

Theoretical Exam 1 Answer keys

Question	Answer	Question	Answer	Question	Answer	Question	Answer	Question	Answer
1	F	42	T	83	F	124	T	165	F
2	T	43	F	84	F	125	F	166	F
3	T	44	F	85	T	126	T	167	F
4	F	45	T	86	T	127	T	168	T
5	T	46	T	87	F	128	F	169	F
6	T	47	F	88	F	129	F	170	F
7	F	48	T	89	T	130	F	171	T
8	T	49	F	90	F	131	T	172	T
9	T	50	F	91	T	132	T	173	F
10	F	51	T	92	T	133	T	174	T
11	T	52	T	93	F	134	F	175	T
12	T	53	T	94	F	135	T	176	T
13	T	54	T	95	F	136	T	177	F
14	T	55	T	96	T	137	F	178	T
15	F	56	T	97	T	138	T	179	T
16	T	57	T	98	F	139	F	180	F
17	T	58	F	99	F	140	T	181	T
18	F	59	F	100	F	141	F	182	F
19	T	60	F	101	F	142	T	183	F
20	F	61	T	102	F	143	F	184	F
21	F	62	T	103	T	144	F	185	F
22	T	63	F	104	F	145	T	186	T
23	F	64	F	105	T	146	F	187	F
24	F	65	F	106	F	147	T	188	T
25	T	66	T	107	T	148	T	189	F
26	F	67	F	108	F	149	F	190	F
27	F	68	F	109	F	150	F	191	F
28	F	69	T	110	T	151	T	192	T
29	T	70	T	111	F	152	F	193	T
30	F	71	T	112	T	153	T	194	F
31	F	72	F	113	F	154	F	195	F
32	T	73	F	114	F	155	T	196	F
33	F	74	T	115	T	156	F	197	T
34	T	75	T	116	T	157	F	198	F
35	T	76	F	117	T	158	T	199	T
36	T	77	F	118	T	159	F	200	F
37	T	78	T	119	F	160	T	201	F
38	F	79	T	120	F	161	F	202	T
39	F	80	T	121	F	162	F		
40	F	81	T	122	T	163	F		
41	T	82	T	123	F	164	T		

Each correct answer scores 1 marks, each incorrect or missing answer score 0 marks.

Biochemistry

Q1(citrate and aconitase)

No.	1	2	3	4
Answer	False	True	True	False

Answers: A, F; B, T; C, T; D, F

Citrate is not chiral but stereochemically recognized by aconitase. The two $-\text{CH}_2\text{COO}^-$ groups of enzyme-bound citrate can be distinguished by aconitase, because the substrate-binding site of the enzyme is not symmetric. This was a critical issue when the citric acid cycle was proposed but later questioned by Krebs himself, and “TCA (tricarboxylic acid) cycle” was proposed instead of the “citric acid cycle”. Now we know the first product, citric acid, can be stereochemically recognized by aconitase.

Q2 (respiratory control)

No.	5	6	7	8
Answer	True	True	False	True

Answer: A, T; B, T; C, F; D, T

From the graphs, intact mitochondria respire.

The major role of the mitochondria is to produce ATP. The enhanced respiration by addition of ADP can be regarded as coupling of ATP synthesis and respiration in intact mitochondria. DNP, however, stimulated respiration in the absence of ATP synthesis, because ADP was fully consumed before the addition of DNP. On the other hand, DCCD stopped the respiration before ADP was fully consumed in the figure on the right. These interpretations can be inferred only from the graphs and do not require the knowledge of DNP and DCCD.

As a side note, typical cellular membranes are impermeable to ions like ATP, but mitochondrial membranes possess ATP/ADP translocators. DNP, the uncoupler, acts as a transporter of H^+ ion across the mitochondrial membrane, because both DNP and DNP/H^+

are lipophilic. DCCD binds covalently to the H⁺ channel of the ATP synthase and blocks the ATP synthesis.

Q3 (RubisCO and PEPC)

No.	9	10	11	12
Answer	True	False	True	True

Answers: A, T; B, F; C, T; D, T

Reaction 1 is catalyzed by phosphoenolpyruvate carboxylase (PEPC), which shows specificity to HCO₃⁻ ion. Reaction 2 is catalyzed by ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO), which shows specificity to CO₂.

Cell Biology

Q4 (sedimentation coefficient)

No.	13	14	15	16
Answer	True	True	False	True

The questions are related to the sedimentation coefficient of specimens. The S-value, which indicates the rate of specimen sedimentation during centrifugation, is a parameter that appears in every textbook of biochemistry, but is simply described to be corresponding to specimen size or density. This question is intended to allow the students to gain more detailed insight into how the S-value is a number that changes under other experimental conditions, such as temperature, viscosity, and specimen shape.

Answers

- A. True:** From equation (2), it is clear that the S-value is proportional to the density difference between the specimen and the solvent, $\rho - \rho_0$. Thus, we can estimate the density difference of organelles from the S-values if we can fix other parameters, such as V_m (molar volume) and r (estimated radius).
- B. True:** From equation (2), it is clear that the S-value is proportional to V_m/r . This parameter changes depending on the size of the specimen (more precisely,

proportional to the surface area), thus we can distinguish the size difference of specimens from the S-values if we can fix the density of specimens, ρ , to be 1.3–1.4.

- C. False:** From equation (2), it is clear that the S-value is proportional to V_m/r , but not to the specimen volume, V_m . Thus, the S-value is not twice as large when the molecular weight is doubled. It is estimated to be of a value of approximately 1.4-fold higher, or the square root of 2, if the globular shape of ribosomes does not change much after forming a compound.
- D. True:** From equation (2), it is clear that the S-value is inversely proportional to η , the viscosity coefficient of the solvent. Thus, the S-value should decrease when η increases at low temperatures.

Questions were prepared with reference to the following document that explains how the sedimentation coefficient relates to the shapes of biomolecules.

References

H. P. Erickson. Shape of protein molecules at the nanometer level determined by sedimentation, gel filtration, and electron microscopy. *Size and Biol. Proc. Online*, **11(1)**:32-51 (2009).

Q5 (Squid axon & Na-pump)

No.	17	18	19	20
Answer	True	False	True	False

A mechanism for maintaining the sodium/potassium ion gradient between the inside and outside of nerve cells is crucial for maintaining the activity of excitation. This question yields a result from classical experiments that determined the efflux rate of sodium ions (Na^+) by the Na^+ pump using the $^{24}\text{Na}^+$ radiation isotope. We can establish whether students understand the basic properties of the Na^+ pump that is supported by ATP derived from mitochondria.

- A. True:** The experiment clearly shows that the activity of Na^+ efflux was inhibited by

DNP, an uncoupler of ATP generation in mitochondria. Thus, the experimental conditions should have been designed to maintain the continuous respiration activity of mitochondria by supplying enough oxygen to the specimen, a squid giant axon.

- B. False:** The observed time course of DNP inhibition indicates that the efflux activity of Na^+ is decreased to approximately 1/40 (inhibited by 98%) of the original activity. Thus, the remaining portion of this Na^+ efflux can partially correspond to the leaking flux of Na^+ (plus efflux depending on the glycolytic ATP production), but it is quite small compared with the pumping activity supported by ATP from mitochondria (without DNP).
- C. True:** The delayed decrease of Na^+ efflux after the application of DNP implies that this chemical does not directly inhibit the activity of the Na^+ pump. Thus, the delayed decrease is reasonable as we can expect that the Na^+ pump activity depends on intracellular ATP remaining inside the nerve axon even if cell respiration is blocked completely.
- D. False:** From the experimental results, we can calculate the rate of Na^+ efflux to be decreased approximately from 50 to 40 (decrease by 20%) in 50 min. This may be corresponding to the decrease or dilution of $^{24}\text{Na}^+$ inside the axon. This experiment does not show any evidence of increasing intracellular Na^+ .

References

K. Schmidt-Nielsen, Animal physiology. 5th ed. Cambridge Univ. Press (1997).
 A.L. Hodgkin & R.D. Keynes, Active transport of cations in giant axons from *Sepia* and *Loligo*. *J. Physiol.*, **128**:28-60 (1955).

Q6

No.	21	22	23	24
Answer	False	True	False	False

- A. False.** Under this condition, the Nernst equilibrium potential of K^+ is -120 mV. Since the membrane potential is -150 mV, the cation K^+ is passively transported into the cell.
- B. True.** Under this condition, the Nernst equilibrium potential of K^+ is -240 mV.

Since the membrane potential is -150 mV, the cation K^+ must be actively transported into the cell.

C. False. Under this condition, the Nernst equilibrium potential of Cl^- is $+60$ mV.

Since the membrane potential is -150 mV, the anion Cl^- must be actively transported into the cell.

D. False. Under this condition, the Nernst equilibrium potential of Cl^- is 0 mV. Since the membrane potential is -150 mV, the anion Cl^- must be actively transported into the cell.

Q7

No.	25	26	27	28
Answer	True	False	False	False

A. True. In wild plants, the outer lamina is bitten by insect larvae less often. On the other hand, in mutant plants that cannot synthesize glucosinolate, both laminae were equally bitten. Therefore, the outer lamina is supposed to accumulate more glucosinolate than the inner lamina.

B. False. This mutant cannot synthesize glucosinolate.

C. False. We cannot judge whether *Arabidopsis* accumulates only glucosinolate as a repellent to this larva, because certain repellents may distribute equally.

D. False. In wild plants, inner lamina is damaged more by this larva. The outer lamina is supposed to be more important for this plant.

Q8

No.	29	30	31	32
Answer	True	False	False	True

A. True. The CO_2 released from malic acid can be used for photosynthesis during daytime. Therefore, more and more malic acid should be stored during night. It is likely that malic acid concentration is the highest just before sunrise.

B. False. Since this is a freshwater environment, there is no reason to believe that water

is scarce.

- C. False.** This plant is not a C4 plant. Therefore, it does not have bundle sheath cells with well-developed chloroplasts.
- D. True.** In shallow waters receiving a high amount of sunshine, CO₂ concentrations decrease due to the activities of other photosynthetic organisms. In other words, CO₂ tends to be scarce during the daytime. Therefore, the strategy of concentrating CO₂ as malate during the night would be advantageous.

Genetics

Q9

No.	33	34	35
Answer	False	True	True

Experiment (1) shows that only the first AUG codon of mRNA may be translated in yeast. From Experiment (2), it is presumed that some condition exists in the AUG codon that functions as the translation initiation in *E. coli*.

In eukaryotes, ribosome binds to the 5' terminal of mRNA, scans the nucleotide sequence, and starts translation from the first AUG codon.

In prokaryotes, translation begins at the AUG codon located approximately 10 bases downstream of the SD sequence which is complementary to the 3' terminal sequence of 16S ribosomal RNA (-CCUCCUA) in the mRNA sequence.

- A. False.** In *E. coli*, the second and subsequent AUG codons in mRNA can also serve as translation initiation codons.
- B. True.** In yeast mRNA, the first AUG codon is the translation initiation codon.
- C. True.** In *E. coli* mRNA, translation begins at the AUG codon linked to the SD sequence.

Q10

No.	36	37	38	39
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Answer	True	True	False	False
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The NH₂ group in the Cytosine base often reacts with H₂O molecule even under natural conditions to be converted into a carbonyl group, and then Cytosine base is converted into an Uracil base. Therefore, all organisms have repair mechanisms that detect Uracil bases in the DNA strands and replaces them with Cytosine bases.

- A. **True.** Since RNA is not transmitted to the next generation, it does not need to be repaired even if there is a chemical change in the base.
- B. **True.** If Uracil base is used in the DNA strand, chemical conversions of Cytosine-to-Uracil base cannot be detected.
- C. **False.** It is the C-G base pairs that are more likely to have mutations in mutant cells with defective Cytosine base repair mechanism.
- D. **False.** Uracil base has no NH₂ group, so chemical changes do not occur easily.

Q11

No.	40	41	42	43
Answer	False	True	True	False

- A. **False.** Alfa is not a dominant allele because A10, which exhibits the trait, is a progeny of A3 and A4, none of which show the trait.
- B. **True.** Charlie cannot deny the possibility of a dominant allele because all the children with the trait have parents with the trait.
- C. **True.** In family B, both B1 and B3 are presumed to be carriers.
- D. **False.** In family C, both C1 and C8 are presumed to be carriers.

Q12

No.	44	45	46	47
Answer	False	True	True	False

- A. **False.** Uracil is an inducer for RutR repressor, and RutR becomes inactive in the presence of uracil. Since the repression by the RutR repressor is derepressed by increasing the concentration of uracil, the expression level of the *rut* operon increases.
- B. **True.** When the affinity between the RutR repressor and uracil decreases, the sensitivity of the RutR to uracil also decreases. Therefore, the RutR repressor is less likely to be in inactive form. As a result, the repression of the *rut* operon is enhanced, and the expression level of the *rut* operon is reduced.
- C. **True.** When the DNA binding affinity of the RutR repressor decreases, repression by the RutR repressor is reduced. As a result, the expression level of the *rut* operon increases.
- D. **False.** When a change occurs in the RutR repressor binding sequence of the P_{rut} promoter, the affinity to the RutR repressor may decrease or increase. Therefore, it cannot be concluded that the expression level always increases.

Q13

No.	48	49	50	51
Answer	True	False	False	True

- A. **True.** Under conditions where glucose is available, lactose metabolism is suppressed.
- B. **False.** The first step in glycogen metabolism is the addition of a phosphate group to glycogen to produce glycogen-1-phosphate. Therefore, glycogen metabolism is suppressed under the phosphate depletion condition.
- C. **False.** The metabolism of fatty acids is an oxidative reaction. Therefore, transcription factors that activate the expression of fatty acid metabolism genes are generally activated in the presence of oxygen.
- D. **True.** Since biofilm formation is promoted by cell adhesion, motility is suppressed.

Q14

No.	52	53	54	55
Answer	True	True	True	True

- A. **True.** In situ hybridization is used to examine mRNA expression and localization in tissues.
- B. **True.** Quantitative RT-PCR can quantitatively analyze the expression levels of specific genes.
- C. **True.** *Bacillus subtilis* cultured in media with different concentrations of nitrogen sources can be screened for genes with altered expression level using the DNA microarray, which can comprehensively measure the expression levels of the entire genome.
- D. **True.** Metagenome analysis is a technology for sequencing genomic DNA extracted from microbial groups in compost for comprehensive analysis of the microbial species.

Q15

No.	56	57	58	59
Answer	True	True	False	False

- A. **True.** Germination of mutant seeds is delayed because gibberellin, a hormone that promotes germination, is not produced.
- B. **True.** In the ent-kaurene synthase knockout mutant, ent-kaurene is not produced, but the downstream biosynthetic genes function normally. Thus, the treated ent-kaurene is converted into gibberellin, and it promotes germination.
- C. **False.** GA20 oxidase is an enzyme acting downstream of ent-kaurene. Therefore the treated ent-kaurene is not converted into gibberellin, and thus germination is not promoted.
- D. **False.** Since the strain of overexpressing the CYP707A enzyme gene showed to promote germination more than the wild type seed, it is considered that 8'-hydroxyabscisic acid has a weaker germination suppressing effect than ABA.

Q16

No.	60	61	62	63
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Answer	False	True	True	False
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- A. **False.** Translation is initiated at the start codon ATG. Protein X mRNA is not translated correctly because of a frameshift.
- B. **True.** Designed correctly.
- C. **True.** Designed correctly.
- D. **False.** The reverse primer, which must be designed starting from the 3'-end in the 3'-to-5' direction to match the complementary strand, is designed in the opposite direction and therefore does not match the template.

Q17

No.	64	65	66	67
Answer	False	False	True	False

- A. **False.** Since the insertion of a single base causes a frameshift, it is considered that the function of the protein encoded by this gene is lost.
- B. **False.** Since the deletion of one base causes a frameshift, it is considered that the function of the protein encoded by this gene is lost.
- C. **True.** One amino acid is missing due to the deletion of 3 bases. If this amino acid is not critical for protein function, the function of the encoded protein may be conserved.
- D. **False.** One codon is added by inserting three bases. Although no frameshift has occurred, it is considered that the function of the encoded protein is lost due to the appearance of the stop codon TAA.

Q18

No.	68	69	70	71
Answer	False	True	True	True

- A. **False.** The transcription/translation control system by attenuation is based on the premise that the rate of ribosome progression is equal to or higher than the rate of

RNA polymerase progression. It has been found that prokaryotic transcription rate is 50–100 bases/second and translation rate is 20 amino acids/second.

- B. True.** When the operator sequence is mutated, the repressor cannot bind to it, so *trp* mRNA is always transcribed. But most transcription events terminate halfway because the translation of the leader peptide is not stagnant.
- C. True.** Even if Trp is depleted in the intestinal bacterium, the translation of the leader peptide ends, so that Region 3 and Region 4 form a pair and transcription of the *trp* operon is stopped.
- D. True.** Since the ribosome is likely to be stagnant in the leader peptide, it is considered that the *trp* operon mRNA is easily transcribed and translated even in the presence of low concentration of Trp, and the intracellular Trp concentration of *E. coli* is increased.

Animal biology

Q19 (Glucagon receptor)

No.	72	73	74	75
Answer	False	False	True	True

The magnitude of hormonal effects in animals depends on hormone concentration. At the same time, it also depends on the amount of receptors in recipient tissues or cells. We provided the students with experimental data measuring the amount of mRNA considered to be corresponding to the expression level of receptors (glucagon receptor, GLR) and asked them to discuss the given results in the context of textbook knowledge.

- A. False:** The liver responds to glucagon and functions as a glucose source. It uptakes and stores glucose in response to another hormone, insulin.
- B. False:** It may be true that brain tissue has little GLR mRNA according to the experimental data (Hansen, 1995), but this does not mean that the brain never needs glucose. I also expect that the examinees know that glucose is the major source of energy for brain tissues.
- C. True:** Hansen's data indicate muscle has little GLR. This is consistent with the properties of muscles such that they just consume ATP (working as ATP sinks) but

need not operate as ATP sources for other tissues/cells.

- D. True:** Hansen's data may be consistent with textbook knowledge indicating that adipose tissue is working as a glucose source in starvation.

Reference

L.H. Hansen et al., Glucagon receptor mRNA distribution in rat tissues. *Peptides*, **16(6)**:1163-1166 (1995).
M.A. Abraham & T.K.T. Lam, Glucagon action in the brain. *Diabetologia*, **59**:1367-1371 (2016).

Q20 (Athlete metabolism)

No.	76	77	78	79
Answer	False	False	True	True

Without any knowledge of exercise physiology, the given table would help the examinees understand that there are multiple energy sources besides stored ATP in our skeletal muscles. Crucial points I wish the students to understand are the differences in the amount and rate of power output depending on the involved metabolic pathways. The questions were prepared to ask examinees about the rationale underlying how the energy source is used during exercise.

- A. False:** For 100-meter sprinters, their muscles require energy sources with a high output rate to maintain running speed, which is sometimes faster than 10 m/s. From the data shown in the table, ATP and CP should be the main energy sources needed for such a high rate of power output. CP would be providing additional ATP molecules as the stored 8 mM ATP inside muscle cells that should be consumed within a few seconds during sprinting. Both are supported by anaerobic process metabolism (without respiration).
- B. False:** In any kind of sport, the substrate for myosin motors during muscle contractions is ATP alone. I intended for the students to infer that some amount of ATP concentration must never be depleted even if the stored ATP and CP are numerically depleted to be zero. It is quite reasonable to expect that ATP converted from stored fat or glycogen are the main energy sources for long-distance runners.

- C. True:** For 1,500 m athletes who need to run longer than 1 min, it is clear that stored ATP and CP are not sufficient. I intended for the examinees to infer that ATP supply from both anaerobic (glycolysis) and aerobic (cell respiration) metabolism are crucial, *i.e.*, an intermediate state of energy consumption between sprinters and marathon runners.
- D. True:** As explained above, ATP enables aerobic metabolism crucial for long-distance runners, thus both stored glycogen and fat should be used during a marathon.

Reference

K. Schmidt-Nielsen, Animal physiology. 5th ed. Cambridge Univ. Press (1997).

Q21 (Huntington Disease)

No.	80	81	82	83
Answer	True	True	True	False

Huntington's disease is a neurological disease that has been known for a long time, but the molecular mechanism of its manifestation was clarified only recently. This question was prepared based on a review of the causative gene mutations (glutamate repeat) in Huntington's disease as well as the results from cell biological research (defects in axonal glycolysis). Contrary to common sense held by examinees, the latter document (Zala et al., 2013) provides clear experimental evidence that axonal transport is mainly dependent on glycolytic ATP. This is a question to assess the examinee's adaptive thinking when exposed to new information that may be contradictory to their textbook knowledge.

- A. True:** The base sequence, CAG (glutamate), is found at the transcriptional initiation site of the *HTT* gene as described in the text. Thus, the N-terminal in translated HTT proteins has some sets of repeated glutamate amino acids.
- B. True:** As described in the text, Zala et al. observed that HTT, motor proteins (FAT transporter), and GAPDH (glycolysis enzyme) were all located within synaptic vesicles. In addition, the FAT of synaptic vesicles cannot be observed in HTT-depleted axons (by RNAi). This evidence implies that HTT helps to maintain the

functioning of both FAT motors and GAPDH in synaptic vesicles. Anchoring by HTT is one of the most possible assumptions.

- C. True:** As described in the text, RNAi of HTT as well as iodoacetate stopped the FAT of synaptic vesicles, but it had no effects on mitochondrial FAT. Thus, ATP produced by mitochondria cannot be utilized by synaptic vesicles, even if there is an adequate amount of ATP in axons, contrary to our textbook knowledge.
- D. False:** As described in the table, iodoacetate cannot stop mitochondrial FAT. Thus, ATP produced by glycolysis is not critical.

References

- D. Zala et al., Vesicular glycolysis provides on-board energy for fast axonal transport. *Cell*, **152**: 479–491 (2013).
- J.A. White II *et al.*, Huntingtin differentially regulates the axonal transport of a sub-set of Rab-containing vesicles *in vivo*. *Human Mol. Genetics*, **24(25)**:7182-7195 (2015)
- F. Saudou & S.Humbert, The biology of huntingtin. *Neuron*, **89**: 910-926 (2016).
- F. Saudou & S. Humbert, The biology of Huntingtin. *Neuron*, **89**:910-926 (2016).

Q22

No.	84	85	86	87
Answer	False	True	True	False

- A. False.** Beaver excretes urine with low concentration. It is expected that the JM/C ratio will be small, thus the answer is False.
- B. True.** Kangaroo rat urine concentration is 5500. At the >4000 plots, they are all over 1.5, so this statement is True.
- C. True.** Medullary salt reabsorption and increased medullary salt concentration promote passive water transport by aquaporins, resulting in decreased urinary water content. Therefore, this statement is True.
- D. False.** It is obvious that the JM/C ratio of animals living in arid climate is higher than that of animals living in freshwater. Therefore, this statement is False.

Q23

No.	88	89	90	91
Answer	False	True	False	True

- A. False.** In Fig. 1, the recording neuron is depolarized from around -50 mV in response to the application of GABA. In Figs. 2 and 3, GABA induced inward current when the membrane potential is -50 mV, and the current direction reversed when the membrane potential is -10 mV. That means the cell is more hyperpolarized. Therefore, this statement is False.
- B. True.** From Table 1, the equilibrium potential of chloride ions is -28 mV, which is higher than the resting membrane potential of recording neurons (approximately -50 mV). Therefore, this statement is True.
- C. False.** GABA treatment induces depolarization of the recording neurons and does not induce action potentials because of the blockade of Na⁺ channels by tetrodotoxin. In this situation, the membrane potential of the recording neurons will approach the equilibrium potential of Cl⁻ (-29 mV) and will not depolarize beyond the equilibrium potential of Cl⁻. Therefore, this statement is False.
- D. True.** In the “other” neuron described in this statement, the membrane potential is hyperpolarized by GABA treatment. In such a situation, Cl⁻ are expected to move inward (this is a “well-known” GABA reaction). Since Cl channels open and Cl ions flow inside, it is suggested that the intracellular Cl⁻ concentration is lower than that of the neurons investigated this time. Therefore, this statement is True.

Q24

No.	92	93	94	95
Answer	True	False	False	False

- A. True.** The nuclear/cytoplasmic ratio increases in both polyspermy and enlargement of the nuclear envelope. At both experiments, the MBT takes place earlier. Thus, this statement is True.

- B. False.** The cell cycle includes interphase after MBT. Thus, it is considered that the earlier the MBT takes place, the longer is the time required for division until the 12th round. Accordingly, this statement is False.
- C. False.** The change in the timing of MBT in the experiments of polyspermy and manipulation of nuclear size indicate that the timing of MBT is not determined by the number of cell divisions. Thus, this statement is False.
- D. False.** MBT occurs earlier as the nuclear size increases. It is possible that the concentration of histones in the nucleus is affected. Because the text states that the amount of histones does not change during the manipulation performed in this experiment, we can assume that MBT occurs when the concentration of histones gets small. The sentence states the opposite: the concentration of histone becomes BIG. Thus, this statement is False.

Q25

No.	96	97	98	99
Answer	True	True	False	False

- A. True.** It is described that the head is disrupted due to the inhibition of organizer formation. GSK3 β inhibits the formation of the organizer because the head defect occurs when GSK3 β is injected dorsally. Accordingly, this statement is True.
- B. True.** Injection with β -catenin on the ventral side induces a secondary axis, and co-injection with GSK3 β has no effect. So, this statement (GSK3 β inhibits β -catenin) is True. The possibility that β -catenin inhibits GSK is also eliminated because DN-GSK3 β /dorsal injection has no effect.
- C. False.** If β -catenin is absent on the ventral side, the secondary axis cannot be formed even if the GSK3 β is suppressed. Thus, the statement is False.
- D. False.** It is expected that suppression of β -catenin causes head defects even if GSK3 β is not activated. Accordingly, this statement is False.

Q26

No.	100	101	102	103
Answer	False	False	False	True

- A. False.** Body temperatures of some ectotherms is higher than those of endotherms.
- B. False.** When the temperature of hypothalamus elevates, it signals to decrease the body temperature of the whole body.
- C. False.** When incubating, the python requires more energy to warm the egg.
- D. True.** Exotherms consume less energy because they rely on extracorporeal temperature.

Q27

No.	104	105	106	107	108
Answer	False	True	False	True	False

- A. False.** From this experiment, it is not clear that introduction of only 3 genes are enough to induce colonies.
- B. True.** This experiment shows requirement of each gene for colony induction.
- C. False.** The long bar in 9 indicates that 9 is unnecessary.
- D. True.** To find the smallest set of genes, we need to add another gene to 14/15/20 to test whether the colonies are actually induced.
- E. False.** This experiment shows that the addition of the gene set into fibroblasts induces colonies, but does not show whether 14, 15, or 20 is expressed.

Q28

No.	109	110	111	112
Answer	False	True	False	True

- A. False.** DNA fragments do not reflect the size of the light chain gene. Also, even if they did, they would be long.

- B. True.** The difference in migration distance depends on the length of hybridized DNA.
- C. False.** The entire DNA fragment that hybridizes with 3'-terminal of mRNA differs between myeloma cells and embryo center cells. But, when considered alone, the hybridized region is the same in myeloma cells and germinal center cells.
- D. True.** Two peaks were observed in the hybrid of full-length mRNA and DNA from embryo center cells. This indicates that there are two DNA fragments that can hybridize with the mRNA sequence.

Plant biology

Q29

No.	113	114	115	116
Answer	False	False	True	True

- A. False.** The absolute humidity of the air is directly reflected in Δw . Therefore, if the absolute humidity determines relative stomatal aperture, it should be the same in the Air and Helox conditions, which are same in Δw , and it should be different in the Air and Helox^{1/2.33} conditions, which are different in Δw . However, this is not the case.
- B. False.** While d_{water} is 2.33 times larger in Helox than in Air, Figure 1 shows that relative stomatal aperture is about 2.33 times smaller in Helox than in Air. The Helox and Air conditions are same in Δw and thus have the same humidity. Taken together, transpiration rate, which is proportional to d_{water} , Δw , and relative stomatal aperture, is calculated to be the same in Helox and normal air at the same humidity.
- C. True.** Relative stomatal aperture decreases as the humidity decreases, CO_2 uptake decreases as relative stomatal aperture decreases, and the concentration of CO_2 in the leaf decreases as CO_2 uptake decreases. Because the rate of photosynthesis is dependent on the concentration of CO_2 in the leaf, the low humidity condition causes a reduction in the rate of photosynthesis.
- D. True.** Figure 1 shows that, even under the conditions with different humidity and Δw , changes of relative stomatal aperture offset such differences to keep the transpiration rate constant. In other words, at a low humidity, Δw increases but the

transpiration rate remains constant as the relative stomatal aperture decreases.

Reference

K. A. Mott, D. F. Parkhurst (1991) Stomatal responses to humidity in air and helox. *Plant, Cell and Environment* 14, 509–515.

Q30

No.	117	118	119	120
Answer	True	True	False	False

- A. True.** Protease inhibitor gene expression is not induced in the wild-type scion grafted to the *spr1* stock, which cannot perceive systemin in the proximity of the wound site.
- B. True.** Protease inhibitor gene expression is not induced in the wild-type scion grafted to the *spr2* stock, which cannot synthesize jasmonates in the proximity of the wound site, while it is normally induced in the *spr2* scion grafted to the wild-type stock.
- C. False.** Protease inhibitor gene expression is induced in the wild-type scion grafted to the *jai1* stock, which cannot perceive jasmonates in the proximity of the wound site.
- D. False.** Protease inhibitor gene expression is induced in the *spr1* scion grafted to the wild-type stock, while it is not induced in the wild-type scion grafted to the *spr1* stock, suggesting that protease inhibitor gene expression is induced only when systemin is perceived in the proximity of the wound site. Therefore, systemin is not likely to be the mobile signaling molecule responsible for long-distance wound signaling.

References

- G. I. Lee, G. A. Howe (2003) The tomato mutant *spr1* is defective in systemin perception and the production of a systemic wound signal for defense gene expression. *The Plant Journal* 33, 567–576.
- L. Li, C. Li, G. I. Lee, G. A. Howe (2002) Distinct roles for jasmonate synthesis and action in the

systemic wound response of tomato. *Proceedings of the National Academy of Sciences of the United States of America* 99, 6416–6421.

J.-Q. Sun, H.-L. Jiang, C.-Y. Li (2011) Systemin/jasmonate-mediated systemic defense signaling in tomato. *Molecular Plant* 4, 607–615.

Q31

No.	121	122	123	124
Answer	False	True	False	True

- A. False.** The results of grafting between *max2* and WT showed that MAX2 function in the shoot is required for the control of branching. This indicates that SL is perceived in the shoot to control branching.
- B. True.** The results of grafting between *max1* and WT showed that branching is normally controlled by SL if either one of the root or shoot has functional MAX1. This indicates that CL is synthesized both in the root and shoot in WT plants. Note that a normal branching phenotype obtained in grafting of the *max1* shoot scion to the WT rootstock indicates that SL synthesized in the root is transported to the shoot.
- C. False.** Grafting of the *max4* shoot scion to the *max1* rootstock resulted in a normal branching phenotype, which indicates that CL synthesized in the root is transported to the shoot and converted to SL. However, grafting of the *max1* shoot scion to the *max4* rootstock resulted in a hyper-branching phenotype, which indicates that CL synthesized in the shoot is not transported to the root. (If CL synthesized in the shoot was transported to the root in this plant, CL would be converted to SL in the root and then transported back to the shoot to control branching. This does not agree with the phenotype.)
- D. True.** As MAX2 functions in the shoot, this grafting creates practically the same situation as grafting of the *max4* shoot scion to the WT rootstock. In this grafted plant, it is expected that SL synthesized in the root is transported to the shoot and controls branching and also that CL synthesized in the root is transported to the shoot and converted to SL in the shoot and controls branching.

Reference

J. Booker, T. Sieberer, W. Wright, L. Williamson, B. Willett, P. Stirnberg, C. Turnbull, M. Srinivasan, P. Goddard, O. Leyser (2006) *MAX1* encodes a cytochrome P450 family member that acts downstream of *MAX3/4* to produce a carotenoid-derived branch-inhibiting hormone. *Developmental Cell* 8, 443–449.

Q32

No.	125	126	127	128
Answer	False	True	True	False

- A. False.** Obvious from comparison of Figure 1(a)·(b) with Figure 1(c).
- B. True.** Obvious from Figure 1(a) and (b).
- C. True.** Zn content in the root was decreased by addition of excess Fe^{2+} , while Zn content in the shoot was not changed by excess Fe^{2+} .
- D. False.** Addition of excess Fe^{2+} did not change Zn content of dry weight basis in the shoot but increased dry weight of the shoot, and therefore increased total Zn amount in the shoot.

Reference

V. Shanmugam, J.-C. Lo, C.-L. Wu, S.-L. Wang, C.-C. Lai, E. L. Connolly, J.-L. Huang, K.-C. Yeh (2011) Differential expression and regulation of iron-regulated metal transporters in *Arabidopsis halleri* and *Arabidopsis thaliana* – the role in zinc tolerance. *New Phytologist* 190, 125–137.

Q33

No.	129	130	131	132
Answer	False	False	True	True

- A. False.** In the inflorescence of sunflower, as is typical for the Compositae inflorescence, florets in the outer region are formed earlier than florets in the inner region. The sunflower florets in the outer region are ligulate and thus bilateral, suggesting that these florets have high expression of gene *G*'.

- B. False.** In variant *x*, the inner florets, which are normally tubular (radially symmetric), are changed to be ligulate (bilateral). This suggests that expression of gene *G'* is increased in variant *x*.
- C. True.** In variant *y*, all florets are tubular (radially symmetric). The disappearance of bilaterality from florets indicates that this variant is a loss-of-function mutant of gene *G'*.
- D. True.** There is an insertion shared by variants *x* and *y*, while there is no common insertion between variants *y* and *z*.

Reference

M. A. Chapman, S. Tang, D. Draeger, S. Nambeesan, H. Shaffer, J. G. Barb, S. J. Knapp, J. M. Burke (2012) Genetic analysis of floral symmetry in Van Gogh's sunflowers reveals independent recruitment of *CYCLOIDEA* genes in the Asteraceae. *PLoS Genetics* 8, e1002628.

Q34

No.	133	134	135	136
Answer	True	False	True	True

The increase of combined dry biomass in the connected ramets compared to the severed ramets showed that the connection released the restriction of photosynthesis by light or nitrogen availability, indicating exchange of nitrogen and photosynthetic assimilates between connected ramets.

- A. True.** In a severed ramet under the high PFD and low nitrogen condition, the absorption of nitrogen in the root limits photosynthesis, and as an adaptive response, the ramet increases the absorption of nitrogen by decreasing the shoot/root ratio with the sacrifice of light absorption in the shoot. However, in a connected ramet under high PFD and low nitrogen condition, nitrogen is not a limiting factor of photosynthesis because of the nitrogen supply from its partner, and such adaptive response does not occur.
- B. False.** In a severed ramet under the low PFD and high N condition, availability of light, instead of nitrogen, limits photosynthesis.

- C. True.** Under uniform conditions, exchange of nitrogen and photosynthetic assimilates between ramets does not complement resource deficiencies in each ramet.
- D. True.** As discussed above, nitrogen and photosynthetic assimilates are exchanged between connected ramets, which is mediated by translocation via stolons.

Reference

D. Friedman, P. Alpert (1991) Reciprocal transport between ramets increases growth of *Fragaria chiloensis* when light and nitrogen occur in separate patches but only if patches are rich. *Oecologia* 86, 76–80.

Q35

No.	137	138	139	140
Answer	False	True	False	True

- A. False.** Mutant x responded to sorbitol in a manner similar to the wild type, indicating that x is normal in sensing and responding to osmotic stress.
- B. True.** While osmotic stress response of mutant x is normal, its response to NaCl was very small, indicating that x is defective in sensing ionic stress. The response of mutant y to NaCl was smaller than the wild type but much larger than mutant x defective in sensing ionic stress. This indicates that mutant y can sense and respond to ionic stress. The reduction in response of mutant y to NaCl is attributable to the defect of osmotic stress sensing, in consideration of the limited response of y to sorbitol.
- C. False.** While both osmotic stress response and ionic stress response are compromised in the $x y$ double mutant, mutant x is defective only in ionic stress response. Because NaCl elicits both osmotic and ionic stresses, the phenotype of the $x y$ double mutant will be more severe than that of the x single mutant.
- D. True.** Because sorbitol elicits only osmotic stress, the phenotype of the $x y$ double mutant will be equivalent to that of the y single mutant, whose response to the osmotic stress is compromised.

Reference

Z. Jiang , X. Zhou, M. Tao, F. Yuan, L. Liu, F. Wu, X. Wu, Y. Xiang, Y. Niu, F. Liu, C. Li, R. Ye, B. Byeon, Y. Xue, H. Zhao, H.-N. Wang, B. M. Crawford, D. M. Johnson, C. Hu, C. Pei, W. Zhou, G. B. Swift, H. Zhang, T. Vo-Dinh, Z. Hu, J. N. Siedow, Z.-M. Pei (2019) Plant cell-surface GIPC sphingolipids sense salt to trigger Ca^{2+} influx. *Nature* 572, 341–346.

Evolution

Q36

No.	141	142	143	144
Answer	False	True	False	False

- A. False.** The frequency of $C^B C^B$ is about 49%. $p^2: 2pq: q^2$, $q^2 = 0.09$, $q = 0.3$, $p = 0.7$, $p^2 = 0.49$
- B. True.**
- C. False.** Random genetic drift is a more plausible explanation.
- D. False.** A small population is more sensitive to random genetic drift than a large population. Therefore, fixation probability of slightly deleterious mutations is higher in a small population than in a large population.

Q37

No.	145	146	147	148
Answer	True	False	True	True

This figure was modified from Zhang et al. 1998 *PNAS*

- A. True.** Tamarin has only the *EDN* gene. In addition, Tamarin *EDN* has a sister-relationship with both of *ECP* and *EDN* genes of all Old World monkeys. That suggests that there is only *EDN* in the common ancestor of primates.
- B. False.** *ECP* genes of human, chimpanzee, gorilla, orangutan, and macaque were derived from only one gene duplication from *EDN* in the common ancestor of them and split into each species according to the speciation.
- C. True.** X to human *ECP* gene: $3+2+0+0+0=5$, X to human *EDN* gene: $3+5+3+0+2=13$.

Number of synonymous substitutions on the branches from X to human *ECP* is smaller than *EDN*.

- D. True.** So many non-synonymous substitutions occurred on the branch of the common ancestor of *ECP* (33) although the number of synonymous substitutions seems to be comparable to the other branches, implying the operation of positive selection.

Q38

No.	149	150	151	152	153	154
Answer	False	False	True	False	True	False

- A. False.** Zebrafish SWS is equally closely related to RH and zebrafish MWS1 to 4. So, Zebrafish SWS is not “most closely related” to RH.
- B. False.** Common ancestor possesses five opsin gene.
- C. True.**
- D. False.** Zebrafish has acquired four opsin genes.
- E. True.**
- F. False.** The ancestor had a gene encoding SWS. The gene was lost in the human lineage, but human acquired a new *SWS* gene via amino acid changes from a *UVS* gene.

Q39

No.	155	156	157	158
Answer	True	False	False	True

This figure was modified from Hoekstra et al. (2006)

- A. True.** MC1R largely contributes to the body color. However, C allele is not fixed in white color population, suggesting that other genes also contribute to the body color. Indeed, such genes were discovered in a latter paper.
- B. False.** Opposite
- C. False.** The mutations in C allele occurred only one time. The existence of the C

alleles in each localities ①-③ was explained by the changes of frequency.

D. True.

Q40

No.	159	160	161	162
Answer	False	True	False	False

This question is in reference to Seehausen et al. (2008) *Nature*.

A. False. Female cichlids choose male cichlids.

B. True.

C. False. It is explained by sexual selection through female mate choice on male body coloration.

D. False. The opsin gene is not differentiated between males and females of the same species because the opsin adapted to light conditions in the habitat of each species.

Q41

No.	163	164	165	166
Answer	False	True	False	False

A. False. More slowly evolving gene should be used to infer the older divergence. Substitution will be saturated in the case of rapidly evolving genes.

B. True.

C. False. We should select organisms that are as closely related as possible.

D. False. Biological species concept does not require the monophyly of the species.

Q42

No.	167	168	169	170
Answer	False	True	False	False

A. False. We cannot judge terrestrial or amphibious in the common ancestor of whales

and hippos.

B. True.

C. False. *Remingtonocetus* and *Miacetus* ate seawater fish, implying the invasion of the oceanic environment whereas their lifestyles were amphibious. This suggests that the acquisition of fully aquatic lifestyle may occur after the invasion of the oceanic environment.

D. False. Loss of enamel gene should be after the divergence of toothed whales and baleen whales. **Q43**

No.	171	172	173	174
Answer	True	True	False	True

This figure is modified from Barton et al. (2007) "Evolution"

A. True.

B. True.

C. False. Loss of *Ubx* in T3 alters the identity of the segment. The loss of *Ubx* in T3 does not have an effect on the other hox genes.

D. True.

Q44

No.	175	176	177	178
Answer	True	True	False	True

This figure was modified from Gilbert (2015) "Developmental Biology"

A. True. Because the freshwater sticklebacks are observed in remote rivers all over the world, they are likely to be independently evolved from oceanic populations in each environment.

B. True. The paper was published to propose this hypothesis.

C. False. Because KO of *Pitx1* results in the loss all organs (ventral spine, thymus and sensory canal), the phenotype will not be the same as in the freshwater population (Indeed, they are lethal).

D. True.

Ecology

Q45

No.	179	180	181	182
Answer	True	False	True	False

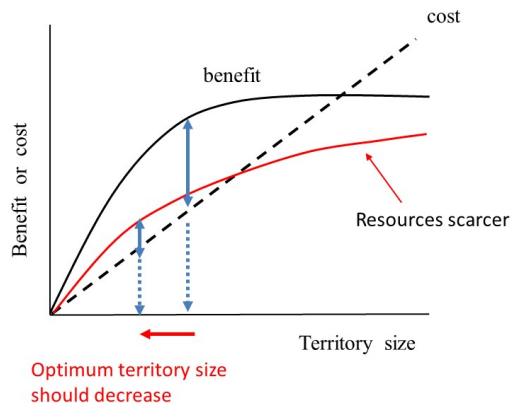
1. **True.** Asexually reproductive species do not need mating partners, but sexually reproductive species need to find a mating partner. Therefore, at low densities, the rate of increase in sexually reproduced species may decrease, but not in asexual reproduction.
2. **False.** A and D are stable equilibrium points, but B is an unstable equilibrium point that disrupts the directions of population changes. Thus, population density is not kept constant at around B in a density dependent manner.
3. **True.** Since there is a positive association between density and growth rate below C, aggregation is beneficial.
4. **False.** Extinction vortex is caused by accelerated reductions of population size with decreasing density. This is more likely to occur in type 2 than in type 1, because population growth rate is negative under low densities in type 2.

Q46

No.	183	184	185	186
Answer	False	False	False	True

- A. **False.** Large territory size does not lead to enhanced food consumption rate and food depletion. Rather, limits on foraging time and food requirement lead to the saturation pattern.
- B. **False.** Optimal territory size is the point at which (benefit - cost) or (benefit/cost) is maximized.
- C. **False.** When resources become scarcer, the slope of the benefit curve becomes less steeper and the intersection of the curve and the cost line should move to the left. Since territory size is determined in a way that maximizes the net benefit gain of individual animals, optimal territory size will become smaller (see below figure).

Notice that this does not mean home range becomes smaller, instead probably becoming larger; this is out of the scope of this question.



- D. True.** The cost line becomes steeper with increasing intensity of competition. Above a certain threshold, the cost is always larger than benefit, which makes territory establishment nonsense. Notice that this does not mean home range becomes smaller.

Q47

No.	187	188	189	190
Answer	False	True	False	False

- A. False.** Comparison of the number of seeds between (1) and (2) reveals the nighttime contributions.
- B. True.** The treatment 4 indicates evidence for selfing.
- C. False.** The treatment 3 shows a large variability in nighttime pollinations.
- D. False.** Comparison of the number of seeds between (5) and (1) indicates pollinator limitation to some degree under natural conditions.

Q48

No.	191	192	193	194
Answer	False	True	True	False

- A. False.** The presence of heterozygotes indicates the presence of the resistance allele.
- B. True.** The increase of the resistance allele frequency indicates natural selection.

- C. **True.** The decrease of allele r after cessation of insecticide application indicates its lower fitness in ordinal conditions.
- D. **False.** The allele frequency of r was 21% in 1990, while it was 94% in 1995.

Q49

No.	195	196	197	198
Answer	False	False	True	False

- A. **False.** The number of species in 50 years was lower than that in the first 2 years.
- B. **False.** The decrease in dominance of species A does not indicate competitive exclusion by itself.
- C. **True.** The abundances of the top 3 species exceed 85% of the total.
- D. **False.** The gradual decrease in the slope of the rank-abundance curve indicates the increase in evenness with time.

Q50

No.	199	200	201	202
Answer	True	False	False	True

- A. **True.** Niche differentiation indicates more efficient use of available nutrients, which results in higher productivity.
- B. **False.** The highest biomass was found at the plot with 9 species.
- C. **False.** Additional 6 species will achieve 0.1 kg increase in a plot on average.
- D. **True.** Sampling effect denotes the higher chance of inclusion of more productive species in species-rich plots, which is an inevitable consequence.