put down your pen immediately when the bell rings at the end of the exam. Enclose the **Answer Sheet** and **Question Paper** in the provided envelope.

Good luck!!!

CELL BIOLOGY

Q.1

The activities of Wee1 kinase and Cdc25 phosphatase determine the state of phosphorylation of tyrosine 15 in the Cdk1 component of M-Cdk. When tyrosine 15 is phosphorylated, M-Cdk is inactive; when tyrosine 15 is not phosphorylated, M-Cdk is active (**Figure Q.1A**). The activities of Wee1 kinase and Cdc25 phosphatase are also controlled by phosphorylation.

The regulation of these activities can be studied in extracts of frog oocytes. In such extracts, Wee1 kinase is active and Cdc25 phosphatase is inactive. As a result, M-Cdk is inactive because its Cdk1 component is phosphorylated on tyrosine 15. M-Cdk in these extracts can be rapidly activated by addition of okadaic acid, which is a potent inhibitor of serine/threonine protein phosphatases. Using antibodies specific for Cdk1, Wee1 kinase, and Cdc25 phosphatase, it is possible to examine their phosphorylation states by changes in mobility upon gel electrophoresis (**Figure Q.1B**). Phosphorylated forms of these proteins generally migrate more slowly than their nonphosphorylated counterparts.

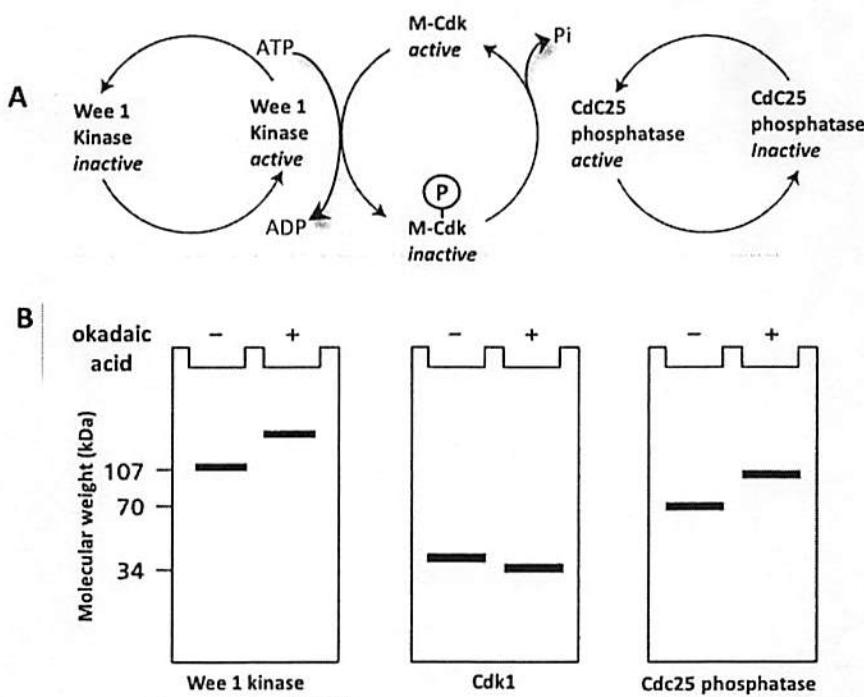


Fig.Q.1. (A) Control of M-Cdk activity by Wee1 kinase and Cdc25 phosphatase;
 (B) Effects of okadaic acid on the phosphorylation states of Cdk1, Wee1 kinase, and Cdc25 phosphatase

Indicate in the **Answer Sheet** if each of the following statements is True or False.

- A. Wee1 kinase is active if it is phosphorylated.
- B. The protein kinases and phosphatases that control the phosphorylation of Wee1 kinase and Cdc25 phosphatase are specific for tyrosine side chains.
- C. Okadaic acid directly affects the activation of Cdk1.
- D. If M-Cdk is able to phosphorylate Wee1 kinase and Cdc25 phosphatase, a small amount of active M-Cdk would lead to its rapid and complete activation.

Answer key:

- A. False; B. False, C. False, D. True

Explanation:

- A. False.** In the absence of okadaic acid, Wee1 kinase is active. As can be seen from Figure Q1B, Wee1 kinase move faster in the absence of okadaic acid => Wee1 kinase is active if it is not phosphorylated .
- B. False.** The protein kinases and phosphatases that control phosphorylation of Wee1 kinase and Cdc25 phosphatase must be specific for serine/threonine side chains because they are affected by okadaic acid, which inhibits only serine/threonine phosphatases.
- C. False.** Okadaic indirectly affect the activation of Cdk1 by controlling Wee1 kinase and Cdc25 phosphatase. Okadaic acid has no direct effect on Cdk1 phosphorylation because it is phosphorylated on a tyrosine side chain. Tyrosine phosphatases are unaffected by okadaic acid.
- D. True.** Some active M-Cdk phosphorylate Wee1 kinase and Cdc25 phosphatase, inactivating the kinase and activating the phosphatase. The resulted decrease in Wee1 kinase activity and increase in Cdc25 phosphatase activity would lead to dephosphorylation (and activation) of more M-Cdk.

Reference: *Molecular Biology of the cell*. B. Alberts et al

Q.2

The translational rate of an mRNA can be estimated from sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). In this experiment, a tobacco mosaic virus (TMV) mRNA, that encodes a 116 kDa protein, was translated in a rabbit reticulocyte lysate in the presence of ^{35}S -methionine. The lysate contained all the components of rabbit reticulocyte translational machinery. Samples were removed at 1-minute intervals and subjected to SDS-PAGE. The separated translation products were visualized by autoradiography. As can be seen in the figure below, the polypeptides get larger with time, until the full-length protein appears at about 25 minutes.

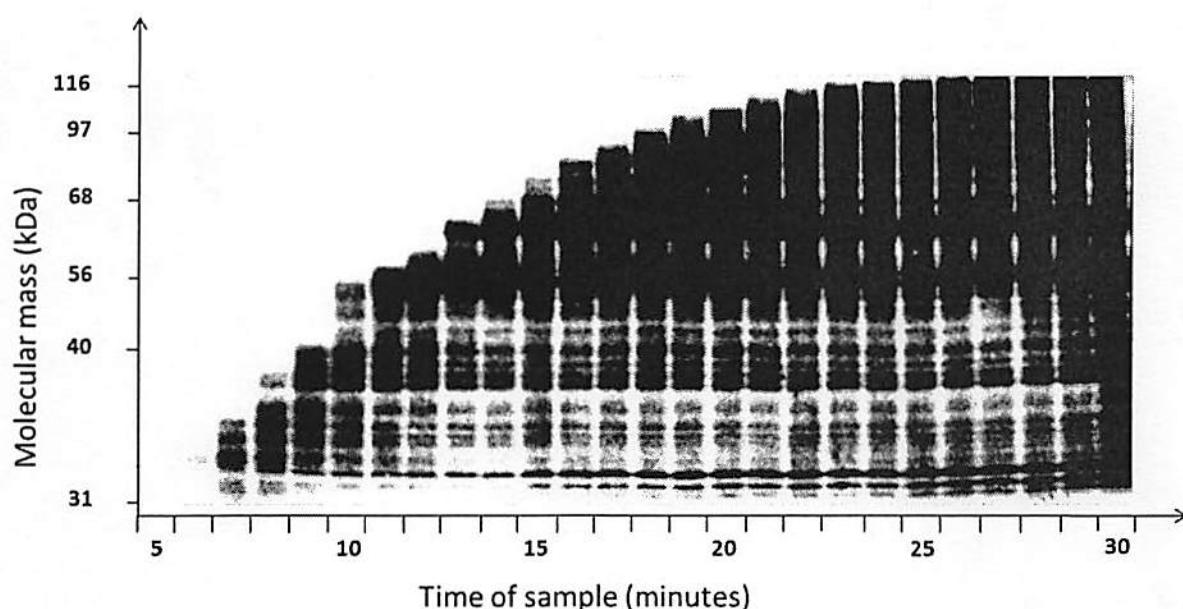


Fig.Q.2. Time course of synthesis of a TMV protein in a rabbit-reticulocyte lysate.

Indicate in the **Answer Sheet** if each of the following statements is True or False.

- The rate of TMV protein synthesis is exponentially proportional to time.
- With an average molecular mass of an amino acid of 110 daltons, the rate of protein synthesis is approximately 35 to 40 amino acids per minute.
- The speed of ribosome movement along the mRNA is constant.
- The mRNA may contain more than two rare codons in its sequence.

Answer key: A. False; B. False, C. False, D. True

Explanation:

A. False. The rate of the protein synthesis is nonlinear with time

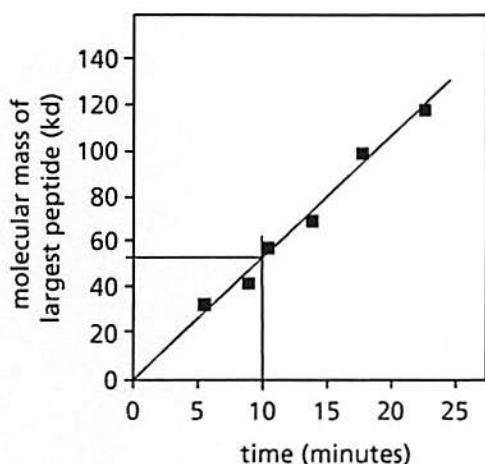
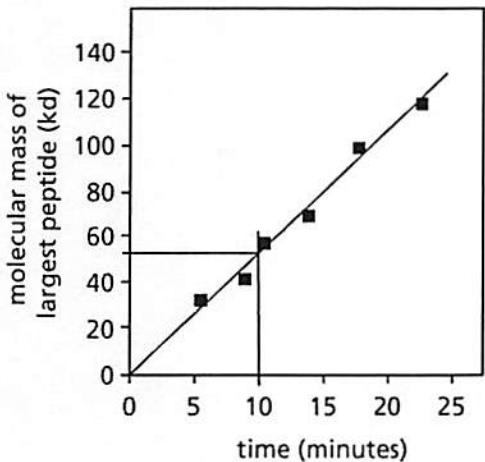


Figure Q2. Rate of synthesis of a TMV protein

B. False. The rate of protein synthesis can be determined from the slope of the line in figure below. This system is synthesizing roughly 52,000 daltons of protein per 10 minutes, or 5200 daltons per minute, which corresponds to about 47 amino acids per minute [(5200 daltons/minute)/(110 daltons/amino acid)].



C. False. The speed of ribosome movement along the mRNA is not constant. Because there are many discrete bands rather than a continuous background fuzz suggests that there are specific hang-up points along the mRNA.

D. True. There are many discrete bands rather than a continuous background fuzz suggests that there are specific hang-up points along the mRNA, perhaps where ribosomes must wait for rare tRNAs.

Reference: *Molecular Biology of the cell*. B. Alberts et al

Q.3

Scientists have isolated three different strains of bacteria ProA⁻, ProB⁻, and ProC⁻ that require added proline for growth. One is cold-sensitive, one is heat-sensitive, and one has a gene deleted. Cross-feeding experiments were carried out by streaking the strains out on agar plates containing minimal medium supplemented with a very low level of proline. In cross-feeding experiments, metabolite leaking from one strain can feed a neighbouring strain. After growth at three temperatures, the results were shown in Figure below.

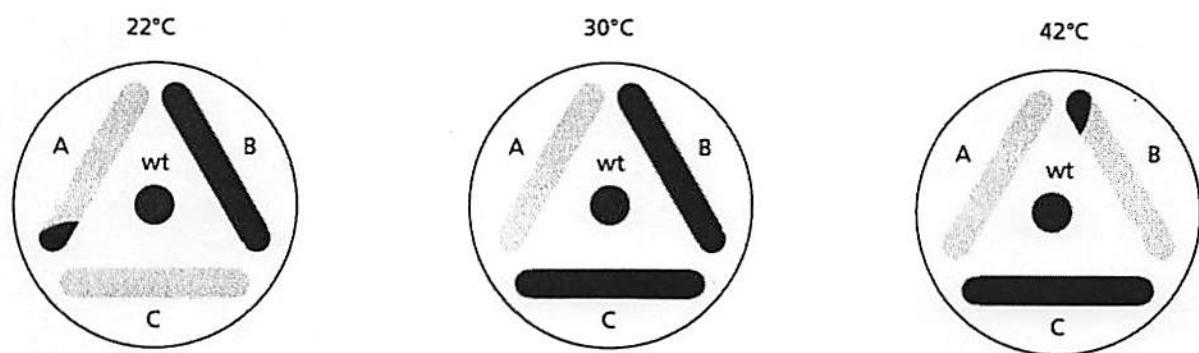


Fig.Q.3. Results of cross-feeding experiments with three strains defective in proline biosynthesis. Dark areas show high cell growth rate; grey areas show low cell growth rate; wt, wild type.

Indicate in the **Answer Sheet** if each of the following statements is True or False.

- A. The intermediate that accumulates in the ProC⁻ strain comes after the block in the ProA⁻ strain.
- B. The intermediate that accumulates in the ProB⁻ strain comes after the block in the ProA⁻ strain.
- C. There are at least three different genes that affect proline biosynthesis.
- D. Under at least one condition, the proline that is produced is rapidly used for protein synthesis and is prevented from being synthesized in excess of needs.

Answer key:

A: True, B: False, C: True, D: True

Explanation:

A: True: At 22°C, the ProC⁻ strain cross-feeds the ProA⁻ strain, indicating that the intermediate that accumulates in the ProC⁻ strain comes after the block in the ProA⁻ strain.

B: False: At 42°C the ProA⁻ strain cross-feeds the ProB⁻ strain, indicating that the intermediate that accumulates in the ProA⁻ strain comes after the block in the ProB⁻ strain.

C: True: The identification of three genes by the cross-feeding experiments shown here indicates that there are very likely to be at least three steps in the pathway.

D: True: The lack of cross-feeding of the ProA⁻ strain by the ProC⁻ strain at 30°C or 42°C, or between the wild-type bacteria and the mutant strains under any conditions, indicates that neither intermediates nor end products accumulate under normal growth conditions.

Reference: *Molecular Biology of the cell*. B. Alberts et al

Q.4

When isolated mitochondria are suspended in a buffer containing ADP, P_i , and an oxidizable substrate, three easily measured processes occur: the substrate is oxidized; O_2 is consumed; and ATP is synthesized. Cyanide (CN^-) is an inhibitor of the passage of electrons to O_2 . Oligomycin inhibits ATP synthase by interacting with subunit F_0 . 2,4-dinitrophenol (DNP) can diffuse readily across mitochondrial membranes and release a proton into the matrix, thus dissipating the proton gradient.

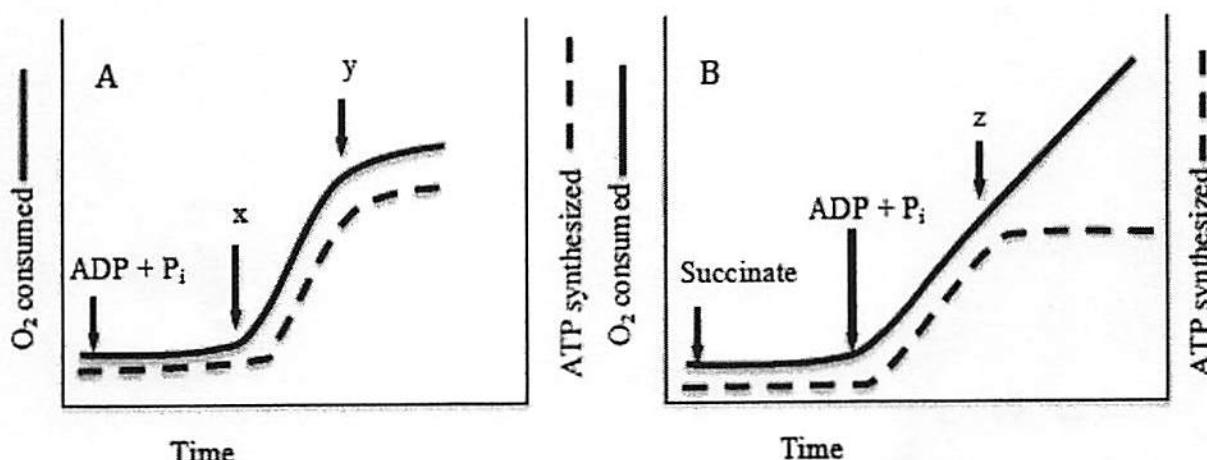


Fig.Q.4. Oxygen consumption and ATP synthesis in mitochondria.

The solid lines indicate the amount of oxygen consumed and the dash lines indicate the amount of ATP synthesized.

Indicate in the **Answer Sheet** if each of the following statements is True or False.

- A. x is the oxidizable substrate.
- B. y may be oligomycin or CN^- .
- C. z is DNP.
- D. If z is a mixture of oligomycin and DNP, the trend of each line in the figure B is not changed.

Answer key:

A: True, B: True, C: True, D: True

Explanation:

The purpose is to test the students' understanding on oxidative phosphorylation and ability to analyze chart data. Electron transfer can be detected by oxygen consumed and

phosphorylation can be detected by ATP synthesised. Electron transfer and ATP synthesis couple with each other.

A. True. In the figure A, x is substrate. Because oxidative phosphorylation require substrates.

B. True. In the figure A, y may be oligomycin or CN⁻. Because coupling of the two processes electron transfer and ATP synthesis, if one of two processes is inhibited the other can not occur. CN⁻ inhibits electron transfer resulting in inhibition of ATP synthesis and oligomycin inhibits ATP synthase resulting in inhibition of electron transfer.

C. True. In the figure B, z is DNP. DNP dissipates the proton gradient across the mitochondrial membrane and thus decreasing proton motive force which is used for ATP synthesis from ADP and P_i by ATP synthase. Because of decrease in proton gradient the outer and the inner membrane, electron transfer still occurs but ATP synthesis can not occur.

D. True. If z is a mixture of oligomycin and DNP, the trend of each line in the figure B is not changed. The presence of DNP causes the inhibition of ATP synthesis with presence or without presence of ATP-synthase inhibitors such as oligomycin. Dissipating the proton gradient across the mitochondrial membrane by DNP results in decreasing proton motive force. Therefore electron transfer still occurs.

Reference

1. Albert L. Lehninger, David L. Nelson and Michael M. Cox, 2008. *Principles of biochemistry*, 5th edition. W.H. Freeman & Company. Page. 714
2. Peter Mitchel, 1961. Coupling of phosphorylation to electron and hydrogen transfer by chemi-osmotic type of mechanism. Nature No. 4784.
3. Peter J. Tummino and Ari Gafni, 1991. A comparative study of succinate-supported respiration and ATP/ADP translocation in liver mitochondria from adult and old rats. Mechanisms of Ageing and Development 59: 177-188.

Q.5

Imagine you are studying a membrane protein represented in the diagram below. You prepared artificial vesicles containing this protein only in the membrane. The vesicles were then treated with a protease cleaving close to the membrane (2) or permeabilised before protease treatment (3). Resulting peptides were subsequently separated using SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis).

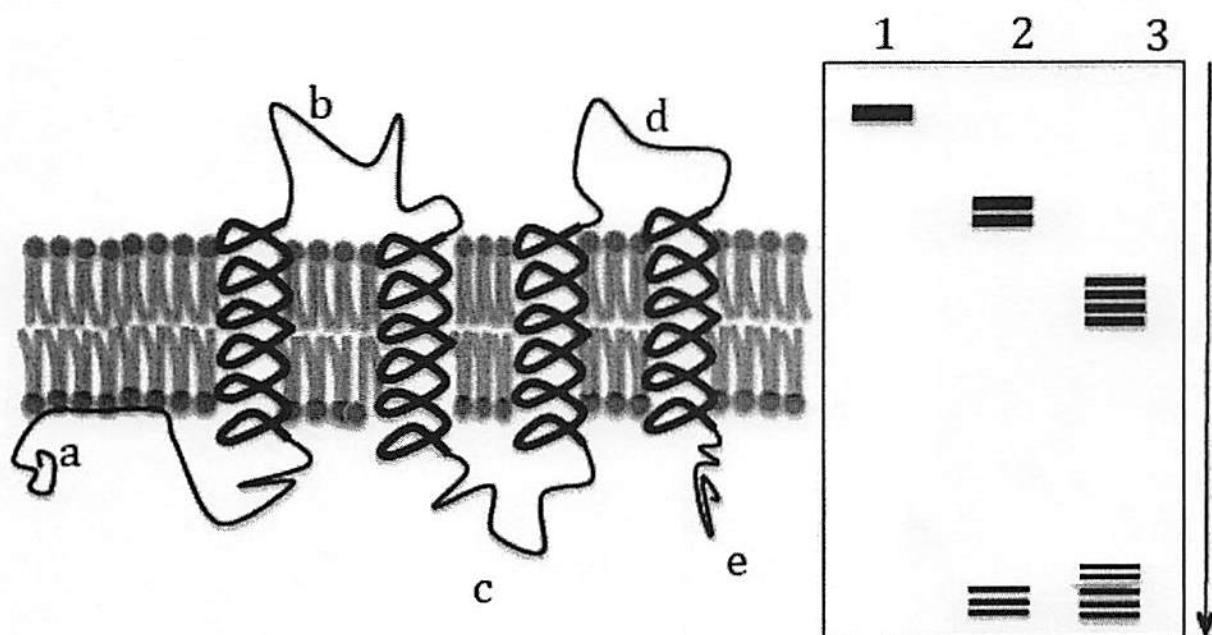


Fig.Q.5. Membrane protein (a, b, c, d, e: domains) and the SDS PAGE gel (1. untreated control, 2. peptides after protease cleavage. 3. peptides after permeabilisation and protease cleavage. The arrow indicates the direction of migration).

Indicate in the **Answer Sheet** if each of the following statements is True or False.

- The bigger fragments in lane 3 are hydrophilic.
- The smaller fragments in lane 2 represent protein domains protruding outside the membrane.
- Domain a is rich in leucine or isoleucine.
- Domains a, c and e protrude into the lumen of vesicles.

Answer key:

A: False, B: True, C: False, D: False

Explanation:

The idea of the question is to test understanding of membrane protein and phospholipid bilayer properties.

A. False. The bigger fragments, which are not cleaved by protease, are the transmission parts of the protein. The transmission parts usually are hydrophobic.

B. True. The smaller fragments in lane 2 represent protein domains protruding outside the membrane. Because protein domains protruding outside the membrane are cleaved into small fragments by protease.

C. False. It is the transmission part not the outside membrane part is rich in leucine or isoleucine. Domain a binds to phosphate areas of the phospholipid, therefore it should be rich in lysine.

D. False. Protease is too large to penetrate the membrane of vesicles. Those parts of the membrane's proteins that are situated on the external side of the lipid bilayer are subjected to digestion by protease, but those parts within the bilayer or lumen face of the membrane are not affected. Under the condition of treatment with permeabilisation and protease, the membrane no longer acts as a barrier to the penetration of the protease, so that the lumen portions of the protein are also subjected to digestion. Under this condition (Lane 3), there were 4 bigger fragments, indicating that domains a, b, c and d were cleaved by protease. Under the condition of treating with protease only, protease could not enter the lumen, and only 2 bigger fragments were, therefore, observed on the gel. This means that it was domain a, c, and e but not domains b and d were cleaved. Therefore domains a, c and e are situated on the external side of the membrane.

References:

1. Gerald Karp, Cell and Molecular Biology: Concepts and Experiments. Chapter 4. Pages: 131-136
2. Dana Boyd, Colin Manoil and Jon Beck with, Determinants of membrane protein topology. Proc Nati.Acad. Sci. USA (1987): 8525-8529

Q.6

Ethanol inhibits microbial growth. Nevertheless, some strains of the yeast *Saccharomyces cerevisiae* can adapt to high concentrations of ethanol. Many studies have documented the alteration of cellular lipid composition in response to ethanol exposure.

In this investigation, we systematically altered the fatty acid composition in *S. cerevisiae* by knocking out *OLE1* gene coding for integral membrane desaturase, responsible for the formation of mono-unsaturated palmitoleic acid (Δ^9 -C_{16:1}) and oleic acid (Δ^9 -C_{18:1}). The knockout strain was then: (1) reconstituted with *OLE1* gene by transformation with YE_p*OLE1* plasmid; (2) transformed with YE_p*OLE1*- Δ^9 Hz, YE_p*OLE1*- Δ^9 Tn, YE_p*OLE1*- Δ^{11} Hz and YE_p*OLE1*- Δ^{11} Tn plasmid containing the open reading frame of *OLE1* ligated to Δ^9 or Δ^{11} desaturases of two lepidopteran insect (moths) *Helicoverpa zea* (Hz) or *Trichoplusia ni* (Tni) via a four codon linker. Fatty acid component and growth curve of each mutant were investigated and shown in table and figure below.

Table Q.6. Composition of major fatty acids of *S. cerevisiae* transformants

at mid-log phase

Transformant	Fatty acid content (%)			
	<u>Saturated</u>		<u>Monounsaturated</u>	
	C _{16:0}	C _{18:0}	C _{16:1}	C _{18:1}
Δ^9				
<i>OLE1</i>	45.5 ± 2.2	4.7 ± 2.4	34.9 ± 0.8	14.9 ± 1.0
<i>OLE1</i> - Δ^9 Hz	45.5 ± 5.5	7.9 ± 2.2	31.7 ± 5.6	11.0 ± 2.0
<i>OLE1</i> - Δ^9 Tn	46.9 ± 4.0	8.6 ± 3.9	12.8 ± 1.9	31.7 ± 5.8
Δ^{11}				
<i>OLE1</i> - Δ^{11} Hz	45.6 ± 3.6	11.9 ± 2.8	42.6 ± 6.3	0
<i>OLE1</i> - Δ^{11} Tn	49.7 ± 4.8	12.5 ± 0.1	41.8 ± 11.8	11.2 ± 1.5

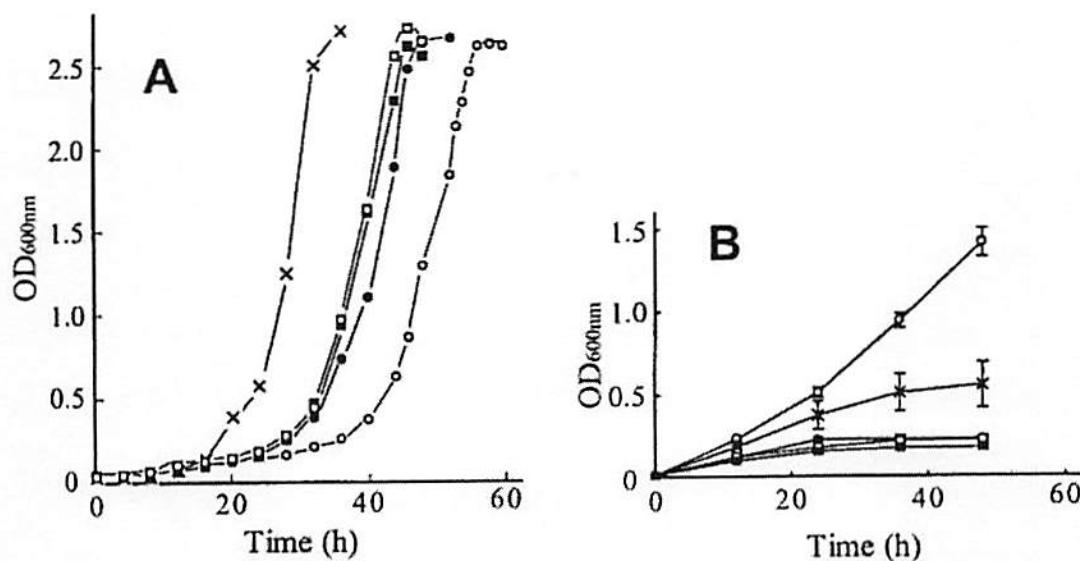


Fig.Q6. Growth curves of *S. cerevisiae* strains transformed with plasmid containing *OLE1* (x), *OLE1-Δ⁹Hz* (●), *OLE1-Δ¹¹Hz* (■), *OLE1-Δ⁹Tn* (○) and *OLE1-Δ¹¹Tn* (□) in YPD medium (A) and in YPD medium containing 5% ethanol (B)

Indicate in the **Answer Sheet** if each of the following statements is True or False.

- A. The lag phase of transformant *OLE1* in YPD medium is shorter than those of *OLE1-Δ⁹Hz*, *OLE1-Δ⁹Tn*, *OLE1-Δ¹¹Hz* and *OLE1-Δ¹¹Tn* due to the presence of native desaturase in yeast cells.
- B. *OLE1* was expressed well in all transformants.
- C. The content of mono-unsaturated fatty acids is a good indicator of the ethanol tolerance in *S. cerevisiae*.
- D. Higher ratio of $\Delta^9\text{-C}_{18:1}$ to $\Delta^9\text{-C}_{16:1}$ causes higher ethanol tolerance in *S. cerevisiae*

Answer key:

A- True; B- False; C- False; D- True

Explanation:

A. **True:** *OLE1* is a nature desaturase of yeast. Although *S. cerevisiae* strain was knocked out *OLE1* gene, it has been reconstituted with *OLE1* gene by transformation with YEPOLE plasmid.

B. **False:** *OLE1* did not express in the case of HzeaPLAQ (Table Q.56)

C. **False:** Based on the result of mono-UFA in Table Q.56, total ratio of mono-UFA is calculated by sum of $C_{16:1}$ and $C_{18:1}$ UFA. Highest mono-UFA is *OLE1* but this

mutant did not show different in the *ethanol tolerance* (Fig.Q.56B). Other mutants have similar ratio of mono-UFA, however, only *TniNPVE* showed different in the *ethanol tolerance* (Fig.Q.56B).

D. True: Higher ratio of $\Delta^9C_{18:1}$ to $\Delta^9C_{16:1}$ is shown in *TniNPVE* (Table Q.56) and this mutant also shows highest *ethanol tolerance* in Fig.Q.56B.

Reference: *Applied and Environmental Microbiology*, 2003, 69(3):1499-1503

Q.7

Poly(3-hydroxybutyrate) (PHB) is a bacterial storage material which is accumulated by various bacteria, usually when grown under limitation of a nutrient such as oxygen, nitrogen, phosphate, sulphur, or magnesium and in the presence of excess carbon. Fig. Q7 shows the PHB synthesis pathway of *Ralstonia eutropha* from acetyl-CoA. In addition, acetyl-CoA can enter the citric acid cycle.

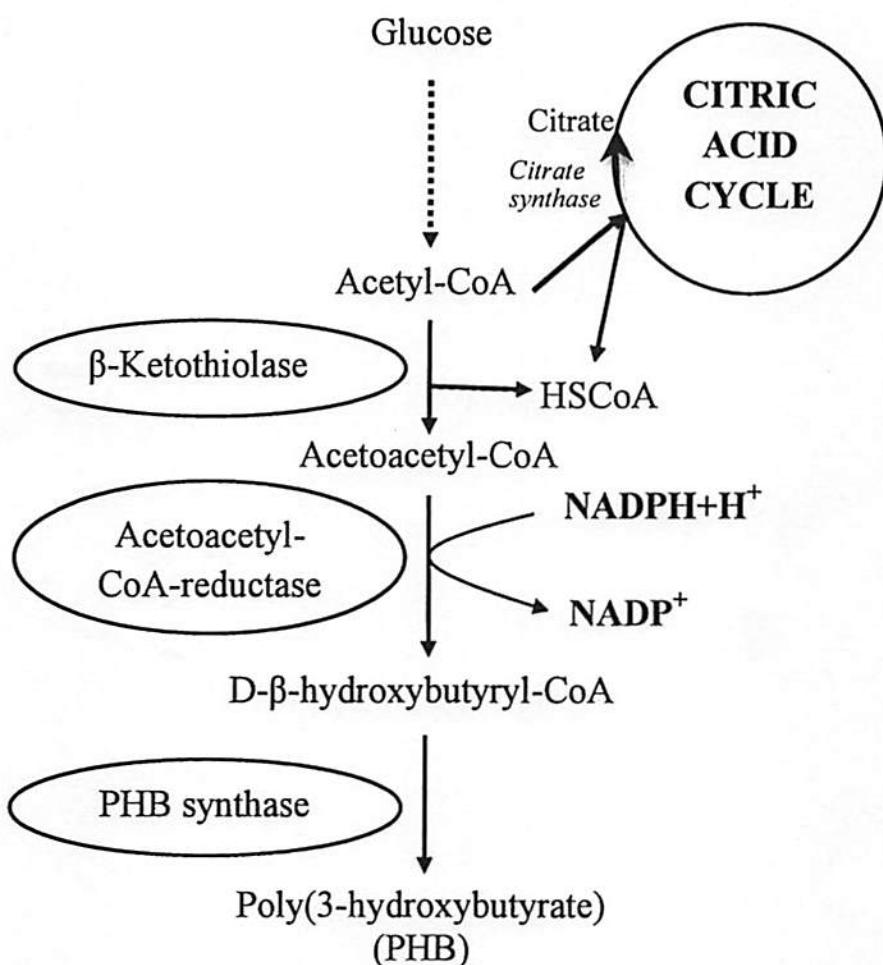


Fig. Q7. PHB synthesis pathway

Indicate in the **Answer Sheet** if each of the following statements is True or False.

- Citrate synthase is an important regulation factor in the PHB synthesis process.
- When the intracellular concentration of HSCoA is high, the rate of PHB synthesis will increase.
- When the rate of PHB synthesis increases, the growth rate of *Ralstonia eutropha* cells will also increase.
- PHB synthesis is stimulated by low ratios of NADPH+H⁺/NADP⁺.

Answer key

A. True, B. False, C. False, D. False

Explanation

- A. True.** Citrate synthase can control the PHB synthesis process based its ability to control carbon flux into the tricarboxylic acid cycle.
- B. False.** β -Ketothiolase is inhibited by high concentration of HSCoA.
- C. False.** The growth rate of *Ralstonia eutropha* cells will decrease because most of Acetyl-CoA enter PHB synthesis pathway.
- D. False.** Acetoacetyl-CoA reductase is stimulated by high ratios of NADPH+H⁺/NADP and high concentration of NADPH+H⁺.

Reference:

Kessler B, Witholt B (2001). Factors involved in the regulatory network of polyhydroxyalkanoate metabolism. Journal of Biotechnology. 86:97-104.

Q.8

Jack has isolated five different polypeptides containing five amino acids (named A, B, C, D, E). He determines the molecular weight and the sequence of each polypeptide. The data, which he obtained is shown on the table below.

Polypeptide	Amino Acids Sequence (in form of the tube containing it before)	Mass (Da)
1	BCDACCDEDCCB	966
2	ABBCAEEDECB	1099
3	BACDAEAECA	1357
4	CACADBACAEB	1279
5	EDDCABCCEE	1014

The mass of individual amino acids are shown in the table below.

Amino Acids	Mass (Da)	Amino Acids	Mass (Da)
Alanine	89	Leucine	131
Arginine	174	Lysine	146
Asparagine	132	Methionine	149
Aspartic Acid	133	Phenylalanine	165
Cysteine	121	Proline	115
Glutamic Acid	147	Serine	105
Glutamine	146	Threonine	119
Glycine	75	Tryptophan	204
Histidine	155	Tyrosine	181
Isoleucine	131	Valine	117

Hint : in polymerization reaction, to form a peptide, when two different ends of amino acids are joined, a water molecule (mass : 18 Da) is released.

Indicate in the Answer Sheet if each of the following statements is True or False.

- A. Amino acid named C is serine
- B. Amino acid named A is tyrosine
- C. Amino acid named E is cysteine
- D. Amino acid named B is glycine

Answer key

A. False, B. False, C. True. D. True

Explanation

- A. False.** Amino acids inside tube C is alanine
- B. False.** Amino acids in tube A is tryptophan
- C. True.** Amino acids inside tube E is cysteine
- D. True.** Amino acids inside tube B is glycine

Students need to solve the simultaneous linear equations:

$$A + 2B + 4C + 3D + E = 1146 \quad (1)$$

$$2A + 3B + 2C + D + 3E = 1279 \quad (2)$$

$$4A + B + 2C + D + 3E = 1537 \quad (3)$$

$$4A + 2B + 3C + D + E = 1459 \quad (4)$$

$$A + 2B + 3C + 2D + 3E = 1194 \quad (5)$$

$$(3) - (2) \leftrightarrow 2A - 2B = 258 \leftrightarrow A - B = 129$$

A must be tryptophan and B must be glycine

Then $(5) - (4) \leftrightarrow D + 2E = 347$

$$2*(2) - (1) \leftrightarrow 5E - D = 500$$

D is serine, E is cysteine and C is alanine.

References:

Nelson DL, Cox MM (2008). Lehninger Principles of Biochemistry. W. H. Freeman and Company. England. pp 73.

Q.9.

Four different bacteria were isolated from the gut of a shrimp to be studied about their probiotic potency through the activity to decrease pathogenicity of *Vibrio harveyi*, a common bacteria infecting shrimp culture. In the first experiment, the four isolated bacteria were inoculated in cross-streak plates to observe inhibition zones against 4 bacterial strains (Fig.9A). In the second experiment, the shrimp survival rate in presence of *Vibrio harveyi* and each bacterial isolate after 5 days incubation was measured (Fig.9B).

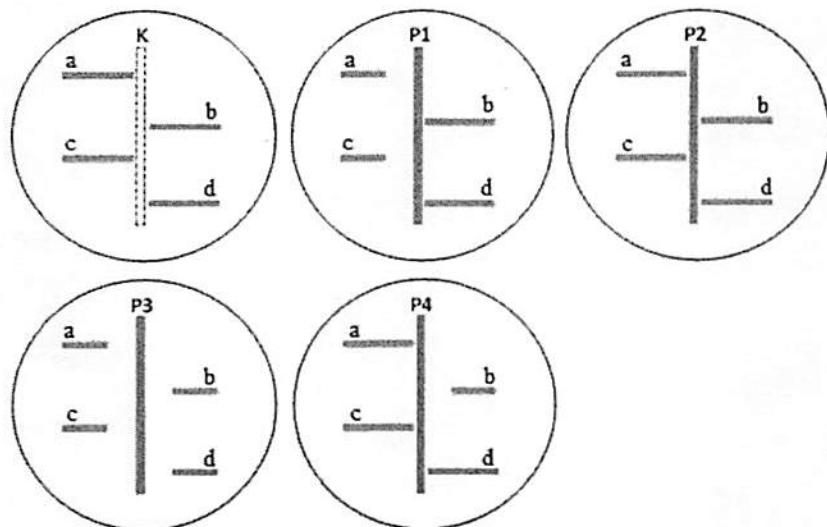


Fig.Q.9A. K = Control (no bacteria streaked on the dash box), P1-P4 = Probiotic candidates 1-4, a = *Streptococcus* sp. (Gram-positive), b = *Vibrio* sp. (Gram-negative), c = *Bacillus* sp. (Gram-positive), d = *Salmonella* sp. (Gram-negative)

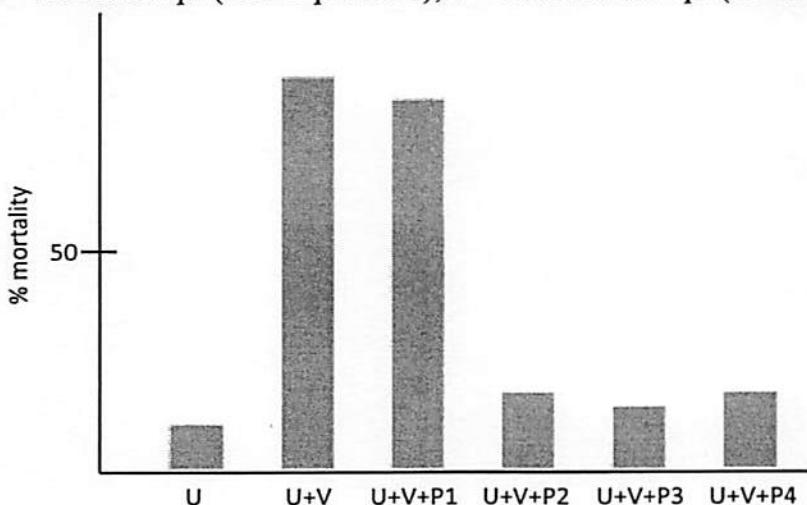


Fig.Q.9B. U = shrimp culture alone, U+V = shrimp culture with addition of *Vibrio* sp., U+V+P1-4 = shrimp culture with addition of *Vibrio* sp. and a specific probiotic candidate P1-4, respectively.

Indicate in the **Answer Sheet** if each of the following statements is True or False.

- A. Candidate No.1 (P1) produced an antimicrobial compound that inhibited Gram-negative and Gram-positive bacteria.
- B. Candidate No.2 (P2) was able to decrease *Vibrio* sp. pathogenicity without killing them.
- C. Candidate No.3 (P3) produced an antimicrobial compound targeting the outer membrane.
- D. Candidate No.4 (P4) had good effect on the shrimp survival by inhibiting Gram-negative bacteria.

Answer key

- A. False, B. True, C. True, D. True

Explanation:

- A. **False.** According to the result of first experiment, P1 only inhibited Gram-positive bacteria.
- B. **True.** P2 bacteria is probably inhibiting *Vibrio* sp. virulence factor, decreasing its pathogenicity (showed in challenge experiment/clinical study) without killing those bacteria (showed in cross streak plate experiment). This model of inhibition has lower selection force to bacterial population than model of inhibition by bacteriocidal agent such as the antibiotics. It tends to keep the existing bacterial population structure instead allowing the raise of resistant strain because of loss of competition with the eliminated bacteria.
- C. **True.** P3 inhibited Gram-negative bacteria so it can attack the outer membrane of bacteria.
- D. **False.** P4 bacteria has activity to produce specific antimicrobial compound inhibiting the *Vibrio*, resulting in the decrease of shrimp mortality.

Reference:

1. Madigan & Martinko, 2006. *Brock Biology of Microorganism*, 11th edition. Chapter 20
2. Paper title: K. Ramesh et al., 2014. *Feasibility of Shrimp Gut Probiotics with Anti-vibrio and Anti-QS in Penaeid Culture*. International Journal of Fisheries and Aquatic Studies; 1(3): 26-34.

Q.10

An experiment was set up to observe cell cycle length of a strain of yeast. Activated yeast cells were subcultured into a new medium with an initial concentration of 10^6 cells/mL. After 40 h, the number of cells increased to 4×10^6 cells/mL. A portion of the culture was taken for a separate experiment. In this experiment, cells were incubated for 15 min into a media containing radioactive thymidine before washing and re-grown on a new media containing non-radioactive thymidine. Cell samples were then taken periodically to measure the percentage of mitotic cells containing radioactive thymidine. **Fig.Q.10** shows the result obtained from the experiment. At each sampling, about 1% of the total cells sampled were undergoing mitosis.

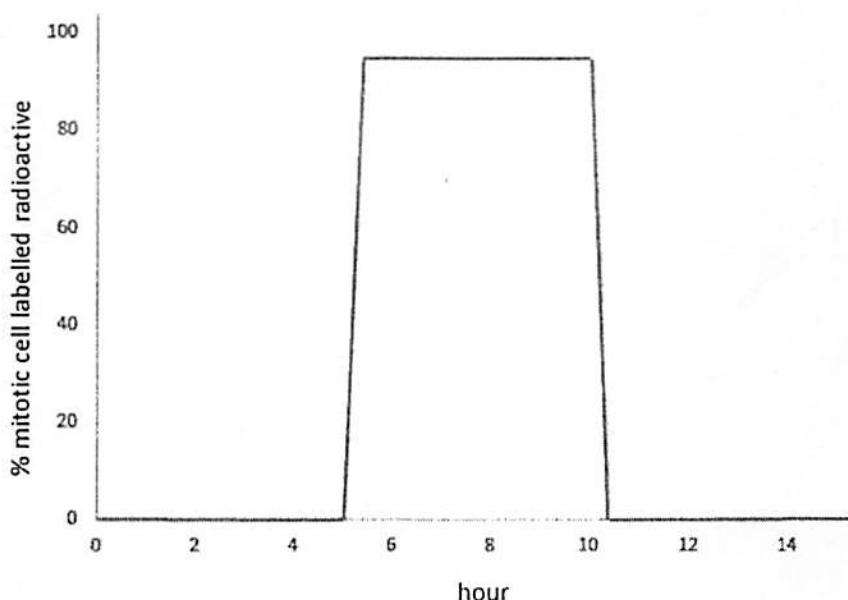


Fig.Q.10. Experiment result of yeast cell culture.

Indicate in the **Answer Sheet** if each of the following statements is True or False.

- G2 phase of the cell cycle takes approximatively 10 hours.
- Most of the yeast cells in the culture are at G1.
- M phase of the cell cycle takes longer than 1 hour.
- Most of the radioactive thymidine is assimilated in the S phase of the cell cycle.

Answer key

- A. False B. True C. False D. True

Explanation

- Length of 1 cell cycle = 20 hours
- Length of M phase = $1\% \times 20 \text{ hours} = 12 \text{ minutes}$

- Length of G2 = 5 hours (the time required for the cells at the end of S phase or entering G2 to reach mitosis and earliest observed mitotic cells to incorporate radioactive thymidine in the chromosome)
- Length of S phase = 4 hours (the time required until the first radioactive mitotic cell is observed until the time the percentage of radioactively labeled mitotic cells decrease. The decrease is observed as movement to non-radioactive media cause cells just entering S phase to incorporate non-radioactive thymidine, reducing the percentage of labeled cells)
- Length of G1 = $20 \text{ hours} - (4 + 5 + 0,2) \text{ hours} = 10,8 \text{ hours}$

Reference:

Alberts et al. Molecular Biology of the Cell, 5th edition (2007). Chapter 17

Karp. Cell and Molecular Biology: Concepts and Experiments, 6th edition (2010). Chapter 14

PLANT ANATOMY AND PHYSIOLOGY

Q.11

Cell walls provide plant cells with a substantial degree of volume homeostasis relative to the large changes in water potential that they experience as the everyday consequence of the transpirational water loss. Water potential (Ψ_w) of a plant cell is composed of solute potential (Ψ_s) and turgor pressure potential (Ψ_p) (Fig.Q11). Relative cell volume is correlated with cell water potential and its components as described in the graph below.

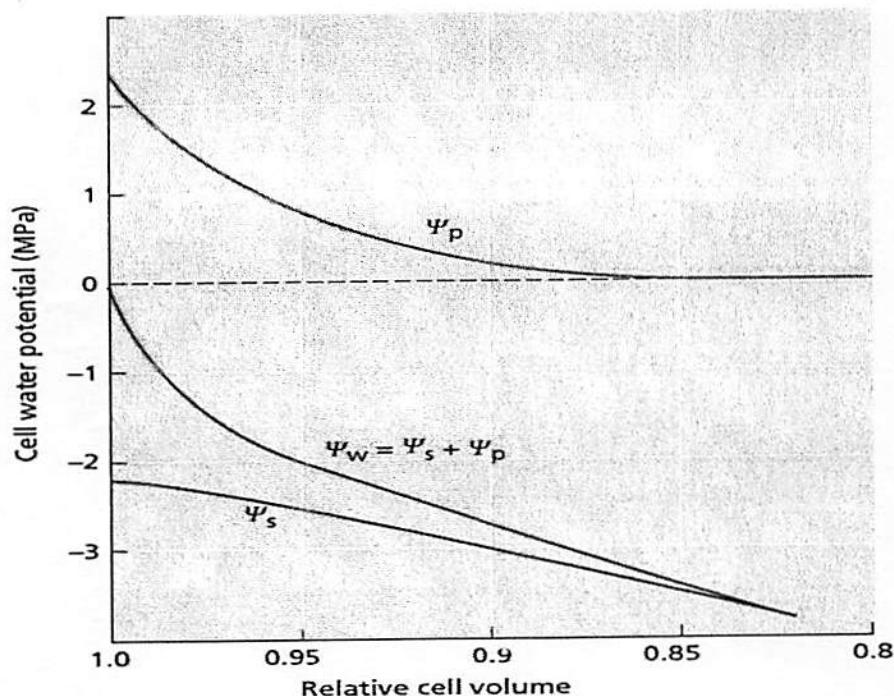


Fig.Q11

Indicate in the **Answer sheet** if each of the following statements is True or False.

- A. Alterations of cell water potential are generally accompanied by a large change in turgor pressure and in cell volume.
- B. Disappearance of turgor pressure indicates the ending point of cell plasmolysis with reduction of approximately by 15% cell volume.
- C. As the cell volume decreases by 15%, most of the change in cell water potential is caused by the drop in cell solute potential together with little change in turgor pressure.

D. During cell rehydration, cell wall expansion stops when cell wall generates pressure equivalent to turgor pressure and the water potential of the cell reach zero.

Answer key

- A. False B. False C. True D. True

Explanation

A. False. Due to fairly rigid structure, the cell wall has limited flexibility to expand or to diminish in response to modifications of water potential and solute potential. Changes in cell water potential are mainly caused by large changes in turgor pressure while changes in cell volume are little as indicated in the figure.

B. False. Plasmolysis begins when turgor pressure of the plant cell disappears. As shown in figure, the cell volume is reduced approximately 15%. The water potential and solute potential are both below minus 3.

C. True. As the cell volume decreases by 15%, the change in water potential (Ψ_w) is mostly on solute potential (Ψ_s) together with little change in turgor pressure potential (Ψ_p).

D. True. The backpressure from cell wall is balanced turgor pressure indicate that plant cell is turgid. At this point, water potential of the cell becomes zero and water no longer enters or goes out the plant cell.

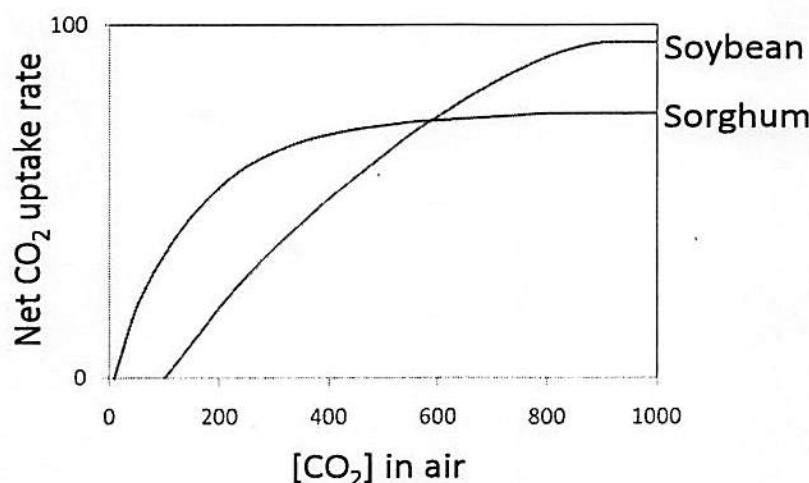
References

Lincoln Taiz and Eduardo Zeiger, Plant physiology textbook, fifth Edition 2010.

Q.12

An experiment was carried out on sorghum (*Sorghum bicolor*) and soybean (*Glycine max*) plants in response to low temperature. Plants were grown at 25°C for several weeks and then at 10°C for three days, while day length and light intensity and ambient carbon dioxide concentration were kept constant throughout the experiment. The net photosynthesis of both plant species are shown in Fig.Q12 below.

Plant	Carbon dioxide uptake per leaf dry mass (mg CO ₂ g ⁻¹)				
	at 25°C (before cooling)	at 10°C (cooling)			at 25°C, days 4 to 10 (after cooling)
		day 1	day 2	day 3	
Sorghum	48.2	5.5	2.9	1.2	1.5
Soybean	23.2	5.2	3.1	1.6	6.4



Indicate in the **Answer sheet** if each of the following statements is True or False.

- A. In 35°C condition, photosynthesis rate of soybean may decrease and that of sorghum may not change.
- B. In cool condition, the biomass of sorghum increases faster than that of soybean.
- C. Soybean plants are likely to have smaller photosynthetic water use efficiency than sorghum.
- D. The reduction of the carbon dioxide uptake in sorghum is mainly due to the decrease of enzyme activity in low temperature.

Answer keys

A. True B. False C. True D. False

Explanation

A. True. As shown in Fig.Q12 and in data table, sorghum shows stable and high photosynthesis rate in high ambient CO₂ concentration compared to soybean, indicating that soybean is C₃ plant and sorghum is C₄ plant. In 35°C condition, the photosynthesis rate of soybean reduces while that of sorghum does not change.

B. False. In cooling condition, photosynthesis rate of sorghum decreases faster than soybean, decreasing plant growth rate which results in low biomass increment.

C. True. Water usage in plant species depends on water absorption through roots and transpiration via opening stomata. In general, sorghum (C₄ plant) has higher water use efficiency than soybean (C₃ plant).

D. False. When returned to a temperature of 25°C for seven days, the carbon dioxide uptake by sorghum (C₄ plant) was not increasing. So the low temperature is not the main reason for reducing the carbon dioxide uptake.

References

Glenn Toole and Susan Toole. Biology in context for Cambridge International A level Text book. Oxford University Press, 2015.

Q.13

The bacterium *Bradyrhizobium japonicum* can infect soybean (*Glycine max*) roots and form nodules. The nitrogen fixation catalyzed by nitrogenase occurs in the nodules and the nitrogenase activity can be measured easily by acetylene reduction instead of nitrogen reduction. Scientists generated a defective mutation of NAD⁺-dependent malic enzyme, the enzyme that generates pyruvate and NADH, and infected soybean seedling roots with wildtype and mutant bacteria. The seedlings were grown in nitrogen-free media. After 14 and 28 days of inoculation, the number and weight of nodules in the seedlings and acetylene reduction activity were recorded (Fig.Q13).

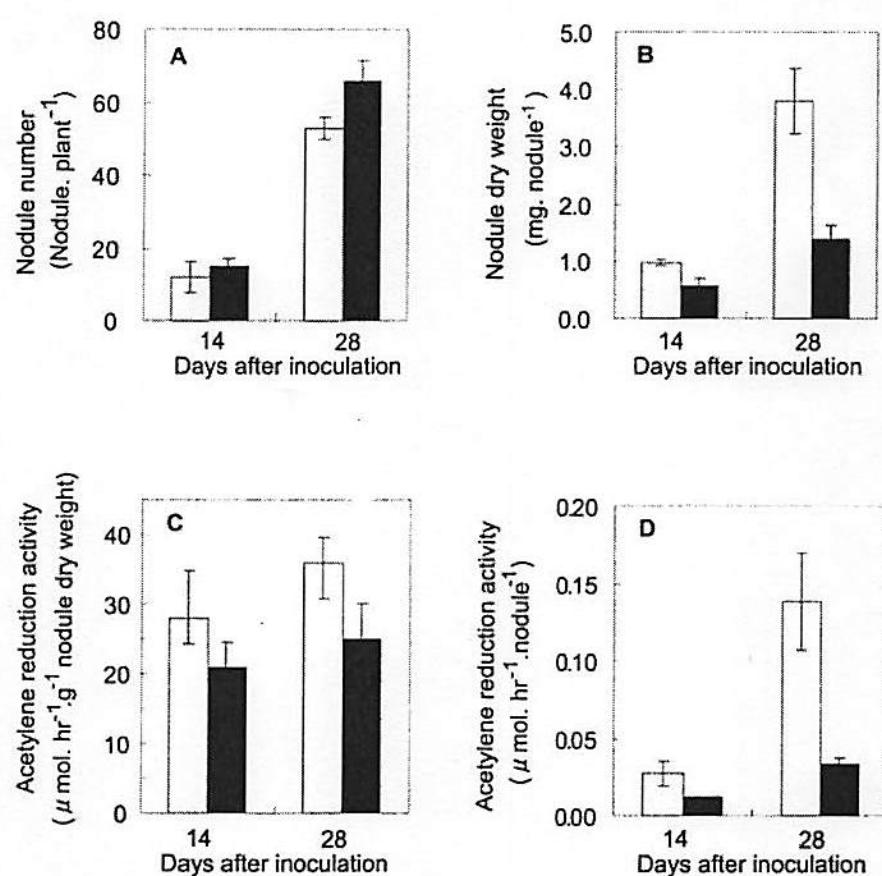


Fig.Q13. Nodule number and dry weight and acetylene reduction acitivity of soybean. Soybean nodules infected with wild-type *B. japonicum* (open bars) and the *dme* mutant (solid bars) are presented.

Indicate in the **Answer sheet** if each of the following statements is True or False.

- A. Nitrogen fixation activity in nodules of the same treatment at 28 days after inoculation is higher than that at 14 days after inoculation.
- B. Both number and size of nodules increase with time from 14 to 28 days after inoculation with *B. japonicum*.
- C. The reduction in nitrogen-fixing activity of nodules infected by the mutant at 28 days after inoculation compared to those at 14 days after inoculation is due to the reduction of nitrogenase activity and nodule formation.
- D. Nitrogen fixation in *B. japonicum*-induced nodule is down-regulated by NAD⁺-dependent malic enzyme.

Answer keys

- A. True B. True C. False D. False

Explanation

A. True. As shown in the figure, in both wildtype and mutant treatments, acetylene reduction activity in nodules at 28 days after inoculation is higher than that at 14 days after inoculation, indicating higher nitrogen fixation activity.

B. True. In both treatments, number and size of nodules are higher at 28 days compared to those at 14 days after inoculation

C. False. Number of nodules at 28 days after inoculation is higher than at 14 days after inoculation

D. False. The mutation of NAD⁺-dependent malic enzyme in the bacteria results in a reduction of acetylene reduction, indicating that the enzyme (in wildtype) up-regulates the nitrogen fixation

Reference

Van Dao T et al., NAD-Malic Enzyme Affects Nitrogen Fixing Activity of *Bradyrhizobium japonicum* USDA 110 Bacteroids in Soybean. *Microbes Environ.* 2008; 23(3):215-20.

Lowe DJ et al., *Klebsiella pneumoniae* nitrogenase: Mechanism of acetylene reduction and its inhibition by carbon monoxide. *Biochem. J.* (1990): 272: 621-625

Q.14

Sucrose is produced in leaves and translocated short and long distance through veins to non-photosynthetic tissues such as roots, stems, flowers and fruits. Two principal pathways include symplast and apoplasm by which sucrose molecules are transported in phloems of leaves as shown in Fig.Q14.

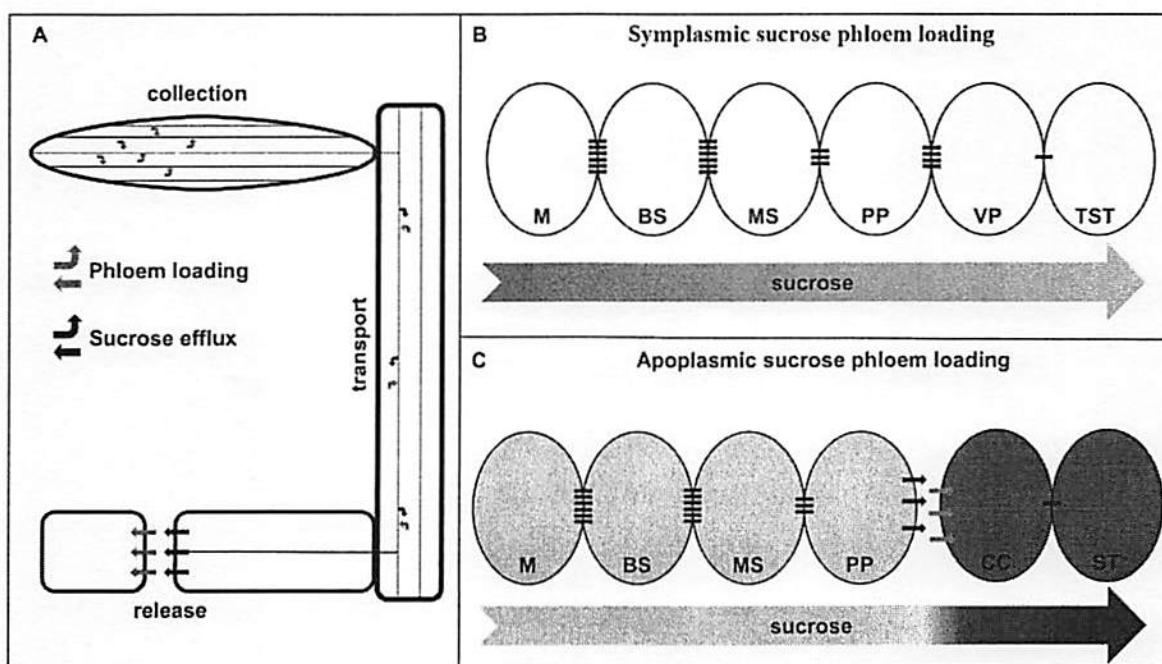


Fig.Q14. Diagram of the whole plant phloem network. M - Mesophyll, BS - Bundle sheath, MS - Mestome sheath, PP - Phloem parenchyma, VP - Vascular parenchyma, CC - Companion cell, TST - Thick walled sieve element, ST - Sieve element.

Indicate in the **Answer sheet** if each of the following statements is True or False.

- Carbon dioxide is synthesized into sucrose in leaves and transported long distance through phloems to sinks under hydrostatic pressure gradient.
- Loading sucrose in apoplastic pathway requires energy in several steps due to the movement across secondary wall of living cells.
- In the symplamic pathway, sucrose molecules are movement as passive loading through plasmodesmata.
- Unloading sucrose molecules at the sinks are always no requirement of energy because of movement down gradient concentration of sucrose.

Answer key

A. True B. False C. True D. False

Explanation

A. True. Sucrose entry into phloem increases the solute concentration that draws water from adjacent xylem. This creates hydrostatic pressure in the phloem to transport sucrose from source to sink over the long distances in plants.

B. False. In the apoplasmic phloem loading, sucrose molecules are transported via plasmodesmata (PD) from mesophyll cells (M) to the phloem parenchyma cells (PP). Sucrose molecules are subsequently imported across the plasma membrane of the companion cells (CC) against their concentration gradient.

C. True. Sucrose molecules are present at high concentrations in mesophyll cells (M) and move down a concentration gradient to enter thick walled sieve element (TSE). This mechanism does not require energy for sucrose to enter TSE.

D. False. As with phloem loading process, phloem unloading occurs through both symplast and apoplast depending on the type of sinks. Sucrose unloading is typically symplastic in growing and respiring organs such as meristematic tissues, young leaves. In storage organs, sucrose unloading is known to occur through apoplast and require energy for several steps.

Reference

David M. Braun, Lu Wang and Yong-Ling Ruan, 2014. Understanding and manipulating sucrose phloem loading, unloading, metabolism, and signalling to enhance crop yield and food security. *Journal of Experimental Botany*, Vol. 65(7), pp. 1713–1735, doi:10.1093/jxb/ert416

Q.15

A student measured length and height of rhizophores of *Rhizophora mangle* plant (Fig.Q15-1). She also made cross sections of rhizophores and observed their anatomical characteristics. The results are shown in Q15-2 and Q15-3.

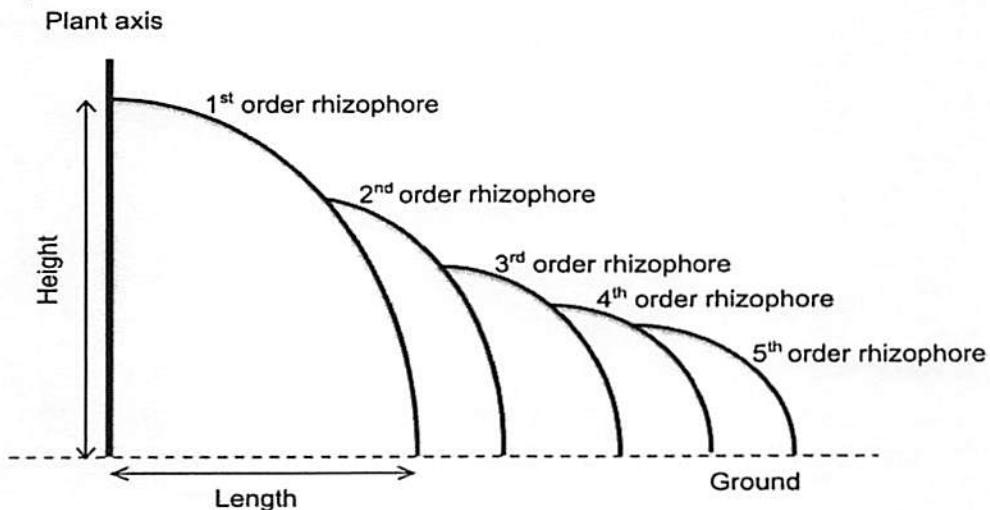


Fig.Q15-1. Rhizophore height and length measurement.

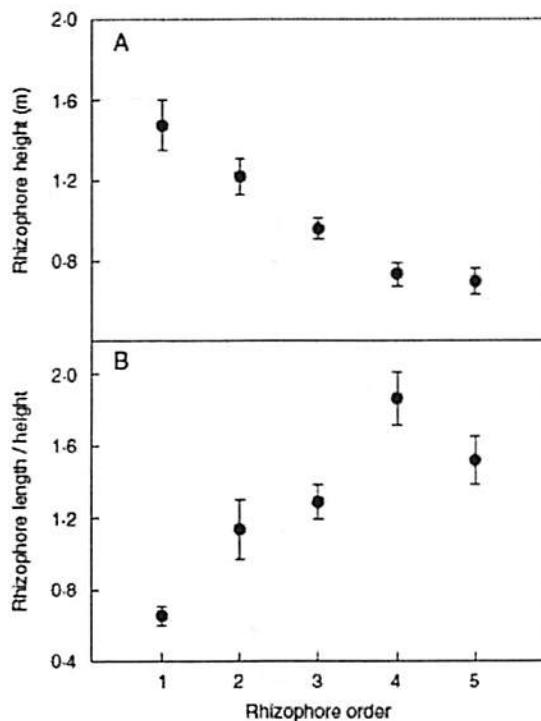


Fig.Q15-2. Change in height and in length/height proportion in five sequential orders of rhizophores from *Rhizophora mangle* plants.

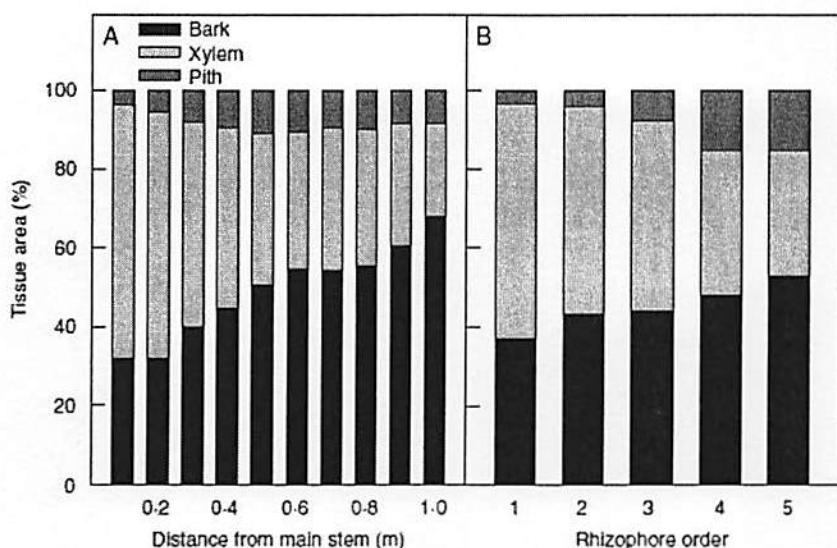


Fig.Q15-3. Relative proportions of bark (including aerenchyma), xylem and pith along the length of individual first-order rhizophores (A), and at the base of rhizophores of sequential orders (B) *Rhizophora mangle* mangrove trees.

Indicate in the **Answer sheet** if each of the following statements is True or False.

- A. There are monotonic decreases in rhizophore height and the length/height proportion in the rhizophores as a function of the rhizophore order.
- B. Within first-order rhizophores, the xylem proportion in the cross-section is larger when closer to the main stem, and decreases progressively as the rhizophore approached the ground while increasing the proportion of bark and pith.
- C. When rhizophore order decreases, bark and pith proportion decreases, while xylem proportion increases.
- D. The supportive function is likely enhanced in the first-order rhizophores, with lower length/height proportion, and higher proportion of xylem compared with bark and pith.

Answer key

- A. False B. True C. False D. True

Explanation

- A. **False.** There is a monotonic decrease in rhizophore height (Fig.Q15-2A) and an increase in the length/height proportion in the rhizophores as a function of the rhizophore order (Fig.Q15-2B).
- B. **True.** Within the first-order rhizophore (Fig.Q15-3A), when the cross-sections are far from main stem, the xylem proportion decreases, while the proportion of pith and bark increases.
- C. **False.** Xylem and bark proportion in the cross-section decreases, while pith proportion increases when rhizophore order increase (Fig.Q15-3B).
- D. **True.** First-order rhizophores have higher xylem proportion and lower length/height ratio, which could increase supportive ability.

Reference

Méndez-Alonso, Rodrigo, et al., Annals of botany 115.5 (2015): 833-840.

Q.16

Arsenic (As) in the soil has become an environmental concern worldwide because it is difficult to remediate and can adversely impact human health. The fern, *Athyrium yokoscense* is well known as a Cd hyperaccumulator as well as a Cu, Pb and Zn tolerant plant. However, no information is available on As accumulation by *A. yokoscense*, although it often grows in soils containing high levels of several heavy metals and As. To understand As accumulation in *A. yokoscense*, a student conducted an experiment in which young ferns collected from a mining area were grown in media containing As-spiked paddy soils or mine soil in a greenhouse for 21 weeks. Before transplanting fern biomass was 0.26 ± 0.08 g plant $^{-1}$ DW and As concentrations of young and old fronds were 7.8 ± 0.3 and 57.7 ± 2.2 mg kg $^{-1}$, respectively.

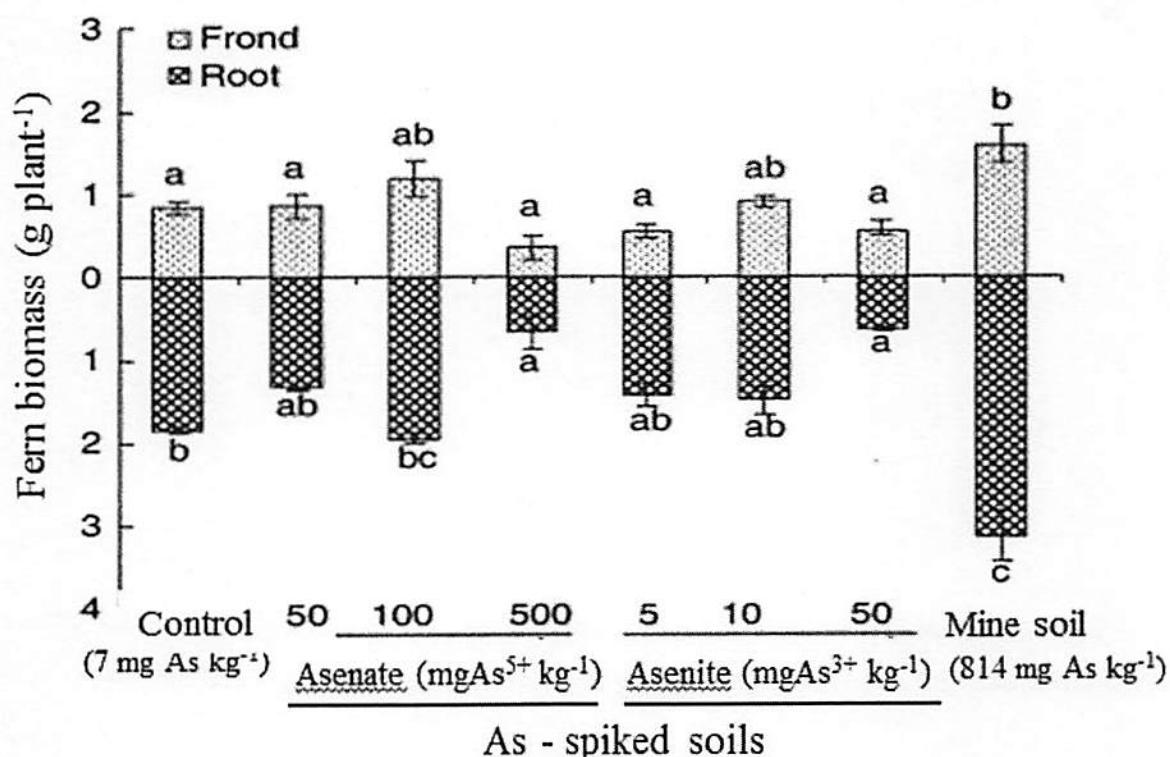


Fig.Q16- 1. Dry biomass of *A. yokoscense* after 21 weeks of cultivation in a greenhouse

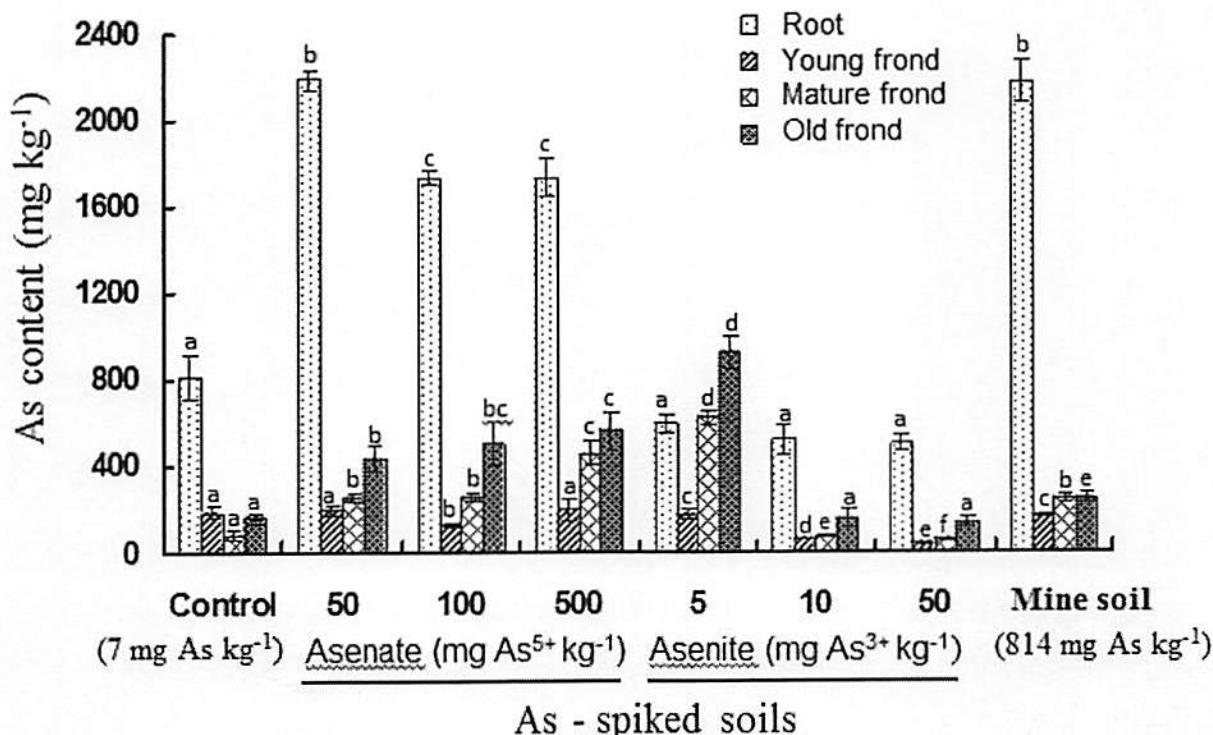


Fig.Q16-2. Arsenic concentration in different parts of *A. yokoscense* cultivated in As-spiked soils and mine soils. a,b,c letter above each bar indicate the significant difference of the same plant parts

Indicate in the **Answer sheet** if each of the following statements is True or False.

- A. Moderate As levels in soils might promote the growth of ferns.
- B. Concentration of As in root grown in arsenite-spiked media is lower than those in arsenate treatment, resulting in the increase of total biomass.
- C. Arsenic concentration increases from young to old fronds and is correlated to As levels in the soil
- D. The transfer of As from root to frond of *A. yokoscense* in mine soil is similar with those in arsenate-spiked soil.

Answer keys

- A. True B. False C. False D. False

Explanation

A. True. Fig.Q16-1 indicated that at arsenate at 100 mg kg^{-1} and arsenite at 10 mg kg^{-1} increased dry biomass of fern.

B. False. The ferns biomass in arsenate and arsenite spiked soils were not significant difference even As concentration in root fern grown in arsenite-spiked media was lower than those in arsenate treatment

C. False. Arsenic concentration in fronds increased from young to old fronds and As accumulation related to As levels in the media containing As-spiked soil as arsenate however, in case of arsenite addition, higher As concentration in soil (at treatment As^{3+} 10 and 50 mg kg^{-1}) reduced As accumulation in both roots and fronds compared to that in treatment As^{3+} 5 mg kg^{-1} .

D. False. Mine soil reduced the transfer of As from root to fronds of *A. yokoscense*, resulted in lower As concentration in fronds in comparison to that growth in arsenate-spiked media. Another reason might be is the fern grown in the mine soil had a greater biomass production.

Reference

Tran Khanh Van , Yumei Kang , Takahiro Fukui , Katsutoshi Sakurai , Kōzō Iwasaki , Yoshio Aikawa & Nguyen Minh Phuong. Arsenic and heavy metal accumulation by *Athyrium yokoscense* from contaminated soils. *Soil Science and Plant Nutrition*, Vol. 52(6), p.701-710. DOI: 10.1111/j.1747-0765.2006.00090

Q.17

To study the effect of phytohormone on fruit maturation, researchers used abscisic acid (ABA) and ethephon to treat of sweet cherry fruits and afterward to evaluate the expression of PacNCED1 gene which encodes 9-cis-epoxycarotenoid dioxygenase, a key enzyme in ABA biosynthesis. They also checked the expression of PacACO1 gene encoding 1-aminocyclopropane-1-carboxylic acid oxidase enzyme that involves in ethylene biosynthesis. The transcript of PacACT1 (one β -actin cDNA fragment was cloned and designated as PacACT1, accession number FJ560908) was used to standardize for all expression (Fig.Q17-D).

Treatment	Firmness of pulp	Soluble solids content/titratable acidity	Anthocyanin(U.g^{-1})
Control	20.3a	14.4a	13.4a
Ethepron	19.6a	15.3a	14.4a
ABA	11.9b	16.8b	23.8b

(a and b show values that are significantly different).

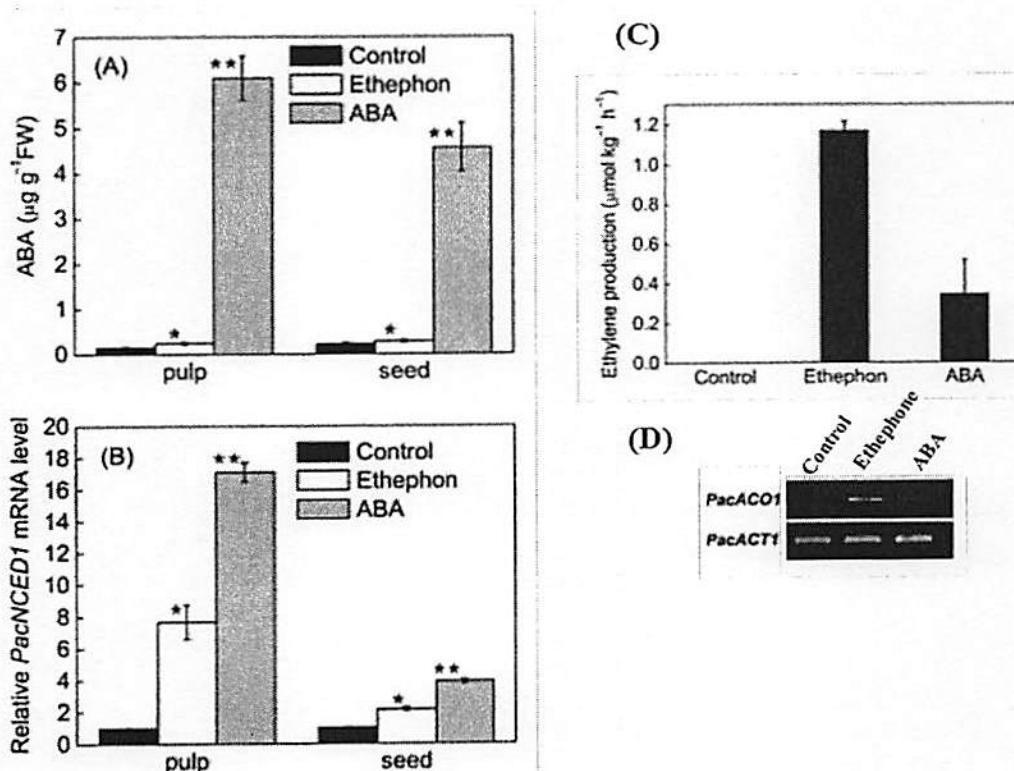


Fig. Q17. Effects of ABA and ethephon application on ABA content (A), accumulation of *PacNCED1* (B), ethylene production during ripening (C) and *PacACO1*(D).

Indicate in the **Answer sheet** if each of the following statements is True or False.

- Both ABA and Ethepron stimulate the expression of *PacACO1* and *PacNCED* genes in sweet cherry fruit.
- The expression of *PacNCED1* and ABA accumulation in pulp are higher than in the seed in the treatments of ABA and ethephon.
- ABA induces the maturation of sweet cherry fruit via stimulation of ethylene production.

D. Ethephon shows lower effect on anthocyanin and indigenous ABA production than exogenous ABA does.

Answer key

- A. True B. True C. False D. True

Explanation

A. True. As shown in fig. B and D, the expression of two genes is higher in the treatments with ABA and ethephon.

B. True. AS shown in fig.A and B, in the treatments with exogenous ABA and ethephon, the ABA content and PacNCED1 expression are higher than in the seed.

C. False. ABA induces the maturation of fruit (table), but it induces ethylene less than ethephon while ethephon does not stimulate fruit maturation.

D. True. In the treatment with ethephone, ABA content and anthocyanin content are lower than those in the treatment with exogenous ABA.

Reference

J Ren , P Chen , SJ Dai , P Li , Q Li , K Ji , YP Wang & P Leng, 2011.Role of abscisic acid and ethylene in sweet cherry fruit maturation: molecular aspects. New Zealand Journal of Crop and Horticultural Science, Vol. 39(3), 161-174,. DOI: 10.1080/01140671.2011.5634

ANIMAL ANATOMY AND PHYSIOLOGY

Q.18

A 55-year-old man has a resting cardiac output of 7000 mL/minute. His arterial pressure is 125/85 mmHg. His heart rate is 100 beats/min and his body temperature is normal. Figure Q.18 represents the changes in left ventricular pressure and blood volume during a cardiac cycle.

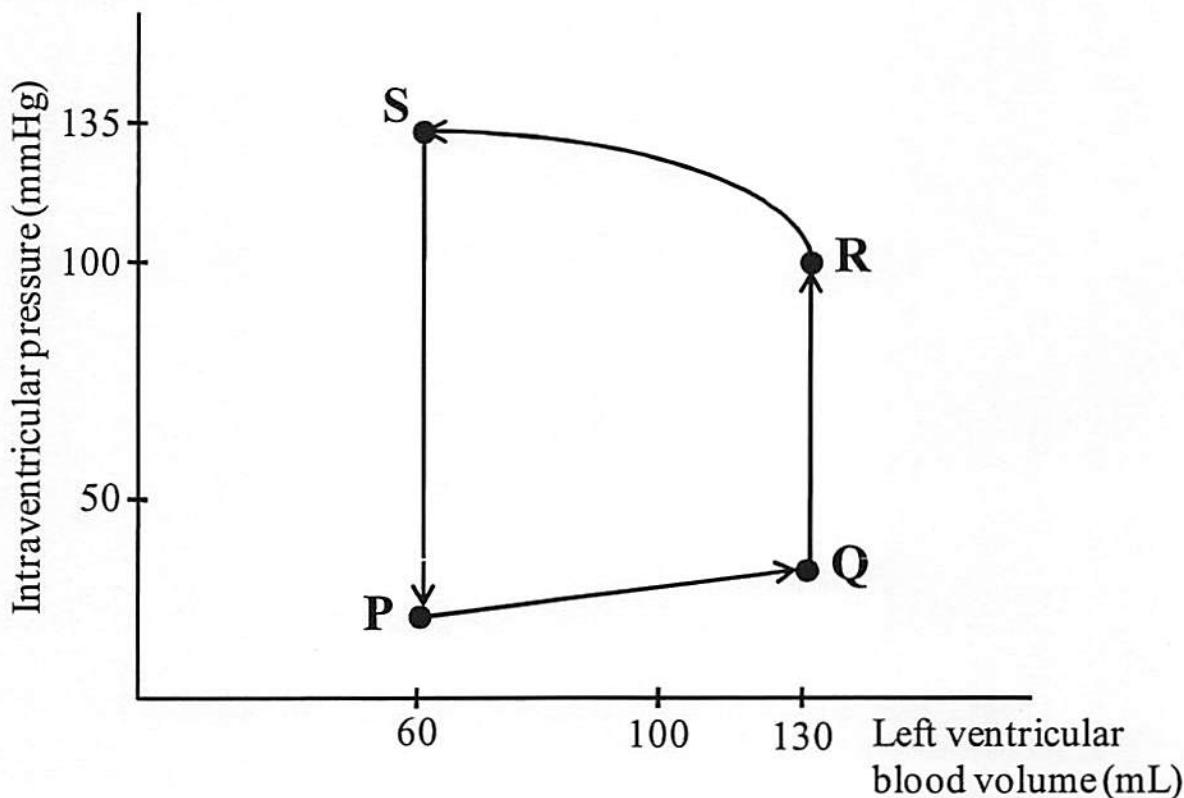


Figure Q.18

Indicate in the Answer sheet if each of the following statements is True or False.

- A. At Q, the left atrioventricular valve is opened.
- B. Ventricular ejection ends at S.
- C. The distance from P to S should be longer if there is aortic valve stenosis.
- D. In period R-S, blood does not flow into both atria and ventricles.

Answer key

- A. False
- B. True
- C. True
- D. False

Explanation

- A. False.** At Q, the left atrioventricular valve is closed after the ventricle is filled with blood and intraventricular pressure is low (P to Q is the period of ventricular filling).
- B. True.** Ventricular ejection ends at S because R – S is the period of ventricular ejection.
- C. True.** Aortic valve stenosis causes decreasing blood flows into the aorta and increasing pressure in the left ventricle. Thus, the distance from P to S is longer.
- D. False.** R-S is the period of ventricular contraction and atrial relaxation, blood does not flow into ventricles and flows into atria.

Reference

Guyton, A.C and Hall, J.E, *Physiology Review*, 2006, pp25

Q.19

Cardiac output (CO) is the volume of blood pumped by the heart in one minute. Cardiac output is affected by the stroke volume (SV) and the heart rate (HR).

Cardiac output can be measured indirectly using the Fick's equation: $CO = Q/(A-V)$, where Q is the rate of oxygen consumption (mL/min), A-V is the difference between oxygen concentration in the arterial blood (A) and in the venous blood (V).

The data below were measured from a healthy person before and during physical exercise.

Parameters	Before Exercise	During Exercise
Rate of oxygen consumption (Q)	250 mL/min	1500 mL/min
Oxygen difference (A-V) blood	50 mL/L blood	150 mL/L blood
Heart rate (HR)	60 beats/min	120 beats/min

Indicate in the Answer sheet if each of the following statements is True or False.

- A. Cardiac output increased by two-fold during exercise.
- B. Stroke volume during exercise was higher than that before exercise.
- C. Physical exercise caused a reduction in hemoglobin's affinity for oxygen, resulting in a three-fold increase in the amount of oxygen released to tissues.
- D. From the data above, it can be concluded that change in cardiac output during exercise is caused by the changes in both heart rate and stroke volume.

Answer key

- A. True
- B. False
- C. True
- D. False

Explanation

A. True. Cardiac output before exercise = $250 \text{ mL/min} / 50 \text{ mL/L blood} = 5 \text{ L blood/min}$.

Cardiac output during exercise = $1500 \text{ mL/min} / 150 \text{ mL/L blood} = 10 \text{ L blood/min}$.

Physical exercise increased cardiac output by $10 \text{ L blood/min} / 5 \text{ L blood/min} = 2$ times.

B. False. Stroke volume = CO/HR. The value was the same ($= 0.083 \text{ L}$) for both before and during physical exercise.

C. True. Most of the oxygen was carried by hemoglobin. Reduction in hemoglobin's affinity caused an increase in the amount of oxygen released from the blood to tissues. From the data, it can be seen that there was a 3 time increase in oxygen released to the tissues during exercise ($150 \text{ mL/L blood} / 50 \text{ mL/L blood}$).

D. False. From the data above, it can be concluded that the change in cardiac output during exercise was caused by the change in heart rate (120 beats/min compared to 60 beats/min) but not caused by the change in stroke volume (Stroke volume = CO/HR. The value was the same for both before and during exercise = 0.083 L).

Reference:

Guyton, A.C and Hall, J. E., 2006. Textbook of medical physiology. Elsevier Saunder.

Q.20

Figure Q.20 shows the models of four types of common human congenital heart defects.

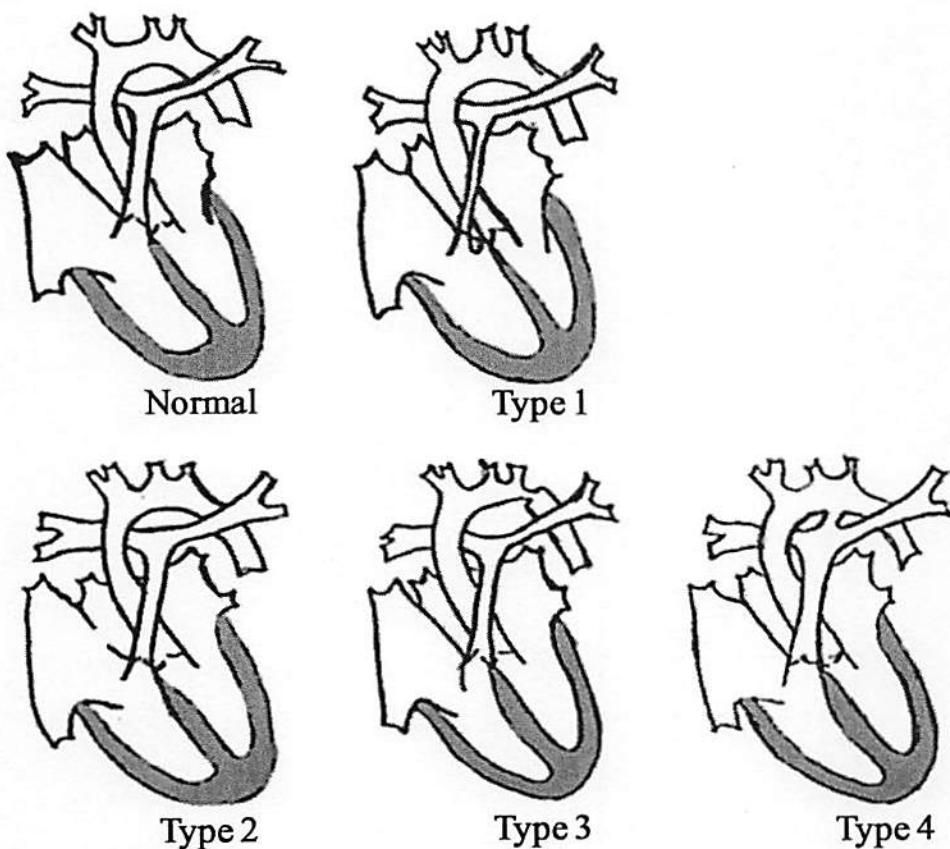


Figure Q.20.

Indicate in the Answer sheet if each of the following statements is True or False.

- A. In type 1, the blood volume going to the lungs is lower than normal.
- B. In type 2, the stroke volume of the left ventricle is increased.
- C. In type 3, the systolic blood pressure at the arms is higher than that in the normal type.
- D. In type 4, the pulmonary blood pressure is increased.

Answer key

- A. True B. False C. True D. True

Explanation

- A. True. Because of the narrow pulmonary artery, the blood going to the lungs is lower than normal.

- B. False. Type 2 shows a hole in the wall between the two atria. Hence, the blood flows from the left atrium to the right atrium, resulting in decreased stroke volume of the left ventricle.
- C. True. In type 3, there is a narrow region in aorta at the position behind the arteries to the arms. Thus, it increases the blood pressure in aorta in front of the narrow position.
- D. True. The blood from aorta goes through the ductusarteriosus to the pulmonary artery inducing increased pulmonary blood pressure.

References

1. Robert L, 2007. Genetics in medicine 7th edition. Saunders, Elsevier Inc. p. 169-171.
2. Bernard Thébaud, 2010. Patent ductusarteriosus in premature infants: A never-closing act. Paediatr Child Health, 15 (5), p. 267-270.

Q.21

Figure Q.21 shows how lung ventilation is affected by physical activity. As the intensity of exercise increases, humans respond to the increased need for gas exchange in two ways: increase in ventilation rate and increase in tidal volume. The experimental data for a runner on a treadmill are shown in Figure Q.21.

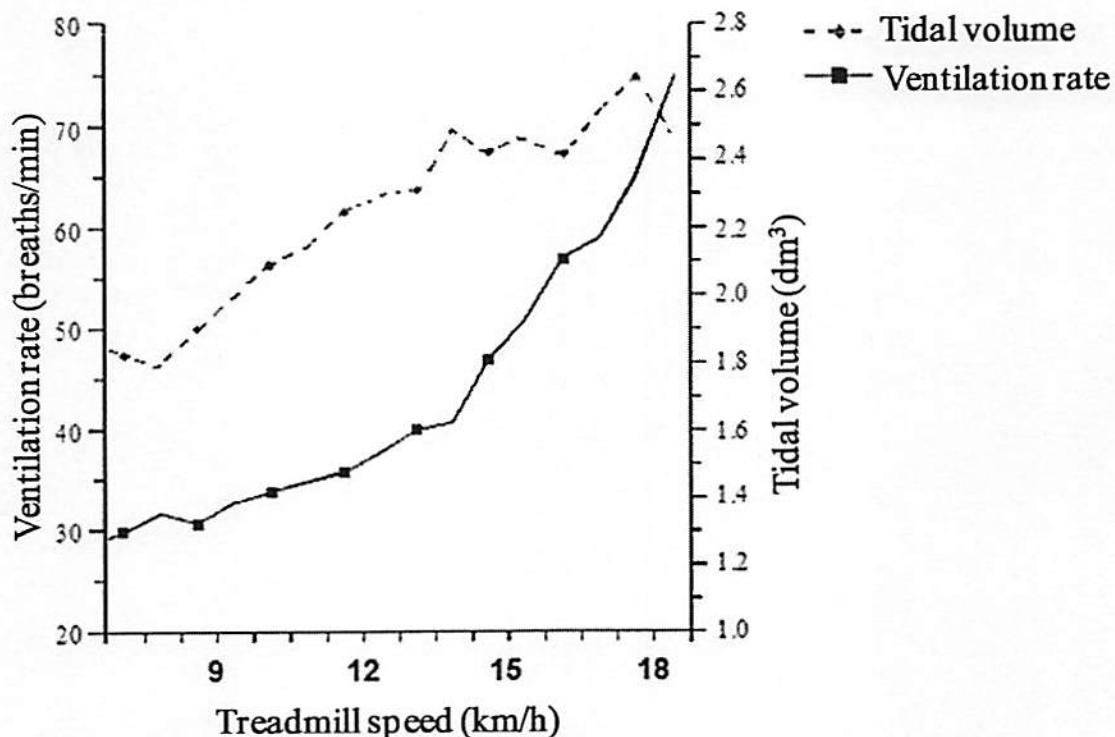


Figure Q.21

Indicate in the Answer sheet if each of the following statements is True or False.

- A. At the onset of physical exercise, the rate of ventilation increases before the depth of ventilation increases.
- B. In intense physical exercise (> 15 km/h treadmill speed), the increase in the ventilatory minute volume is mainly caused by increased ventilation rate.
- C. At the treadmill speed of 15 km/h, the ventilatory volume per minute is approximately 120 dm³.
- D. In an adult human, a tidal volume of 200 cm³ and ventilation rate of 30 breaths per minute can provide equally effective gas exchange as a tidal volume of 600 cm³ and a ventilation rate of 10 breaths per minute.

Answer key

- A. True B. True C. True D. False

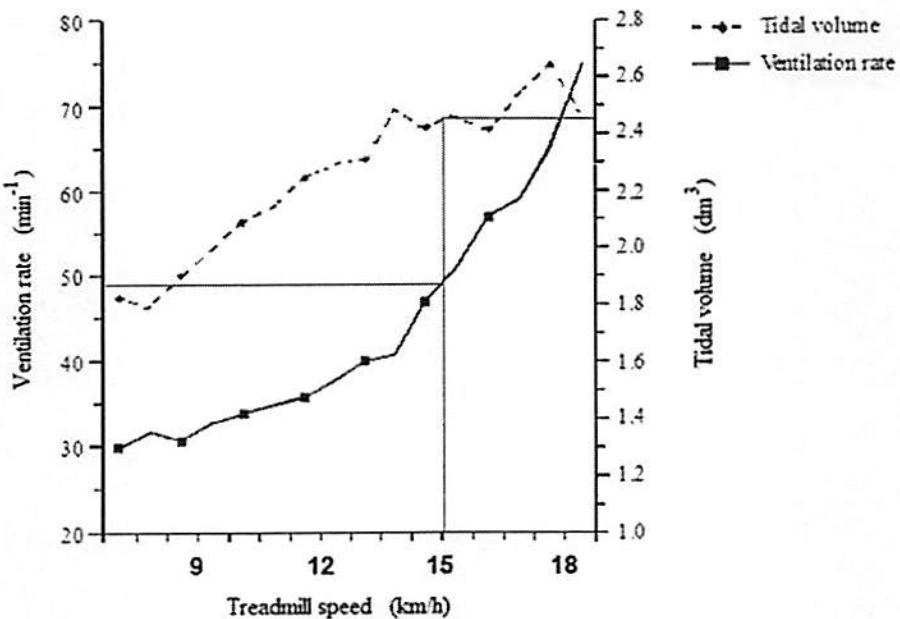
Explanation

A. True. At the onset of physical exercise, the rate of ventilation increases before there is an increase in depth of ventilation.

B. True. As can be seen in the diagram, it is the tidal volume that increases first at onset of exercise. At intense physical activity the increase in ventilatory volume per minute is mainly an effect of increased ventilation rate.

C. True. The tidal volume increases to a “plateau” at 2.4 dm^3 and an increase in ventilation rate is the only way of increasing the gas exchange.

At a treadmill speed at 15 km/h, the ventilatory volume per minute is approximately 120 dm^3 .



As can be read out from the diagram $2.46 \text{ dm}^3 \times 49 \text{ min}^{-1} = 120.5 \text{ dm}^3 \text{ min}^{-1}$

D. False. In the human lungs, a tidal volume of 200 cm^3 and ventilation rate of 30 per minute provides for an equally ventilatory volume per minute as a tidal volume of 600 cm^3 and a ventilation rate at 10 per minute.

Since the dead space of adult human respiratory tracts is about 150 cm^3 , ventilation of the alveoli is not as efficient at shallow breathing.

Ventilation rate	30 / min	10 / min
Tidal volume	200 cm ³	600 cm ³
Minute volume	6 000 cm ³	6 000 cm ³
Alveolar ventilation	1 500 cm ³	4 500 cm ³

Reference

Cycling Article: Physiology. Lung Physiology. pp.2

Q.22

In an experiment, a researcher isolated a neuron and placed it in the standard Ringer solution (isotonic physiological solution). He measured the resting membrane potential of the axon, then stimulated the axon and measured its action potential (Record A, Figure Q.22).

Subsequently, he repeated the experiment several times, each with a different modified Ringer solution. Figure Q.22 shows some of the results (Records B to E).

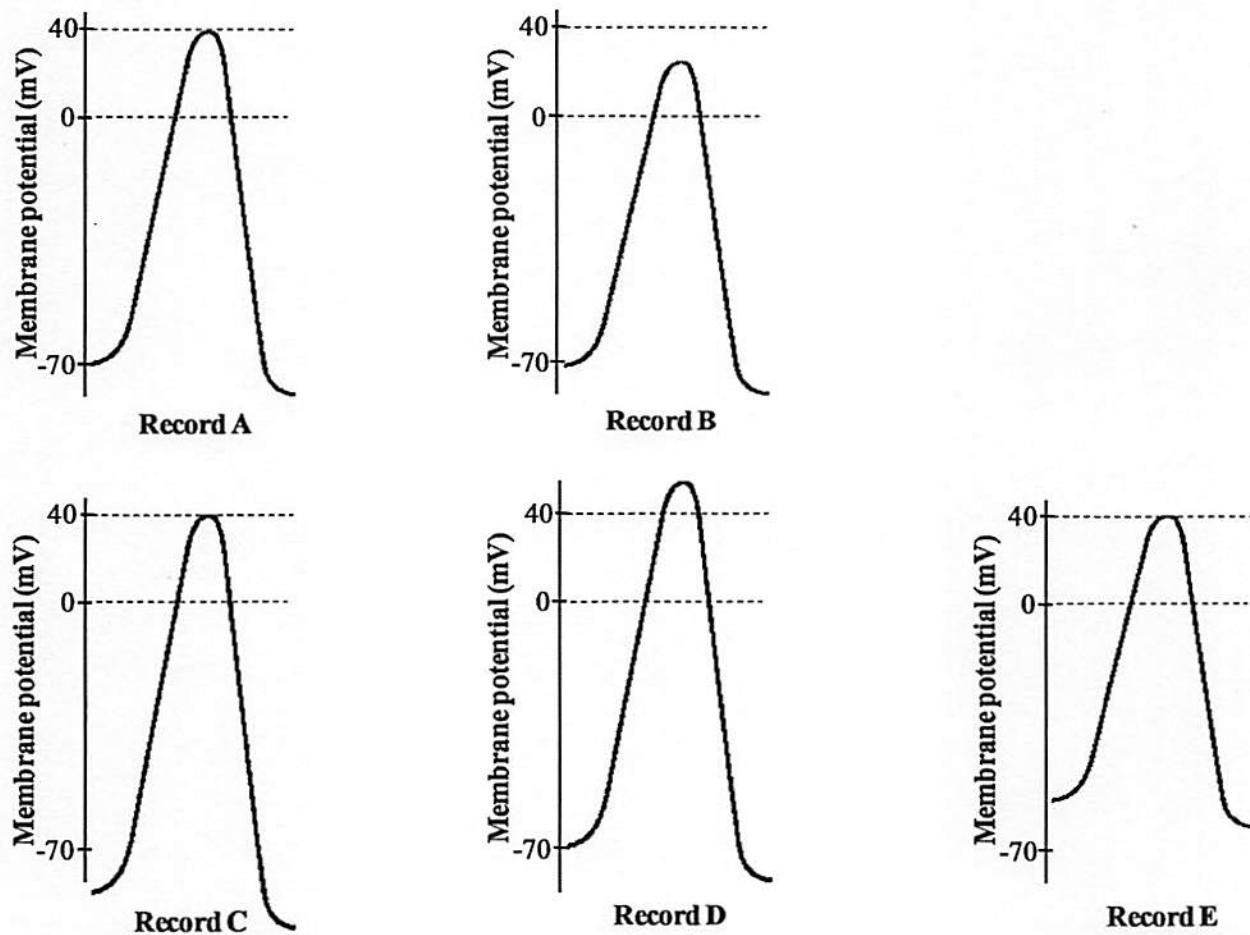


Figure Q.22

Indicate in the Answer sheet if each of the following statements is True or False.

- A. If the modified Ringer solution contained a lower concentration of Na^+ than the standard Ringer solution, the action potential is Record B.
- B. If the modified Ringer solution contained a lower concentration of K^+ than the standard Ringer solution, the action potential is Record C.

- C. If the modified Ringer solution contained a substance that increased membrane permeability to K^+ , the action potential is Record D.
- D. If the modified Ringer solution contained a substance that increased membrane permeability to Cl^- , the action potential is Record E.

Answer key

- A. True B. True C. False D. False

Explanation

- A. True.** Record B had a reduced overshoot (20mV vs 40mV). This is caused by a reduced concentration of Na^+ in the extracellular environment. Less Na^+ enters into the neuron.
- B. True.** Low K^+ concentration caused more negative resting potential. This is caused by more K^+ leaving the neuron.
- C. False.** Increased membrane permeability to K^+ caused more negative resting potential (because more K^+ leaving out the neuron), but did not cause an increased overshoot.
- D. False.** Increased membrane permeability to Cl^- led to more Cl^- enter to neuron resulting in more negative resting potential.

Reference

D. Penney, *Physiology Review*, 2004, pp18.

Q.23

Bats and birds are typical animal groups with flying form of locomotion. Compared with non-flying mammals of similar size and weight, birds and bats have a number of different physiological characteristics.

Indicate in the Answer sheet if each of the following statements is True or False.

- A. Despite having higher daily energy needs, birds have statistically shorter intestines compared to those of the non-flying mammals.
- B. The ratio of lung weight per body weight of birds is greater than that of the non-flying mammals.
- C. The speed of water absorption and excretion via the digestive tracts of bats is faster than that of the non-flying mammals.
- D. Although bats and the non-flying mammals have similar blood volume and hemoglobin's affinity for oxygen, bats have higher hematocrit level.

Answer key

- A. True B. False C. True D. True

Explanation

- A. True.** Despite having higher daily energy needs, birds have plenty amount of digestive enzymes allowing shorter their intestines compared to the non-flying mammals.
- B. False.** In birds, the characters of ventilation and lung structure make the gas exchange highly efficient. These are associated with the smaller the ratio of lung weight per body weight of birds compared to that in the non-flying mammals.
- C. True.** Bats rapidly absorb and excrete water. This is related with optimizing body weight in flying locomotion.
- D. True.** Bats have higher hematocrit level, leading to more oxygen concentration in the blood. This supplies more oxygen for flying activity.

References

1. Digestive Adaptations of Aerial Lifestyles. PHYSIOLOGY 30: 69 –78, 2015.

2. The structural and functional respiratory refinements in birds and bats. *The Journal of Experimental Biology* 203, 3045–3064 (2000).

Q.24

Polycystic ovarian syndrome (PCOS) is a common disorder of women characterized by increased levels of testosterone and by chronic failure in ovulation. The ovary can be stimulated to produce more testosterone when insulin levels in the blood are high.

Indicate in the Answer sheet if each of the following statements is True or False.

- A. PCOS patients are more likely to have acne than healthy people.
- B. PCOS patients have progesterone level higher than healthy people.
- C. Obese women have a higher risk of PCOS than normal-weight women.
- D. Follicle-stimulating hormone (FSH) and Luteinizing hormone (LH) can be used to increase the probability of PCOS women to conceive.

Answer key

- A. True
- B. False
- C. True
- D. True

Explanation

- A. True.** PCOS patients can become hyperandrogenic which causes acne.
- B. False.** The ovaries produce progesterone at the time of ovulation. Ovulation does not occur in PCOS patients.
- C. True.** PCOS develops due to the overproduction of testosterone from the ovaries. Obesity is usually accompanied by increased plasma insulin level, intensifying PCOS.
- D. True.** In PCOS women, an increase in FSH and LH can result in development of a mature follicle in the ovary and ovulation. Therefore, FSH can be used to increase the probability of PCOS women to conceive.

References

1. Andrea dunaif, 2013. Insulin Resistance and the Polycystic Ovary Syndrome: Mechanism and Implications for Pathogenesis. *Endocrine Reviews* 18(6): 774–800.
2. GambineriA, Pelusi C, Vicennati V, Pagotto U, Pasquali R, 2002. *Obesity and the polycystic ovary syndrome*. Int J ObesRelatMetabDisord, 26(7), p883-896.

3. Homburg R and Howles CM, 1999. *Low-dose FSH therapy for anovulatory infertility associated with polycystic ovary syndrome: rational, results, reflections refinements.* Hum. Reprod. Update 5 (5), p493-499.

Q.25

In the gills of freshwater teleost fishes, the blood plasma is separated from the freshwater by a thin epithelium so that the blood plasma tends to lose ions such as Na^+ and Cl^- to the ambient water across the gill epithelium and H_2O tends to enter the plasma from the ambient water across the gill epithelium. There are transport mechanisms by which inorganic ions and water cross the gill epithelium and they help to maintain the difference in the ion composition between the plasma and the ambient water. Figure Q.25 shows the transportation of four ions across the gill epithelium.

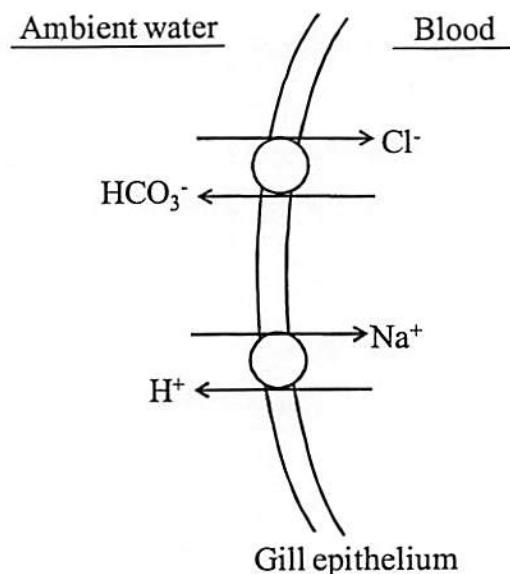


Figure Q.25

Indicate in the Answer sheet if each of the following statements is True or False.

- Inhibition of the Cl^- pump leads to an increase in blood pH.
- An increase in CO_2 produced by catabolism leads to an increase in Na^+ and Cl^- transports across the epithelium cell.
- A substance blocking the electron transport chain causes a decrease of Na^+ influx, but does not affect HCO_3^- outflux at the gill epithelium.
- During alkalosis, the epithelial cell increases the synthesis of a key $\text{Cl}^-/\text{HCO}_3^-$ countertransport protein.

Answer key

- A. True B. True C. False D. True

Explanation

- A. True.** Inhibition of the Cl^- pump causes an increase of HCO_3^- in the blood, leading to increased blood pH.
- B. True.** HCO_3^- and H^+ ions are generated by reaction of metabolically produced CO_2 with H_2O . An increase in CO_2 will lead to an increase in countertransports of H^+/Na^+ and $\text{HCO}_3^-/\text{Cl}^-$ across the gill epithelium.
- C. False.** When the electron transport chain is blocked, ATP production is decreased causing a decrease of Na^+ influx and HCO_3^- outflux, because both Na^+/H^+ and $\text{HCO}_3^-/\text{Cl}^-$ pumps use ATP.
- D. True.** During alkalosis, the epithelial cell increases the synthesis of a key $\text{Cl}^-/\text{HCO}_3^-$ countertransport protein that exports HCO_3^- from the body fluids in exchange for Cl^- .

Reference

R.W.Hill, G.A.Wyse, Animal Physiology, 2004. pp 688-689.

Q.26

Two groups of students separately performed an experiment on kidney function. Thirty minutes before the experiment, each student in one group was instructed to drink 500 mL of water, while each student in the other group was instructed to drink 100 mL of water. At $t = 0$ minute, each student in both groups was instructed to drink 750 mL of water. Each student was then asked to urinate as he or she would normally do without attempting to manipulate the speed or flow in any way at the different time points shown in Figure Q.26. An electronic uroflowmeter was used to measure the urine flow rate. The Cl^- concentration in each urine sample was measured. Figure Q.26 shows the data of the experiment.

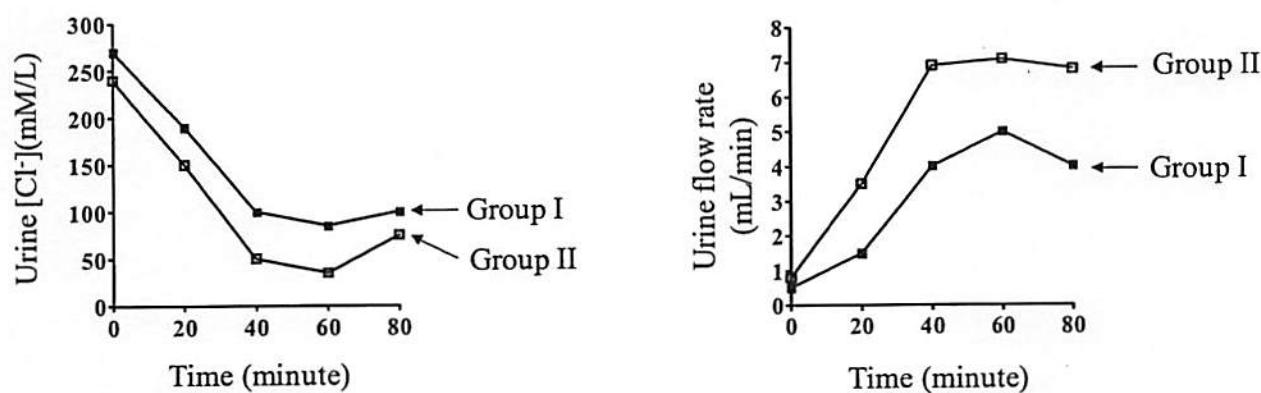


Figure Q.26

Indicate in the Answer sheet if each of the following statements is True or False.

- The reabsorption of water by nephrons of the students in Group II at $t = 60$ min was lower than that of the students in Group I.
- The plasma aldosterone concentration of the students in Group I was the highest at $t = 60$ min.
- The students in Group I drank 500 mL of water 30 minutes before the experiment.
- The students in Group II produced about 140 mL of urine during the period between $t = 40$ min and $t = 60$ min.

Answer key

- A. True B. False C. False D. True

Explanation

A. True. The urine flow rate of the students in Group II at $t = 60$ min was higher than that of students in Group I. This is consistent with the possibility of reabsorption of water by nephrons of the students in Group II was lower than that in the students of Group I.

B. False. At $t = 60$ min, the urine flow rate of the students in Group I was the highest. This was associated with the low level of aldosterone.

C. False. The urine flow rate was lower in Group I compared to that in Group II throughout the experiment. Furthermore, the urine Cl^- concentration was higher in Group I compared to that in Group II throughout the experiment. Therefore, it is most likely that the students in Group I drank less water than the students in Group II before the experiment.

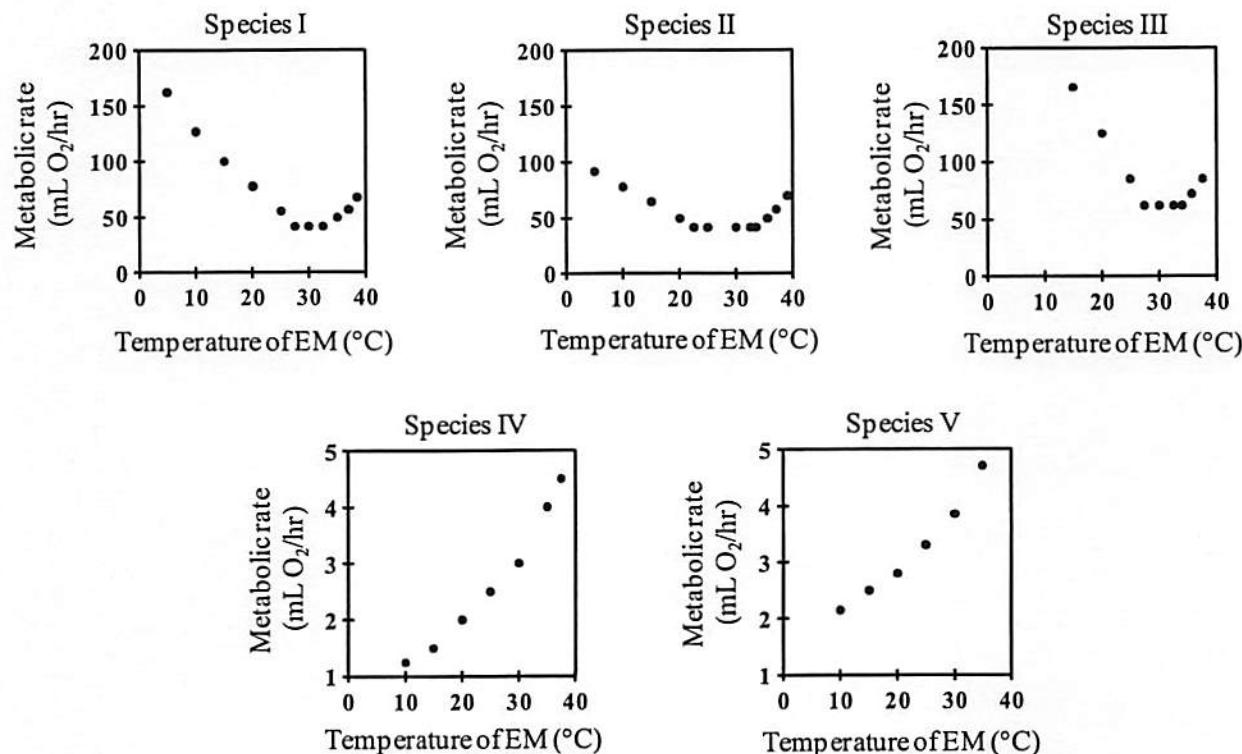
D. True. During the period $t = 40$ to 60 minute, the urine production rate was 7 mL/min. Therefore, urine output generated approximately $= (60 - 40) \times 7 = 140$ mL.

Reference

Yadi Xu. Urinary Function Lab Report. BIOL 302 Section 12 March 1, 2012.

Q.27

Figure Q.27 shows the changes in metabolic rates of adult individuals of five animal species in response to temperature changes of the external environment (EM). The individual body weights of the five species were similar (about 30 grams). The data were measured from the animals when they were staying at rest.

**Figure Q.27**

Indicate in the Answer sheet if each of the following statements is True or False.

- A. Species IV was an ectothermic animal.
- B. Species II had the highest thermo-insulating ability among the five species.
- C. Species III possessed the highest basal metabolic rate among the five species.
- D. An increase in body temperature in species V was mainly dependent on metabolism.

Answer key

- A. True B. True C. True D. False

Explanation

- A. True.** The metabolic rate of species IV was positively related to the changes in temperature of the external environment.
- B. True.** Species II showed the least fluctuation in metabolic rate in response to the changes of environmental temperature. This means that species II had the highest thermo-insulating ability among the five species.
- C. True.** The basal metabolic rate is the minimum metabolic rate to maintain the basal function of the cells and the bodies. Among the five species above, species III had the highest minimum metabolic rate.
- D. False.** Species V was an ectothermic animal whose the increase in body temperature was mainly dependent on the increase in temperature of external environment.

References

1. D. Sadava, 2012, Life: the science of biology. 9th edition, pp 1028.
2. John R. Speakman. Measuring energy metabolism in the mouse – theoretical, practical, and analytical considerations. Frontiers in Physiology, 2013. pp 9.

Q.28

BDNF, a brain protein, is crucial for neuron activities. BDNF is activated during the activation of neurons of the limbic system. An important function of BDNF in mammals is shown in Figure Q.28. The signal via the catecholamine receptor (β -AR) is specific for the browning process and thermogenesis.

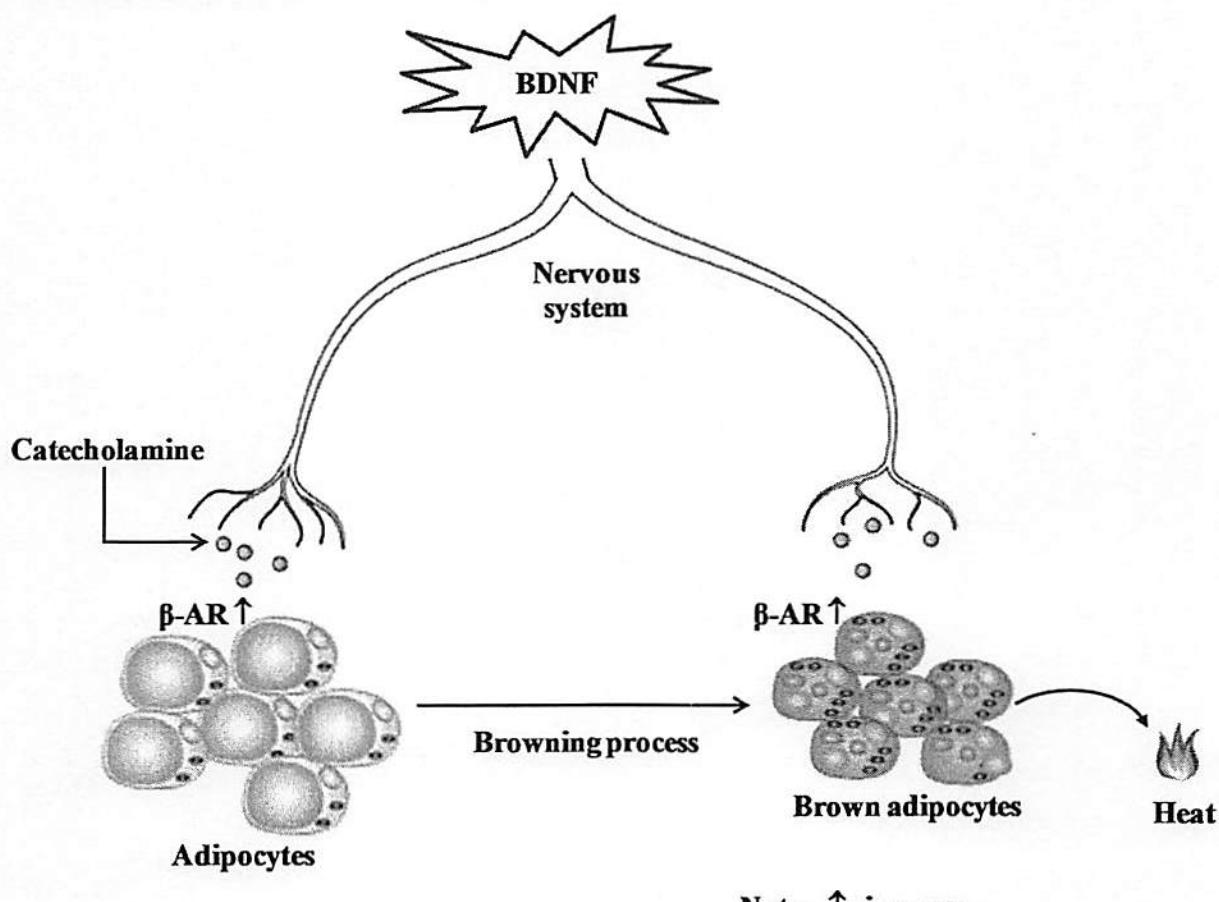


Figure Q.28.

Indicate in the Answer sheet if each of the following statements is True or False.

- A. Regularly learning and memorizing activities help to increase the number of brown fat cells.
- B. Inhibition of BDNF expression reduces the size of white adipose tissue.
- C. Psychological anxiety increases the manifestation of the β -AR.
- D. β -AR gene-deleted mice will display decreases in the plasma levels of free fatty acids.

Answer key

A. True B. False C. True D. False

Explanation

A. True. Regularly learning and memorizing activities activate neurons of the limbic system. This activation enhances the activity and expression of BDNF. Therefore, it increases browning process.

B. False. Inhibition of BDNF expression reduces the switch of white fat into brown fat. Thus the accumulation of white fat increases.

C. True. Psychological anxiety activates the sympathetic nervous system which in turn releases catecholamines, leading to increased expression of β -AR.

D. False. β -AR gene-deleted mice do not have signal to activate brown fat cells to use free fatty acids, leading to increase in plasma levels of free fatty acids. Moreover, increased white fat mass liberates more free fatty acids.

References

1. Kataoka N. *et al.* Psychological stress activates a dorsomedial hypothalamus-medullary raphe circuit driving brown adipose tissue thermogenesis and hyperthermia. *Cell Metab.* 2014 Aug. 520(2): pp.346–58.
2. Lei Cao *et al.* White to Brown Fat Phenotypic Switch Induced by Genetic and Environmental Activation of a Hypothalamic-Adipocyte Axis. *Cell Metab.* 2011 Sep.14 (3), pp.324–338.

Q.29

The skeletal muscle fibers are divided into three types depending on the speed and energy sources of muscle contraction:

- Type I: Slow twitch, oxidative muscle fibers.
- Type IIa: Fast twitch, oxidative muscle fibers.
- Type IIb: Fast twitch, glycolytic muscle fibers.

Figure Q.29 shows the correlation of mRNA expression levels of the genes *Myh7*, *Myh2*, and *Myh1* specific for muscle fiber types I, IIa, and IIb, respectively, in human skeletal muscles of legs: quadriceps, gastrocnemius, and soleus muscles.

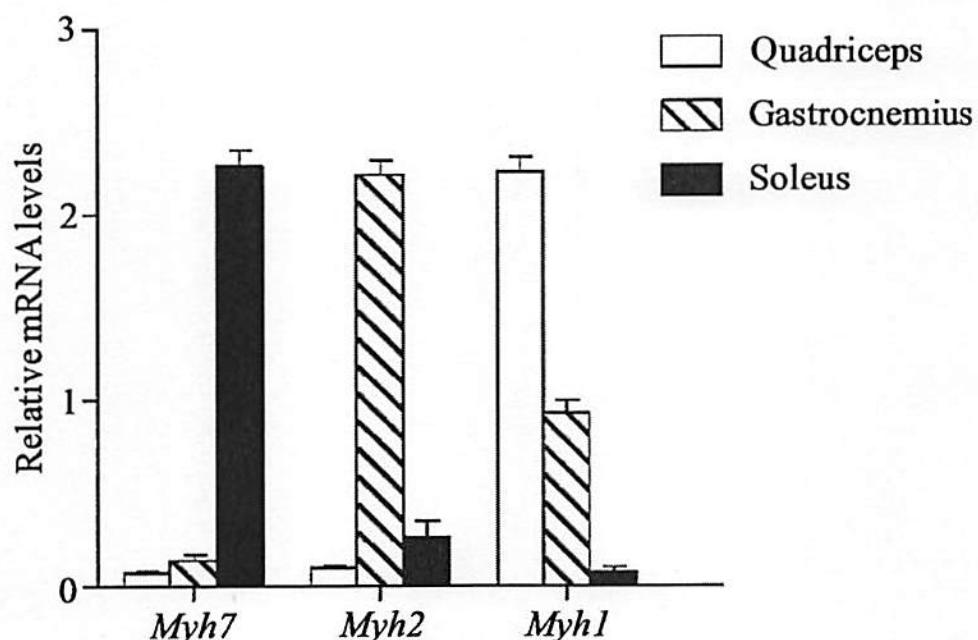


Figure Q.29.

Indicate in the Answer sheet if each of the following statements is True or False.

- A. The soleus muscles of the sprinter athletes are prone to be more developed than those in the marathon athletes.
- B. The ratio of mitochondria number per muscle mass in the quadriceps muscle is less than that in the gastrocnemius muscle.
- C. The soleus muscle contains less sarcoplasmic reticulum than the gastrocnemius muscle does.

D. Regularly doing the endurance exercise for a long period of time can increase the number of glycolytic muscle fibers in the gastrocnemius muscles.

Answer key

A. False B. True C. True D. False

Explanation

A. False. The soleus is slow twitch muscle that displays the highest *Myh7* gene level. This muscle, thus, is well developed in the athletes with endurance exercise such as marathon athletes.

B. True. The expression level of *Myh2* in the quadriceps is lower than that in the gastrocnemius. Hence, the ratio of mitochondria number per muscle mass in the quadriceps muscle is less than that in the gastrocnemius muscle.

C. True. The soleus muscle contains mainly slow twitch muscle fibers characterized by relatively fewer endoplasmic reticulum than does the gastrocnemius muscle that contains mainly fast twitch muscle fibers.

D. False. Regularly doing the endurance exercise after a long period of time can induce the muscle fiber switch from glycolytic to oxidative metabolic phenotype.

References

1. BMC Genomics. 2010 Mar 15;11:176. doi: 10.1186/1471-2164-11-176.
2. Nature vs. nurture: can exercise really alter fiber type composition in human skeletal muscle? Journal of Applied Physiology Published 1 November 2004 Vol. 97 no. 5, 1591-1592

Q.30

Figure Q.30A shows an insulin secretion and the mechanism by which insulin stimulates glucose absorption into cell. The mechanism includes four steps depicted by the four circled digits 1 to 4.

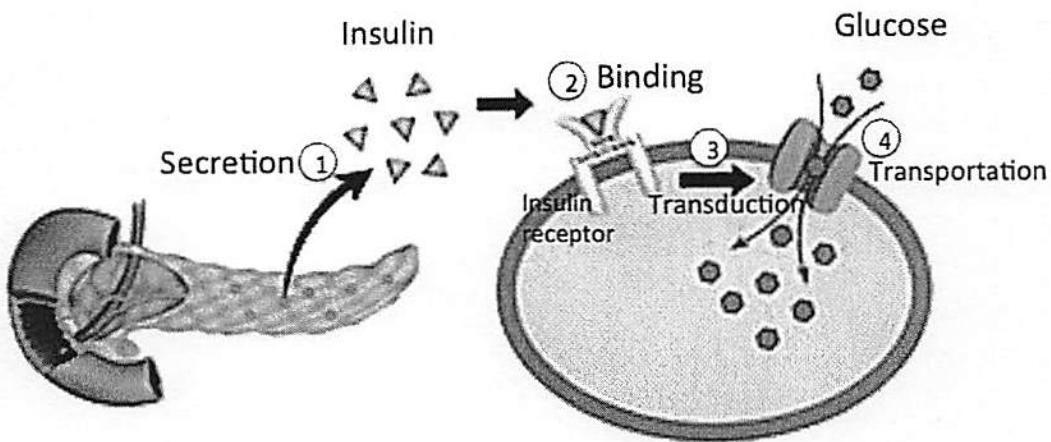


Figure Q.30A.

Four patients (E, F, G and H) had a defect each in a single step of the above mechanism. Patients E, F, G and H had defect in steps 1, 2, 3 and 4, respectively. These patients were given two tests:

- Test 1: Muscle cells from each patient were isolated and the percentage of cells in different concentrations of insulin was determined (Figure Q.30B).
- Test 2: Each patient was injected with same insulin quantity related to their body mass and their plasma glucose concentrations were then measured at various times after injection (Figure Q.30C).

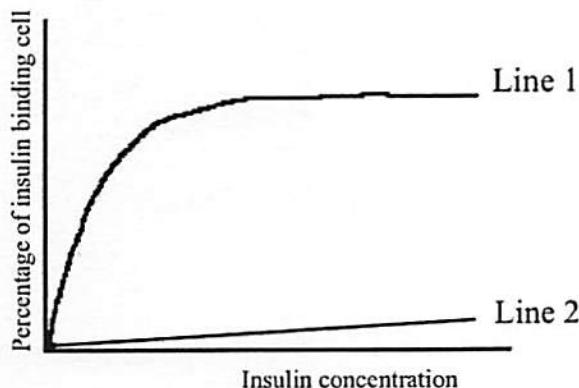


Figure Q.30B.

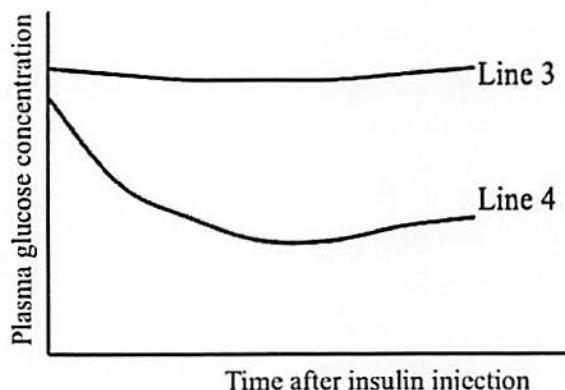


Figure Q.30C.

Indicate in the Answer sheet if each of the following statements is True or False.

- A. The result of Test 1 of Patient G could be shown in Line 1.
- B. Lines 2 and 3 show the results of Tests 1 and 2, respectively, of Patient F.
- C. Line 3 shows the tested result of Patient E.
- D. Lines 1 and 4 show the results of Tests 1 and 2, respectively, of Patient H.

Answer key

- A. True
- B. True
- C. False
- D. False

Explanation

A. True. The binding between insulin and its receptor was normal in Patient G. Therefore, the percentage of insulin-binding cells increased rapidly when the insulin concentration increased. However, the percentage did not elevate later even though the insulin concentration increased since most of the receptors were already bound to insulin (Line 1).

B. True. The binding between insulin and its receptor was defective in Patient F. Therefore, the percentage of insulin-binding cells was lower than normal as the same insulin concentration (Line 2). Thus, insulin failed to decrease plasma glucose in this patient (Line 3).

C. False. The insulin secretion was defective in Patient E. Hence, the plasma glucose level should have reduced after insulin was injected. This means that Line 3 is not the tested result of Patient E.

D. False. The binding between insulin and its receptor was normal in Patient H (Line 1). The glucose transportation into the cells was defective in Patient H. Therefore, plasma glucose level should not have reduced markedly after insulin was injected. That means Line 4 is not the tested result of Patient H.

Reference

Gisela Wilcox, 2005. Insulin and Insulin Resistance. *ClinBiochem Rev.* 2005, 26 (2), p. 19–39.

ETHOLOGY**Q.31**

The territorial behavior of the red-whiskered bulbul (*Pycnonotus jocosus*) was studied during pre-nesting and nesting periods. Songs of decoys were played from ten different locations around a farmhouse, where the test birds lived, and the time taken to display territorial behavior by the resident male measured at the different locations (Table Q.31). The resident bird defended the territory with aggressive calls, threat display and tried to attack the decoy.

Based on this information, indicate in the answer sheet if each of the following statements about the behavior of the birds is true or false.

Table Q.31

Stations (code, distance, direction)										
C	I	K	G	H	B	F	J	D	E	
20 m	50 m	110	30 m	50 m	19 m	46 m	72 m	29 m	38 m	
N	N	m	S	S	E	E	E	W	W	
N										
Pre-nesting										
Territory	+	+	-	+	-	+	+	-	+	-
defense	1	3	> 10	5	> 10	1	3	> 10	4	> 10
Time spent										
Nesting										
Territory	+	+	-	+	-	+	+	-	+	-
defense	0.2	1	> 10	0.7	> 10	0.3	2.5	> 10	1	> 10
Time spent										

Mean times spent = time taken to defend territory (min). (+) signifies territory defense whereas (-) stands for "no territory defense". N= north, S= south, E= east and W = west. (Sotthibandhu 2003)

- A. The territory size during the pre-nesting period was smaller than that during the nesting period.
- B. The quadrangle made by GDIF stations marks the territory size of the bird.

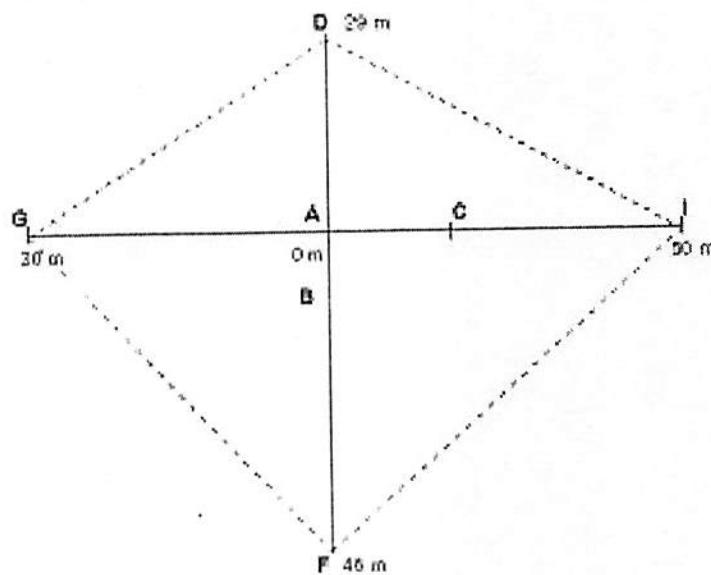
- C. The male responded more rapidly to the decoy within its territory during the nesting period.
- D. The intensity of territorial behavior displayed is dependent on seasonal fluctuation of hormones.

Answer key

A. False B. True C. True D. True

Explanation

- A. False. The territory size during the pre-nesting period and during the nesting period is the same, but the red-whiskered bulbul exhibited a stronger behavior during the nesting period than during the pre-nesting period.
- B. True. Based on data in given table 1, we can see in G station (in the south, 30m far from the farm house) the male bird still defends his territory, but in the H station farther in the south (50 m far from the farm house), the male bird did not defend the territory. Similar situations we can see in the stations F (46 m far from the farm house in the east) and station D (29 m far from the farm house in the west). Therefore, the quadrangle made by GDIF stations could mark the territory size of the bird is as showed below:



- C. True. Data on the table show clearly that the male responded more rapidly to the decoy in the nesting period.
- D. True. Sex hormones play important role in territory behavior.

Reference: Territorial defense of the red-whiskered bulbul, *Pycnonotus jocosus* (Pycnonotidae), in a semi-wild habitat of the bird farm Sunthorn Sotthibandhu Songklanakarin J. Sci. Technol., 2003, 25(5) : 553-563

Q. 32

Female vampire bats live in colonies made up of unrelated females and their offspring, and they feed exclusively on blood of herbivores. The fully-fed bats often share some of the blood that they collected with bats that are starving, and are more likely to receive blood from these individuals when they themselves starve.

Indicate in the answer sheet if each of the following statements about this behavior of bats is mostly true or false.

- A. Kin selection has an important role to play in the evolution of sharing behavior.
- B. Vampire bats are likely to live in colonies only for short periods of time.
- C. The bats showing this behavior have higher indirect fitness than if they would not do so.
- D. Vampire bats are able to recognize and remember other individual bats.

Answer key

- A. False B. False C. False D. True

Explanation

- A. False. Kin selection works in some eusocial species, like bees, ants, where individuals in the colonies are super-sisters. In the case of vampire bats they live in colonies made of unrelated females.
- B. False. The sharing behavior of bats is reciprocal altruism, and so the donors have higher chance to receive a donation of blood from other bats in their colony when they are starving, but only when they have been associated for long and know each other.

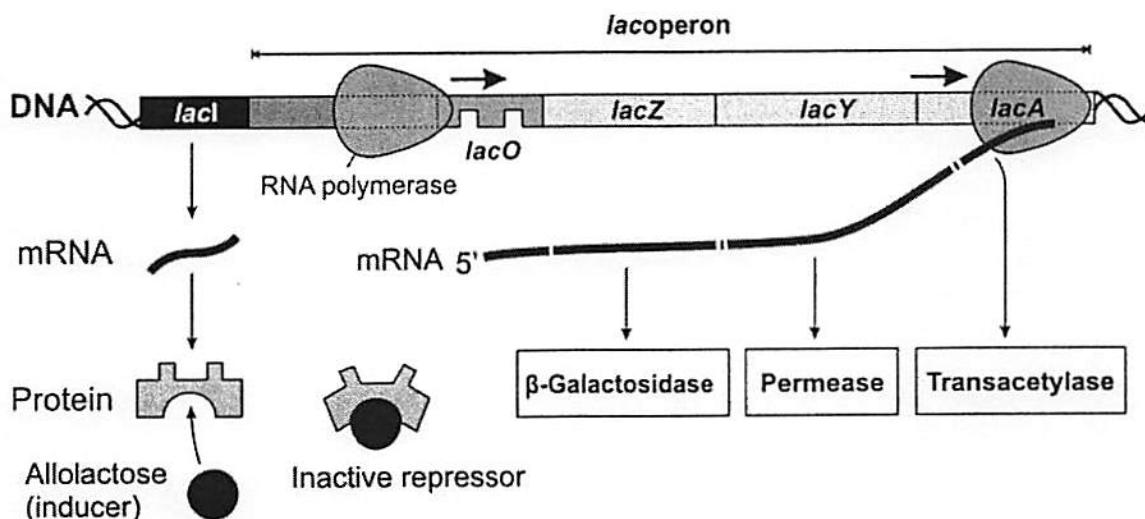
- C. False. Indirect fitness is an individual's fitness plus the additional fitness that it gets by helping genetically related individuals to breed. Vampire bats live in colonies made up unrelated females, and so only have direct fitness if they can survive in hard times.
- D. True. Vampire bats can recognize and remember other individual bats so that to make sure that other bats can't cheat.

Reference: Greg Krukonis, PhD; Tracy Barr (2008). Evolution for dummies

GENETICS AND EVOLUTION

Q. 33

Long had ten strains of *Escherichia coli* with different mutations in their *lac* operon. Analyzing their DNA, he found that each strain is one of five mutation types: *lacZ*, *lacY*, *lacI*, *lacIS* (the repressor LacI^S can bind to the operator but cannot bind to the inducer), or *lacOc* (the repressor cannot bind to mutated operator *lacOc*). Long also knew that strain number 6 is a *lacZ*⁻ mutant. The *lac* operon is shown below.



Long isolated DNA containing the *lac* operon from each strain (donor) and transformed it into other strains, thus making the strain merodiploid (recipient). Thereafter recipient strains were cultured on minimal medium that contained lactose as the only carbon source. Growth of the strains was recorded in the table below (+ means that the strain is growing, - means that the strain is not growing)

	2	-							
Recipient colonies	3	-	+						
	4	-	+	+					
	5	+	+	+	+				
	6	-	+	+	+	+			
	7	-	+	+	+	+	-		
	8	-	-	-	-	+	-	-	
	9	+	+	+	+	+	+	+	
	10	-	+	-	+	+	+	-	+
	1	2	3	4	5	6	7	8	9
Donor colonies									

Indicate in the answer sheet if each of the following statements is true or false.

- A. Strain number 7 is a *lac Z* mutant.
- B. Strain number 3 is a *lac Y* mutant.
- C. Strains number 2 and 4 are of the same mutation type.
- D. If strain number 5 receives DNA from itself, the transformed bacteria cannot grow.

Answer key:

- A. True
- B. True
- C. True
- D. False

Explanation

When both glucose and lactose are not present, repressor LacI binds to *lacO* and represses transcription of *lac* operon. However, repression is not complete and the *lac* operon is still expressed at extremely low level, producing several molecules of three enzymes in each bacterial cell. When lactose becomes available, it is transported into the cell by permease and converted to allolactose by β -galactosidase. Allolactose binds to repressor and prevent it from binding to *lacO* thus derepressing *lac* operon.

Mutated repressor LacI^S cannot bind to operator *lacO* therefore *lac* operon is not repressed. Repressor LacI^S cannot bind to allolactose (*lac* operon inducer) but still can bind to *lacO* thus *lac* operon is always repressed. In merodiploid *lacI^S/lacI⁺* or *lacI^S/lacI*, repressor LacI^S still binds to *lacO* and represses *lac* operon. *lacI^S* is dominant, *lacI*

and $lacI$ are recessive. Mutated operator $lacO^c$ is not bound by LacI, LacI^r, or LacI^s therefore lac operon is not repressed.

Colonies are mutated in only one of four genes/sequences $lacI$, $lacO$, $lacZ$ or $lacY$, other genes/sequences are normal. When DNA from a *lac* mutant is transformed in to another *lac* mutant, it may complement function of mutated gene of recipient cell. In that case, the mutated genes of the two mutants are different genes. Otherwise mutants A and B are mutated in the same gene. In case of regulatory genes or sequences, nature of mutations may complicated interpretation of complementation test results. $lacI^s$ mutant, for instance, cannot be complemented by any of the above mentioned mutations but $lacO^c$. Presence of DNA from colony number 5 or 9 conferred all cells ability to utilize lactose, therefore, colony number 5 and 9 are $lacO^c$ mutants. Extra copy of $lacO^c$ mutant's DNA does not affect colony number 5 ability to grow on lactose, therefore, **statement D is false.**

With the exception of colonies number 5 and 9, presence of DNA from colony number 1 represses *lac* operon expression in all other colonies, thus colony number 1 is a $lacI^r$ mutant.

With the exception of colonies number 5 and 9, colony number 8 was not complemented by any other colonies' DNA, therefore colony number 8 is also a $lacI^s$ mutant.

With the exception of colonies number 1 and 8 ($lacI^s$ mutants), presence of DNA from colony number 2 or 4 enables cells using lactose, thus colonies 2 and 4 are $lacI$ mutants.

Statement C is true.

DNA from colony number 6 (a $lacZ$ mutant) did not complement mutated gene of colony number 7 while that of other colonies did (colony number 7 is not a $lacI^s$ mutant), therefore colony number 7 is a $lacZ$ mutant. **Statement A is true.**

DNA from colony number 3 complemented $lacZ$ gene of colony number 6 and 7, and did not complement $lacI^s$ of colony number 8, suggesting that colony number 3 is neither $lacZ$ nor $lacI^s$ mutant. DNA from colony 1 (a $lacI^s$ mutant) suppressed *lac* operon of colony number 3, thus colony number 3 is not a $lacO^c$ mutant. Colony number 3 is either $lacY$ or $lacI$ mutant. *lac* operon of colony number 10 is expressed when receiving DNA from a $lacZ$ mutant (colony number 6 or 7) but not that from colony number 3. Therefore, colony number 3 is not a $lacI$ mutant. Colony number 3 is $lacY$ mutant.

Statement B is true.

References:

McCauley, S. J., Rowe, L., & Fortin, M.-J. (2011). The Deadly Effects of “Nonlethal” Predators. *Ecology*, 92 (11), 2043-2048.

Q. 34

Long analyzed DNA samples from three families using six loci of short tandem repeat (STR) on six different autosomal chromosomes. Each STR locus has two alleles which are usually different from each other (the number in the table below e.g 3/5). In the first family, the father is Hung, the mother is Huong and their son is Dung. In the second family, the father is Nhan and his two sons are Tin and Nghia. In the third family the father is Phu, and his son is Quy. Long also included a DNA sample from Dat who is unrelated to any of the three families. Tubes were randomly encoded with number but the key was lost except for Huong's sample.

STR loci

	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6
DNA samples						
Huong	3/5	2/2	5/6	3/3	1/2	2/6
669	3/5	2/7	4/9	5/8	3/7	4/5
297	1/5	3/3	3/3	6/9	2/7	4/8
653	1/5	2/2	6/6	3/7	2/9	4/5
735	5/7	2/4	5/5	3/4	2/2	1/2
130	5/7	7/7	5/9	3/8	2/7	4/5
860	1/6	2/3	3/5	6/7	1/7	2/8
938	3/7	4/5	5/6	4/4	2/3	1/2
264	3/7	7/7	1/4	5/9	7/9	3/4

Indicate in the answer sheet if each of the following statements is true or false.

- A. Sample 735 is Dat's DNA
- B. Sample 669 is Nhan's DNA
- C. Sample 938 is Hung's DNA
- D. Sample 297 can be Phu's DNA

Answer key

- A. False
- B. True
- C. True
- D. True

Explanation

STR locus is composed of tandem repeats of several-nucleotide units flanking by two conserved sequences where PCR primers annealing to. Different STR alleles are different in numbers of repeated units, thus different in length. Each STR locus usually has multiple alleles. Combination of several polymorphic STR loci can be used as an individual's DNA fingerprint. With a good combination of STR loci, it is extremely rare for two unrelated people have the same STR record.

By studying presence of two alleles at each STR locus, it is found that sample 735 is related to Huong and sample 938. Therefore sample 735 cannot be Dat, who is not related to anyone. **Statement A is False.**

Sample 669 is related to sample 130 and 264. There are only two families with three members among analyzed samples. One is Hung, Huong and Dung family and the other is Nhan, Tin and Nghia family. Therefore samples 669, 130 and 264 belong to Nhan, Tin and Nghia. Tin or Nghia each received an allele from Nhan and Nhan must be able to pass different alleles to his two sons. At locus 3, sample 669 has two alleles 4 and 9, sample 130 has 5 and 9, and sample 264 has 1 and 4. Sample 669 possibly pass allele 9 to sample 130 and allele 4 to sample 264. Neither sample 130 nor 264 would be able to pass two different alleles to the other two. Similarly for locus 4, only sample 669 would be able to pass two different alleles to samples 130 and 264. Therefore, sample 669 is Nhan. **Statement B is true.**

As mentioned above, samples 735, sample 938 and Huong are related. Dung is Hung and Huong's son. For each locus, Dung will receive one allele from Huong and the other allele from Hung. Two alleles at each locus of sample 735 can be accounted for by alleles from sample 938 and Huong. While sample 938 cannot receive allele 5 of locus 2, allele 3 of locus 5 from either Huong or sample 735, therefore sample 938 is Hung's and sample 735 is Dung's. **Statement C is true.**

Samples 297 and 860 are related. However, without a third related person, it is impossible to identify which sample belongs to the father, Phu, or to the son, Quy. Therefore, sample 297 can be either Phu or Quy. **Statement D is true.**

References:

- McCauley, S. J., Rowe, L., & Fortin, M.-J. (2011). The Deadly Effects of "Nonlethal" Predators. *Ecology*, 92 (11), 2043-2048.

Q.35

Centromere position can be mapped using linear tetrads in some fungi. If there is no cross-over between gene E and the centromere, four spores are arranged in sequence of eeEE or EEee (Fig.Q.35A). If there is a cross-over between gene E and the centromere, four spores are arranged in sequence of eEeE or EeEe (Fig.Q.35B).

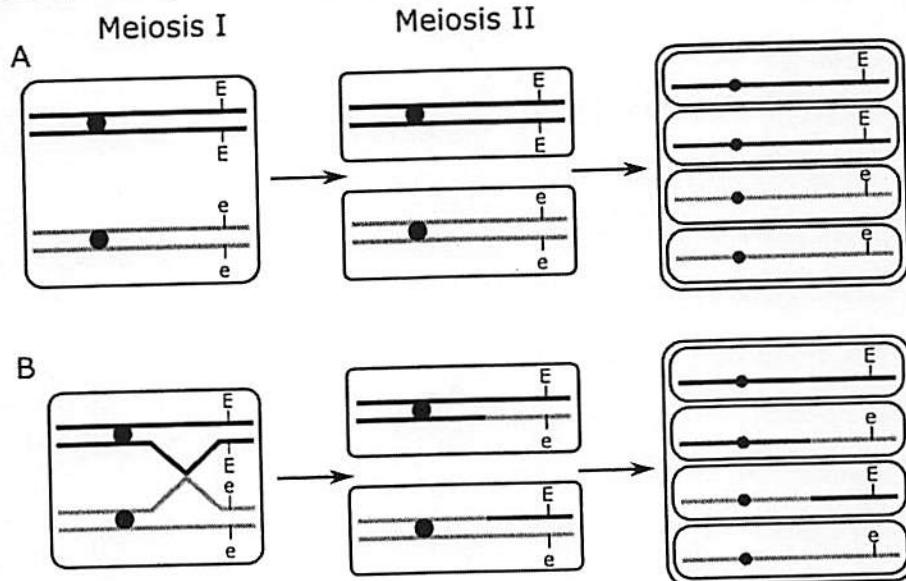


Figure Q.35

Strain ab was crossed to strain $a^+ b^+$ and 100 linear tetrads were isolated. Six classes in the following proportions were identified:

Class 1	Class 2	Class 3	Class 4	Class 5	Class 6
ab	ab	ab	ab	ab^+	ab
$a^+ b$	ab^+	ab	$a^+ b^+$	$a^+ b$	$a^+ b^+$
ab^+	$a^+ b$	$a^+ b^+$	ab	ab^+	ab^+
$a^+ b^+$	$a^+ b^+$	$a^+ b^+$	$a^+ b^+$	$a^+ b$	$a^+ b$
15	29	47	2	2	5

Indicate in the answer sheet if each of the following statements is true or false.

- A. Locus a and b are located on the same arm of chromosome.
- B. Class 4 involves a 4-strand double crossover.
- C. Class 6 involves a 3-strand double crossover, with one between gene a and the centromere and between the centromere and gene b.
- D. Class 5 involves a 2-strand double crossover.

Answer key

A. False

B. False

C. True

D. False

Explanation

A. False. Distance between the centromere and gene a is $100 * \frac{1}{2} * (\text{class 1} + \text{class 4} + \text{class 5} + \text{class 6})/100 = \frac{1}{2} * (15 + 2 + 2 + 5) = 12 \text{ cM}$

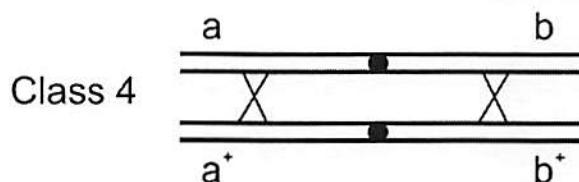
Distance between the centromere and gene b is $100 * \frac{1}{2} * (\text{class 2} + \text{class 4} + \text{class 5} + \text{class 6})/100 = \frac{1}{2} * (29 + 2 + 2 + 5) = 19 \text{ cM}$

Distance between gene a and gene b is $100 * (\text{class 5} + \frac{1}{2} * (\text{class 1} + \text{class 2} + \text{class 6})) / 100 = 2 + \frac{1}{2} * (15 + 29 + 5) = 26.5 \text{ cM}$

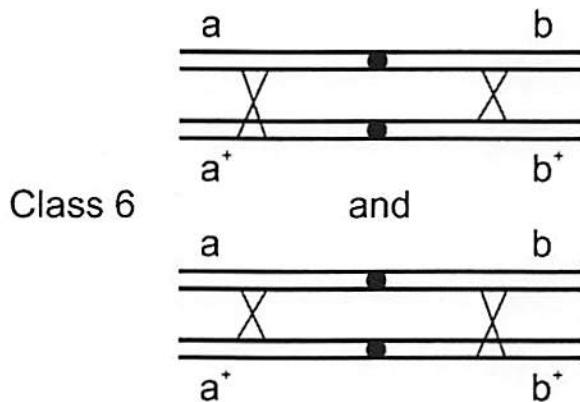
If two genes are on the same chromosome arm, distance between them should be about $19 - 12 = 7 \text{ cM}$, which is much smaller than calculated distance of 26.5 cM.

Therefore two genes must be on two different chromosome arms.

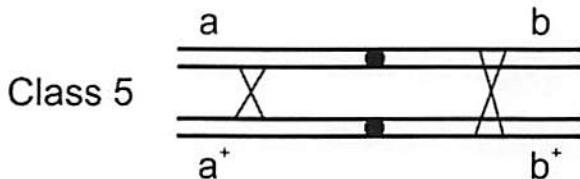
B. False. As in Fig.Q.35II, class 4 is resulted from a double cross-over between the centromere and gene a, and between the centromere and gene b of two chromatids.



C. True. As in Fig.Q.35II, class 6 is resulted from a double cross-over between one chromatid of a chromosome with two chromatids of the other chromosome.



D. False. As in Fig.Q.35II, class 5 is resulted from two single crossovers between two pairs of chromatids.



References: Anthony J.F. Griffiths; Jeffrey H. Miller; David T. Suzuki; Richard C. Lewontin and William M. Gelbart. An introduction to genetic analysis.

Q. 36

Heritability is a statistical measure of how strongly the phenotype of the offspring resembles the phenotype of the parents. Heritability in broad sense- H^2 is the ratio of total genetic variance (V_g) to total phenotypic variance (V_p): $H^2 = V_g / V_p$.

$V_p = \sum (X_i - X_{\text{mean}})^2 / (n-1)$. The phenotypic variance is often divided into three components: the genetic variance (V_g), environmental variance (V_e) and the interaction variance (V_i), $V_p = V_g + V_e + V_i$. Value of H^2 range from 1 to 0. The genetic variance is divided into three components: The additive genetic variance (V_a), the dominant genetic variance (V_d), and the genetic interaction variance (V_i), $V_g = V_a + V_d + V_i$. Heritability in narrow sense (h^2) = V_a/V_p .

Indicate in the answer sheet if each of the following statements about heritability is true or false?

- A. A low H^2 value of a trait indicates that the trait is determined mainly by the environment.
- B. Genetic variance depends on the environment to which the population is exposed.
- C. The artificial selection for a trait with higher H^2 is more successful than that for a trait with lower H^2 .
- D. Two pure strains of been that produce seeds with different weights were crossed. The variances of seeds (V_p) from the parental, F_1 , and F_2 plants are: $P_1 = 1.7$; $P_2 = 2.1$, $F_1 = 2.0$ and $F_2 = 4.1$. If we ignore interaction effects, H^2 of seeds is 0.54.

Answer key

- | | | | |
|----------|---------|----------|---------|
| A. False | B. True | C. False | D. True |
|----------|---------|----------|---------|

Explanation

- A. False. Low value of H^2 indicates that there is a little genetic variance in the population.
- B. True. Genetic variance has been changed by shifting the environment and the environmental variance has been changed by a change in genotype. In general, genetic variance depends on the environments to which the population exposed and environmental variance depends on the frequency of the genotypes.

- C. False. The artificial selection for a trait with high h^2 (narrow heritability) is more successful than that for a trait with low h^2 but not for H^2 .
- D. True. The value of the environmental variance can be determined by averaging the variances of parents and the F_1 because in pure strains and in F_1 the phenotype variance is considered as the environmental variance. $V_e = (1.7 + 2.1 + 2.0)/3 = 1.9$. The genetic variance (V_g) for the F_2 population is: $4.1 - 1.9 = 2.2$. $H^2 = V_g/V_p = 2.2/4.1 = 0.54$.

Reference: Anthony J.F. Griffiths; Jeffrey H. Miller; David T. Suzuki; Richard C. Lewontin and William M. Gelbart. An introduction to genetic analysis.

Q. 37

F.D. Enfield selected for increased body size in the flour beetle *Tribolium castaneum*. He started with a population of beetles that had a mean weight of 2.4 g and variance of 4.0 g². For each generation, the selection differential (S) was 0.022g. The initial value of h^2 (heritability in narrow sense- h^2 is the ratio of the additive variance to the total phenotypic variance) for body size in the original population was 0.3, so the response to selection (R) was 0.0066 g. For the first 50 generations, the mean weight of the selected population increased steadily, showing a response to selection close to the predicted 0.0066. After 125 generations of selection, the mean weight had increased to 5.8g, more than twice the original mean, and additional selection did not result in a further increase in size. The lightest individuals in the selected population were heavier than the heaviest individuals in the original population. Enfield determined h^2 for the selected population after 125 generations and discovered that it was only slightly less than for the original population.

Based on above information, indicate in the answer sheet if each of the following statements is true or false.

- A. The failure of the population to respond to further selection was because the population genetic variation is exhausted.
- B. The reason why the mean of the population could be shifted to a value outside the original range of the population is that the selection for the increased the body size is a selection favoring heterozygotes.
- C. After 125 generations of selection, additional selection can result in a further increase in size if we increase the selection differential (higher than 0.022g).
- D. If selection was stopped after 125 generations of selection then the body size would decrease.

Answer key

- A. False
- B. False
- C. False
- D. True

Explanation

- A. False. The h^2 after 125 generations of selection only slightly less than h^2 in the original population means that the population still contained a great deal of additive genetic variance for the body size.
- B. False. The reason why the mean of the population could be shifted to a value outside the original range of the population is that additive alleles have both positive and negative effects on body size, and the mean weight of the original population represented a balance between alleles that increased body size and alleles that decreased it. During the selection experiment, these additive alleles segregated, producing some progeny with fewer negative alleles and more positive alleles than any individuals in the original population. Consequently, these individuals were larger than any individuals in the original population.
- C. False. Increasing the selection differential do not work in this case because the selected population reached the point where no combinations of the alleles in that population could give larger beetles that were also fertile.
- D. True. If selection was stopped after 125 generations of selection then the body size would be dropped, and fertility increased.

Reference: The science of genetics. Alan G. Atherly; Jack R. Girton; John F. McDonal and Genetics from genes to genomes, 4th Hartwell 2011. P. 679

Q. 38

A mutated male mouse that is phenotypically normal shows reproductive anomalies when compared with a normal male as shown in table Q.38.

Table Q.38

Mating	Embryo (mean No.)			
	Implanted in uterine wall	Degenerating after implantation	Normal	Degeneration (%)
Mutated ♂ X normal ♀	8.7	5.0	3.7	57.5
Normal ♂ X normal ♀	9.5	0.6	8.9	6.5

Study carefully data given in the table and indicate in the answer sheet if each of the following statements is true or false.

- A. The mutated male mouse could have a chromosome deletion.
- B. The mutated male mouse can be a chromosomal translocation heterozygote.
- C. The mutated male mouse can be a chromosomal inversion heterozygote.
- D. The genetic defect in mutated male could be verified by cytological observation of meiotic cells in the mouse.

Answer key

- A. False B. True C. True. D. True

Explanation

- A. False. The mutated mouse had 51% degeneration more than normal then it cannot be a deletion heterozygote because a viable deletion heterozygote has normal reproduction.
- B. True. The mutated mouse had 51% degeneration more than normal then it can be a translocation heterozygote. Translocation heterozygote is often semi-sterile.
- C. True. The mutated mouse had 51% degeneration more than normal then it can be an inversion heterozygote. Inversion heterozygote is often semi-sterile.
- D. True. A cross-figure can be seen in meiotic cells of translocation heterozygote due to paring of translocated chromosomes with their normal partners. A loop-figure can be seen in meiotic cells of inversion heterozygote due to paring of a chromosome with a deletion with its normal partner.

Q. 39

Speciation rates are variable in different lineages of organisms. Some lineages have many species; others have only a few.

Indicate in the answer sheet if each of the following statements about the factors influencing speciation rates is true or false.

- A. The larger the number of species in a lineage, the larger number of opportunities for new species to form.
- B. Animal-pollinated plant families have, on average, more species than closely related families pollinated by wind.
- C. Animals with complex behavior are likely to form new species at a low rate.
- D. Oscillations of climates may increase the speciation rate.

Answer key

- A. True B. True C. False D. True

Explanation

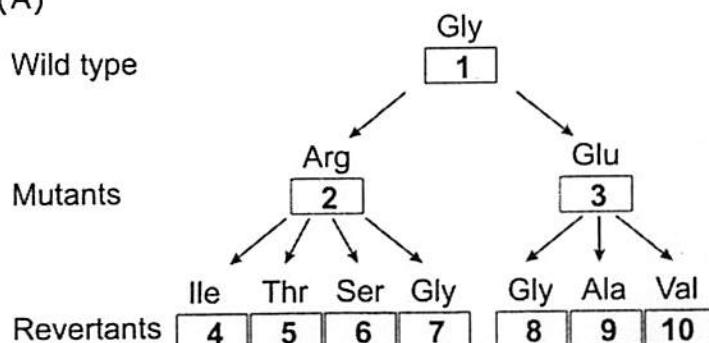
- A. True. For example speciation by polyploidy, the more plant species in a lineage, the more species are available to hybridize with one another to form new species.
- B. True. Animal-pollinated plant families have, on average, have more many species than their closely related families pollinated by wind have.
- C. False. Animals with complex behavior are likely to form new species at a high rate because they make sophisticated discriminations among potential mating partner. Such discriminations can greatly influence which individuals are most successful in producing offspring and may lead to rapid reinforcement of reproductive isolation between species.
- D. True.

Reference: Life science of Biology 7th edition.

Q. 40

About 50 years ago, Charles Yanofsky studied the sequence of the tryptophan synthetase of *E. coli*. The wild type protein (1) has a glycine in position 38. Yanofsky isolated two inactive *trp* mutants: 2 and 3. Mutant 2 had Arg instead of Gly at position 38, and mutant 3 had Glu at position 38. Mutants 2 and 3 were plated on minimal medium (without tryptophan). Colonies appearing correspond to spontaneous mutations that restored tryptophan synthetase function. The amino acid at position 38 was identified as described in figure A. Assume that each amino acid replacement results from a single base-pair change.

(A)



(B)

		Second base					
		U	C	A	G		
First base	U	UUU Phe UUC UUA Leu UUG	UCU UCC UCA UCG	UAU Tyr UAC UAA Stop UAG Stop	UGU Cys UGC UGA Stop UGG Trp	Third base	
	C	CUU CUC Leu CUA CUG	CCU CCC CCA CCG	CAU His CAC CAA Gln CAG	CGU CGC CGA Arg CGG	U	C
	A	AUU AUC Ile AUA AUG Met	ACU ACC ACA ACG	AAU Asn AAC AAA AAG Lys	AGU Ser AGC AGA Arg AGG	U	C
	G	GUU GUC Val GUA GUG	GCU GCC GCA GCG	GAU Asp GAC GAA Glu GAG	GGU GGC GGA Gly GGG	U	C

Indicate in the answer sheet if each of the following statements is true or false.

- A. Mutant 2 results from a base transition at the first position of codon 38.
- B. Strains 7 and 8 likely have the same sequence as the wild type strain.
- C. The codon 38 in strain 10 is 5'GTA3'
- D. The codon 38 in strain 6 is 5'AGC3'.

Answer key

- A. True B. True C. True D. False

Explanation

A. True. Glycine is encoded by four codons GGN, arginine is encoded by 6 codons CGN and AG(A/G). A single point mutation must be happened at the first nucleotide of the codon 38 to change glycine in wild type 1 to arginine in mutant 2. The first nucleotide G can be replaced by either A (transition mutation) or C (transversion mutation).

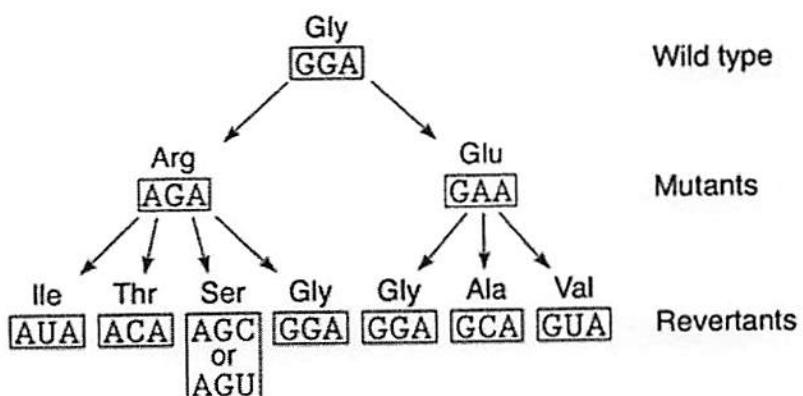
Arginine in mutant 2 is changed to isoleucine (encoded by AU(A/C/U)) in strain 4 and to threonine (encoded by ACN) in strain 5. Because each amino acid change is caused by a single nucleotide change, the first nucleotide of the codon 38 in mutant 2 must be A.

Therefore the first nucleotide of the codon 38 is changed from G in wild type 1 to A in mutant 2, a transition mutation.

B. True. Since the first nucleotide of codon 38 in mutant 2 is A, the arginine-encoding codon must be AG(A/G). Because arginine 38 (encoded by AG(A/G)) is reverted to glycine (GGN) in strain 7, a single nucleotide change must be happened at the first nucleotide of the codon 38 (A to G). Therefore codon 38 in strain 7 should be identical to wild type 1 and is GG(A/G).

Because glycine 38 (encoded by GG(A/G)) in wild type 1 is changed to glutamine (encoded by GA(A/G)) in strain 3, a single nucleotide change must be happened at the second nucleotide of the codon (G to A). Because glutamine 38 (encoded by GA(A/G)) in mutant 3 is reverted to glycine (encoded by GGN) in strain 8, a single nucleotide change must also be happened at the second nucleotide of the codon (A to G). Therefore codon 38 in strain 8 should be identical to wild type 1 and is GG(A/G).

- C. True. Because arginine 38 (encoded by AG(A/G)) in mutant 2 is changed to isoleucine (encoded by AU(A/C/U)), a single nucleotide change must be happened at the second nucleotide of the codon (A to U). The third nucleotide of the codon must be unchanged and common to both mutant 2 and strain 4. Only a nucleotide A is common to both set of codons therefore codon 38 is AUA in strain 4 and AGA in mutant 2. It is GGA in wild type, strain 7 and strain 8; and GAA in mutant 3. Because glutamine 38 (encoded by GAA) in mutant 3 is changed to valine (encoded by GUN) in strain 10, a single nucleotide change must be happened at the second nucleotide of the codon (A to U) and the third nucleotide should be unchanged. Therefore the codon 38 in strain 10 is GUA.
- D. False. Serine is encoded by six codons UCN and AG(C/U). The serine 38 in strain 6 is resulted from a single nucleotide change of codon AGA (arginine in mutant 2). The single nucleotide change should be happened at the third nucleotide and A can be changed to C or U. The codon 38 in strain 6 can be either AGC or AGU.



References:

Charles Yanofsky, Edward C. Cox and Virginia Horn, 1966. The Unusual Mutagenic Specificity of an *E. coli* Mutator Gene. *Proceedings of the National Academy of Sciences of the United States of America*, 55 (2): 274-281

Peter J. Russell (2010). iGenetics: a molecular approach 3rd edition. Pearson Education

Q. 41

Frequencies of the human ABO blood group alleles in a population are $p(I^A) = 40\%$, $p(I^B) = 40\%$ and $p(i) = 20\%$. If the population is in Hardy-Weinberg equilibrium, indicate in the answer sheet if each of the following statements is true or false.

- A. In this population, the number of persons with the blood groups A and B should be equal.
- B. In this population, the number of persons with the blood groups A and AB should be equal.
- C. In this population the frequency of persons with anti-B antibodies is 64%.
- D. Locus ABO is localized on an autosomal chromosome because the blood group frequencies are the same for men and women.

Answer key

- A. True B. True C. False D. True

Explanation

A. True : $p(A) = p(I^A I^A) + p(I^A i) = 0.4 * 0.4 + 2 * 0.4 * 0.2 = 0.32$ (32%)
 $p(B) = p(I^B I^B) + p(I^B i) = 0.4 * 0.4 + 2 * 0.4 * 0.2 = 0.32$ (32%)

B. True: $p(AB) = p(I^A I^B) = 2 * 0.4 * 0.4 = 0.32$ (32%)

C. False. Anti-B antibodies should occur in people with blood groups A and O. The sum of this blood groups is $32\% + 4\% = 36\%$. Because $p(A) = p(I^A I^A) + p(I^A i) = 0.4 * 0.4 + 2 * 0.4 * 0.2 = 0.32$ (32%) and $p(O) = p(ii) = 0.2 * 0.2 = 0.04$ (4%)

D. True. In case of Y-chromosomal localization of ABO locus only males could have different blood groups. In case of X-chromosomal localization of ABO locus males could not follow the Hardy-Weinberg law and could not have AB blood group. In case of mitochondrial DNA localization of ABO locus all population could not follow the Hardy-Weinberg law.

Q. 42

Frequency-dependent selection is an evolutionary process where the fitness of a phenotype depends on its frequency relative to other phenotypes in a given population. In **positive** frequency-dependent selection, the fitness of a phenotype increases as it becomes more common. And in **negative** frequency-dependent selection, the fitness of a phenotype decreases as it becomes more common.

Indicate in the answer sheet if each of the following statements is true or false.

- A. Plant self-incompatibility alleles are an example of negative frequency-dependent selection.
- B. Spreading of a newly emerged virus in a human population is controlled by negative frequency-dependent selection.
- C. Prevalence of *Papilio memmon* whose females resemble unpalatable *Papilio coon* is an example of positive frequency-dependent selection.
- D. Spread of genes responsible for warning coloration in toxic or distasteful organisms in population is controlled by positive frequency-dependent selection.

Answer key

- A. True
- B. True
- C. False
- D. True

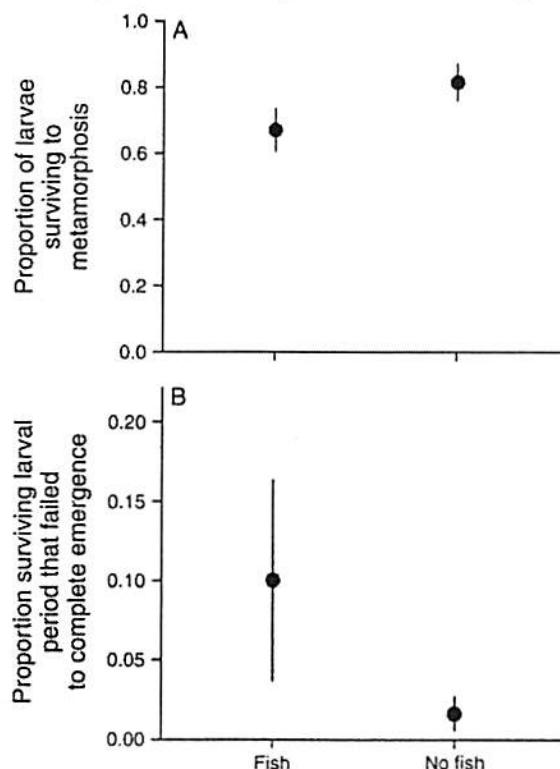
Explanation

- A. True. When two plants share the same incompatibility allele, they are unable to produce viable zygotes. Thus, a plant with a new (and therefore, rare) allele has more success at mating, and its allele spreads quickly through the population.
- B. True. Once a particular flu virus strain has been common in a human population, most individuals would have developed an immune response to that strain. But a rare, novel strain of the flu virus would be able to spread quickly to almost any individual. This is an example of negative dependent selection.
- C. False. This is an example of negative frequency-dependent selection.

D. True. Positive frequency dependent selection gives an advantage to common phenotypes. This means that new alleles can have a difficult time invading a population, since they don't experience significant benefit until they become common. This has been proposed as a difficulty in the evolution of aposematic (or warning) coloration in toxic or distasteful organisms. The presumed advantage of the aposematic coloration is that predators have learned to avoid potential prey with that color pattern. But when the pattern is rare, the predator population does not become 'educated' and the pattern has no benefit. Therefore the warning color is only advantageous once it has become common.

ECOLOGY**Q. 43.**

A population of dragonfly larvae (*Leucorrhinia intacta*) is separated into two groups. In both groups, larvae populations are put inside a cage with no food limitation. The first group is exposed to a fish predator that can swim freely, but cannot enter the cage. The second group is a control with no fish. The proportion of larvae surviving and the proportion of live larvae failing to metamorphose in the two groups are shown below:

**Figure Q.43**

Indicate in the **Answer sheet** if each of the following statements is True or False.

- A. One of the causes of high failure rate of metamorphosis of the larvae upon exposure to a non-lethal predator is cannibalism.
- B. The high mortality of larvae in the first group is due to predator-induced stress.
- C. In the predator treatment, the percentage of individuals that survived the larval stage completed emergence to the adult stage is lower than the percentage of those in the fishless treatment.
- D. The survival of dragonfly before metamorphosis is dependent on the predator while those during metamorphosis is not.

Answer key:

- A. False B. True C. True D. False**

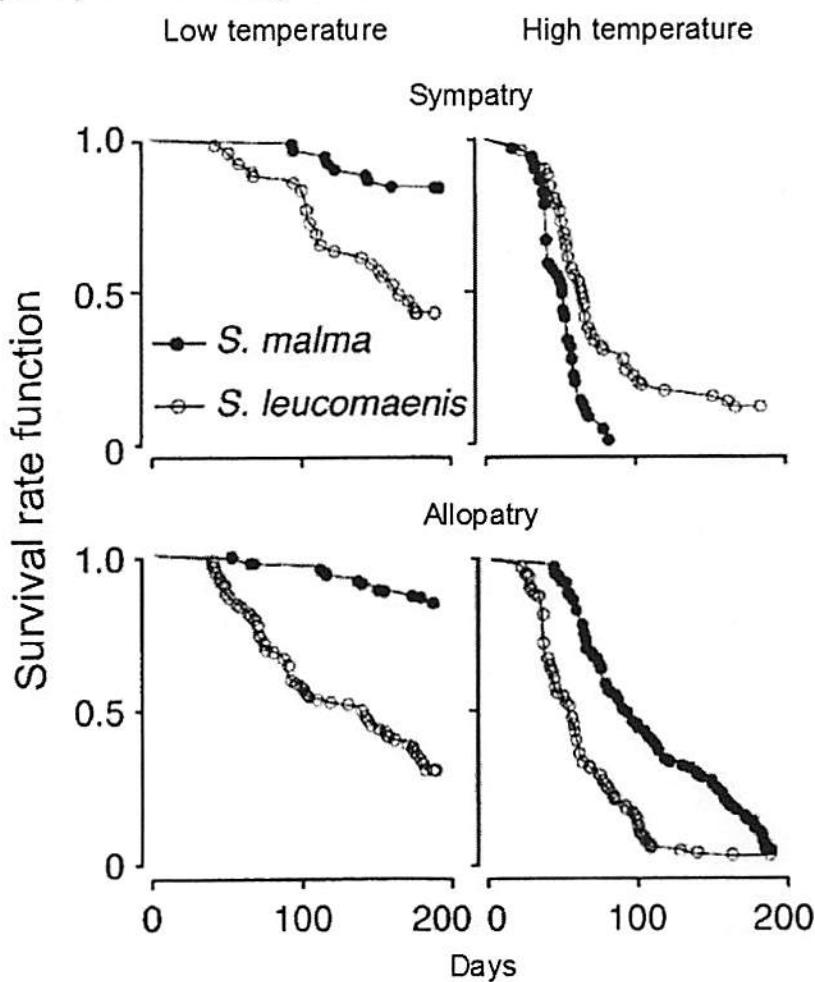
Explanation:

- A. False.** An additional mortality source, cannibalism, is common in larval odonates, but cannibalism is unlikely in the experiments because of the relatively low mortality of the larvae observed. The original paper did not find any evidence of cannibalism.
- B. True.** Stress response to the presence of predators in the immediate environment has been demonstrated in numerous animals (Hawlena and Schmitz 2010) including damselflies, a group closely related to dragonflies (Slos and Stoks 2008). The findings indicate that a certain amount of prey mortality can be caused by predator-induced stress.
- C. True.** In the predator treatment, approximately 10% of individuals that survived the larval stage died during emergence to the adult stage, while only 2% of the larval survivors died at emergence in the fishless treatment (Fig. B).
- D. False.** The survival of dragonfly in both stages is dependent on the predator.

References: McCauley, S. J., Rowe, L., & Fortin, M.-J. (2011). The Deadly Effects of “Nonlethal” Predators. *Ecology*, 92 (11), 2043-2048.

Q. 44

Laboratory experiments were conducted to examine the effects of temperature on interspecific competition between two stream salmonid fishes, *Salvelinus malma* and *S. leucomaenoides*, with largely allopatric altitudinal distribution. Three combinations of species population, including allopatric populations of *S. malma* and *S. leucomaenoides*, and sympatric populations of both species, were treated with low temperature (6°C) and high temperature (12°C), in which thriving allopatric populations of *S. malma* (6°C) and *S. leucomaenoides* (12°C) are commonly found.

**Figure Q.44**

Indicate in the **Answer sheet** if each of the following statements is True or False.

- Competition of these two species is likely to have been affected by temperature and altitude
- S. malma* may be distributed at higher altitudinal ranges than is *S. leucomaenoides*
- S. leucomaenoides* is likely to be more low-temperature stress-resistant than *S. malma*
- S. malma* has a narrower fundamental niche than does *S. leucomaenoides*

Answer key

- A. True B. True C. False D. False

Explanation:

- A. True.** Highest survival rate was achieved by different species in each temperature treatment. This indicate that competition between these species was affected by temperature and altitude.
- B. True.** As *S. malma* outcompete *S. leucomaenesis* in the lower temperature, *S. malma* is more likely to present in lower range temperature than *S. leucomaenesis*, i.e. area with higher altitudinal range. It also has higher survival rate in lower temperature compared to *S. leucomaenesis*.
- C. False.** Stress is defined as condition that limit species ability to exploit resource, i.e. low temperature. Species with lower metabolic demands at cold temperature will likely more suited on low temperature and thus stress-resistant. As *S. leucomaenesis* survived less than *S. malma* at a lower temperature, this population was not suited to stressful conditions such as low temperature habitat.
- D. False.** According to the allopatric population treatments, *S. malma* has a broader ecological niche.

Reference: Yoshinori Taniguchi; Shigeru Nakano. *Ecology*, Vol. 81, No. 7. (Jul., 2000), pp. 2027-2039.

Q. 45

Guppies (*Poecilia reticulata*) show a complex color pattern polymorphism that varies with predation pressure, reflecting a balance between selection for crypsis by predators and selection for conspicuousness by sexual selection. Three experimental ponds were used to study this phenomenon, mimicking the real condition on the native habitat of the guppies and its predators, *Rivulus hartii* and *Crenicichla alta*. One pond has the control group while the other two ponds were added with one of the two predators. In the field, *C. alta* was observed to be more dangerous than *R. hartii*.

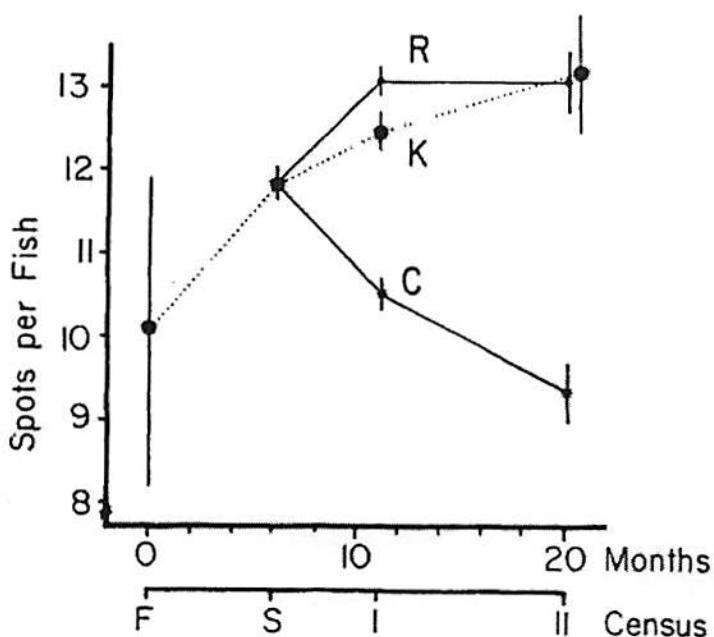


Figure Q.45. Changes in the number of spots per fish during the course of the experiment. Line 'K' stands for pond without predator, 'R' for pond with *R. hartii*, and 'C' for pond with *C. alta*. In the X-axis, 'F' stands for the time when the foundation population was started, 'S' stands for the time when the predators were added, then 'I' and 'II' stands for the numbering of the following censuses.

Indicate in the **Answer sheet** if each of the following statements is True or False.

- A. The color pattern is responsible for the reduced fitness of *P. reticulata*.
- B. Sexual selection for color pattern of the *P. reticulata* cannot be inferred from the data.
- C. The color pattern of the *P. reticulata* may be advantageous in escaping *R. hartii*.
- D. The two predators possibly use two different mechanisms to detect *P. reticulata*.

Answer key

- A. False B. False C. False D. True

Explanation:

- A. False.** Color patterns can be different while still being equally cryptic, so a very high color pattern diversity is possible without a loss in fitness. Moreover, with the absence of predator increases the number of spots per fish (see line 'K').
- B. False.** The sexual dimorphism observed in this species indicate that brighter color pattern in male was preferred by females (it is also given in the text). This can be shown by the increasing number of spots in control ponds.
- C. False.** The number of spots per fish in ponds with *R. hartii* does not differ much from the control ponds, indicating that *P. reticulata* does not use the color pattern to escape *R. hartii*.
- D. True.** Contrasting with the ponds with *R. hartii*, the ponds with *C. alta* experienced decreasing number of spots per fish, indicating that these predators use different mechanisms to detect *P. reticulata*.

Reference: Endler, J. A., 1980. Selection on Color Patterns in *Poecilia reticulata*.
Evolution. 34(1): 76-91)

Q. 46

Poa and *Stipa* are two perennial grasses in the steppes. The former is highly preferred by domestic and wild herbivores, while the latter is relatively unpalatable. A student grew *Poa* plants in different distances to *Stipa* plants, with or without root barrier, in different grazing levels, and then recorded the growth of *Poa* plants.

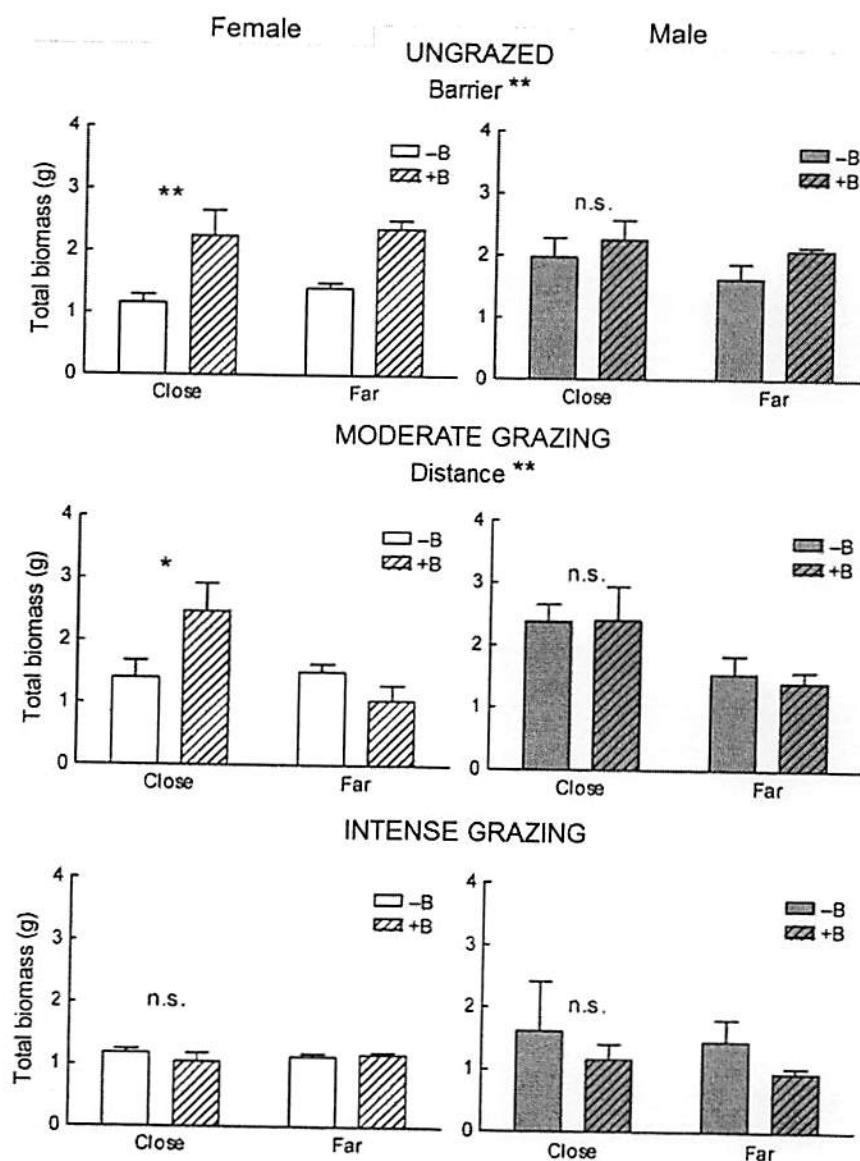


Figure Q.46

Effects of distance (close and far) and barrier (without barrier (-B) and with the barrier present (+B)) to root competition manipulations on *Poa* female (left panels) and male plants (right panels) total biomass, at each grazing intensity level. “**” and “*”: Statistically significant differences; n.s.: non significant.

Indicate in the **Answer sheet** if each of the following statements is True or False.

- A. Distance to the less palatable *Stipa* neighbours significantly affects the *Poa* biomass of females and males at the ungrazed site.
- B. Plants at the moderate grazing site, whether male or female, generally perform better near *Stipa* tussocks than far from them, demonstrating a positive effect of *Stipa* canopies on both genders.
- C. There is a strong below-ground competition between *Poa* females and *Stipa* neighbours at the ungrazed site.
- D. Population sex ratio drift between female and male bias may be influenced by domestic grazing intensity.

Answer key

- A. False B. True C. True D. True

Explanation

- A. False.** As shown in the figure, at the ungrazed site treatment, both the biomasses of *Poa* females and males planted close and far from *Stipa* neighbours are not significantly different.
- B. True.** At the moderate grazing site, plants, whether male or female, generally have higher biomass when planted near *Stipa* tussocks than far from them, indicating a positive effect of *Stipa* canopies on both genders.
- C. True.** Female transplants growing close to neighbours grow twice as large with the barrier than without it, indicating strong below-ground competition with *Stipa* neighbours.
- D. True.** There are differences between *Poa* male and female plants at different grazing intensities.

Reference: Graff et al., *Journal of Ecology* 101.5 (2013): 1146-1157. doi: 10.1111/1365-2745.12114.

Q. 47

Goats are fed with alfalfa and corn stubble. At time 0, they were also fed with *Mimosa* seeds. The presence of viable *Mimosa* seeds in goat faeces was recorded with a germination experiment with egested and control seeds.

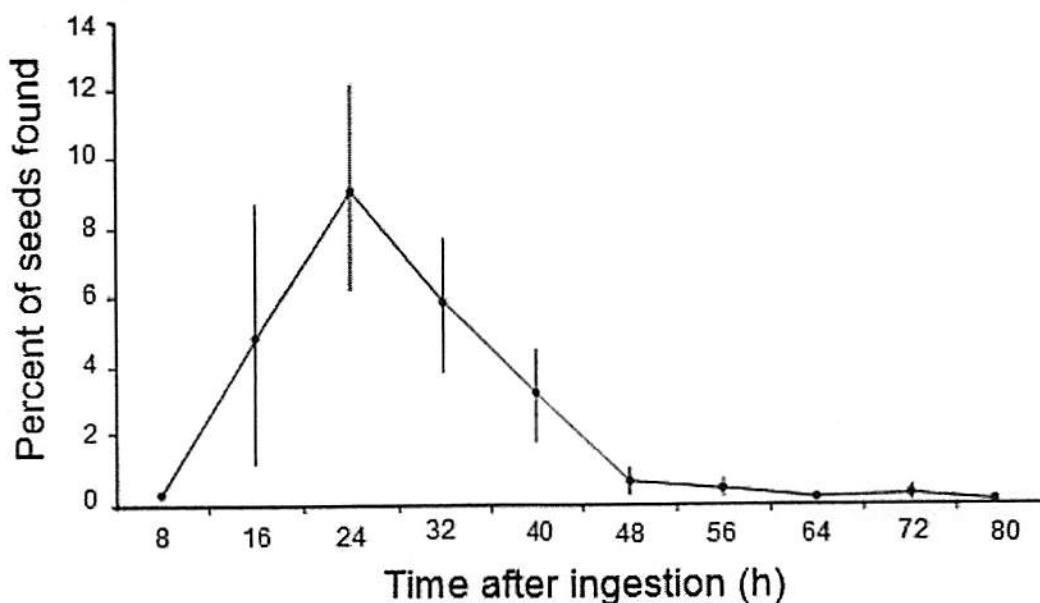


Figure Q.47.1. Percent of seeds of *Mimosa* found in goat faeces as a function of time since seeds were ingested. Goat pellets were collected every 8 hours over a period of 80 hours after ingestion.

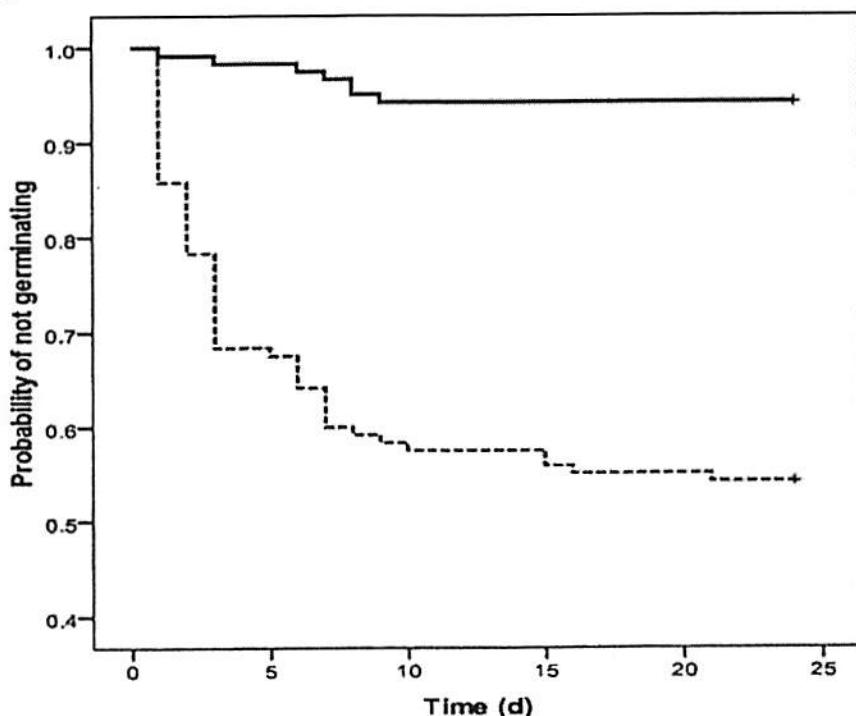


Figure Q.47.2. Probability of not germinating of egested (stippled line) and control (solid line) *Mimosa* seeds.

Indicate in the **Answer sheet** if each of the following statements is True or False.

- A. *Mimosa* seed can survive up to 3 days in the goat digestive system.
- B. The passage through the goat digestive system decreases seed germination.
- C. Number of seeds egested after ingestion is highest after 24 hours.
- D. Goats could act as legitimate disperser of *Mimosa* seeds.

Answer key

- A. True B. False C. True D. True

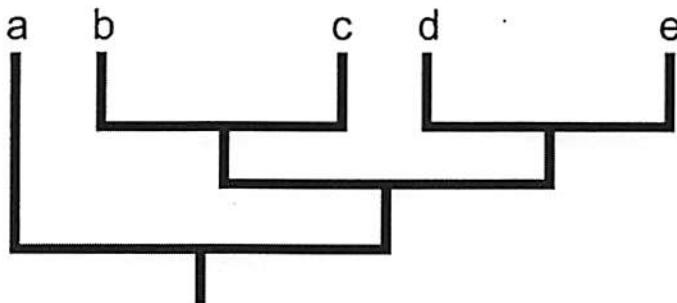
Explanation

- A. **True.** Figure 1 shows some percent of seeds found at 72 hours after ingestion.
- B. **False.** Germination rate of egested seeds is higher than the control (Figure 2).
- C. **True.** Percent of seeds found in pellets in the observation at 24 hours after ingestion is higher than the others (Figure 1).
- D. **True.** Seeds can pass the goat digestive system and germinate with higher rate compared to the control.

Reference: Luca Giordani *et al.*, *Journal of Tropical Ecology*, 2014, DOI: 10.1017/S0266467414000510

BIOSYSTEMATICS**Q. 48**

It is important that you learn how to “read” a phylogenetic tree correctly. Study the cladogram given below and indicate in the answer sheet if each of the following statements is true or false.



- A. Clade (a) and the clade containing (b), (c), (d) and (e) originated at the same time.
- B. The degree of similarity among extant organisms of taxa (b) and (c) is larger than that between (c) and (d).
- C. Taxon (b) is more closely related to taxon (a) than to taxon (e).
- D. The lineage leading to taxon (a) was the first to diverge from the other lineages.

Answer key

- A. False B. False C. False D. True

Explanation

- A. False. Their sister clade relationship indicates that these two groups share a more recent common ancestor with each other than they do with other groups, but this not necessarily means that they originated at the same time. Indeed, while fossil evidence indicates that gymnosperms originated at least 305 million years ago, this does not mean that angiosperms are that old, only that the most recent common ancestor of gymnosperms and angiosperms must be that old. Reference: Campbell Biology in focus, pp: 521.
- B. False. The tree does not illustrate the degree of similarity among the branch tips, but rather shows actual historical relationships. Although closely related organisms tend to be similar to one another, this is not the case if the rate of

evolution is not uniform. For example, crocodiles are more closely related to birds than they are to lizards, even though anyone can see that crocodiles look a lot more like lizards than birds. Reference: Living world 7th, pp. 327.

- C. False. Taxon (b) is actually more closely related to taxon (e) because the last common ancestor of (b) and (e) is a descendant of the last common ancestor of taxon (a) and taxon (b). Reference: Living world 7th, pp. 327.
- D. True. Taxon (a) is a basal. A lineage that evolved early from the root and remains unbranched is called basal taxon.

Reference: Campbell Biology in focus, pp: 521 and Living world 7th, pp. 327.

(Campbell 9, 7 unit 5)

Q.49

Most taxonomists today believe that biological classification systems should reflect the evolutionary relationships of organisms and taxonomic groups should be monophyletic. However, the classification used today still contains many polyphyletic and paraphyletic taxonomic groups.

Indicate in the answer sheet if each of the following statements is true or false.

- A. Polyphyletic taxonomic groups exist in many life trees because all species in the groups are associated by a similar phenotype although their common ancestral characters cannot be identified.
- B. Paraphyletic taxonomic groups exist in many life trees because the classification systems are mainly based on the degree of phenotypically similarity among organisms.
- C. Taxa in the paraphyletic taxonomic group evolved more slowly than some of their descendants not included in the group.
- D. Gymnosperms are a monophyletic taxon.

Answer key

- A. True B. True C. True D. False

Explanation

- A. True. A polyphyletic group is a collection of distantly related OTUs (operational taxonomic units) that are associated by a similar phenotype, but are not directly descended from a common ancestor.
- B. True. A paraphyletic group excludes some of its descendants (for example all mammals, except the Marsupialia taxa). All taxa in the paraphyletic group share many characteristics.
- C. True. Some of their descendants are excluded from the paraphyletic group because they evolved rapidly and have many different characteristics.
- D. False. Gymnosperms contain Cycadophyta, Ginkgophyta, Coniferophyta and Gnetaophyta. The relationships of these four phyla to each other are uncertain.

Reference:

Q.50

Using sequence differences to establish phylogenies has some advantages and possible dangers. An inappropriate choice of molecule could result in molecular trees that greatly distort true phylogenetic relationships. Hence, care must be taken in using this approach.

Indicate in the answer sheet if each of following statements is true or false.

- A. Cytochrome c molecules are very useful for establishing evolutionary relationships between closely related species.
- B. Comparing subunit sequences from many different genes is better than comparing whole-gene sequences of the organisms under phylogenetic study.
- C. Rates of nucleotide substitution are faster in organisms with short generation times than in organisms with long generation times.
- D. The Neutral Theory requires that all polypeptide and DNA sequences evolve at the same rate.

Answer key

- B. False B. True C. True D. False

Explanation

- A. False. Cytochrome c is a slowly evolving protein. Hence, so it is not suitable for establishing evolutionary relationships between closely related species. (The Science of Genetics, Atherly A.G.; Girton J.R.; McDonald J.F. pp. 695).
- B. True. Different parts of the same gene evolve at different rates. Those parts of genes that have the least effect on function tend to evolve at highest rates. The idea of the molecular clock is that individual proteins and genes evolve at a constant rate and that the differences in the sequences of present-day organisms can be used to date past evolutionary events.
Reference: Genetics a conceptual approach. Pp. 740.

- C. True. Short-generation organisms have more rounds of DNA replication per unit of time than long-generation organisms. Hence, the rates of substitution per base per year in short-generation organisms are faster than in organisms with long-generation times.
- D. False. The Neutral Theory does not require that all polypeptide and DNA sequences evolve at the same rate. For some positions within a sequence, all or nearly all mutations will be selectively neutral. However, for other positions, a smaller fraction of all mutations will be selectively neutral, and for some positions, almost no mutations will be selectively neutral. Thus, the Neutral Theory explains the variation in evolutionary rates observed among proteins and DNA regions by invoking differences in functional constraints. The highest rates are observed in molecules or in portions of molecules that are not constrained by selection to preserve a function. The lowest evolutionary rates are observed in molecules where selection pressure is strongest.

Reference: The Science of Genetics, Atherly A.G.; Girton J.R.; McDonald J.F. pp. 695 and Genetics a conceptual approach. Pp. 740.