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IBO 2018: Theoretical exam 1

Duration

This exam lasts three hours.

Topics

Q 1–15 Biochemistry and molecular biology

Q 16–26 Animal physiology and anatomy

Q 27-36 Plant physiology

Q 37–47 Ecology and evolution

Marking

For questions with four statements the following marking scheme is used:

Number of correct answers	0	1	2	3	4
Points	0	0	0	0.5	1.0

For questions with five statements the following marking scheme is used:

Number of correct answers	0	1	2	3	4	5
Points	0	0	0	0.25	0.75	1.25

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This edited version contains typographical, textual and graphical corrections compared with the original exam. This version was approved by the main organizers of IBO 2018.

Q. 1 – Pathway Reconstruction

Compounds X and Y are precursors in the pathway of Z synthesis. Z is essential for growth. Wild type (WT) Neurospora are prototrophs, meaning that they can grow on minimal medium (MM).

Growth Experiment: Four Z⁻ neurospora mutants were isolated, and results of experiments with these mutants are presented below (+ =growth, - =no growth).

Cell Type	ММ	MM + Y	MM + X	MM + Z	Accumulated compound during growth in MM
WT	+	+	+	+	
Mutant 1	-	-	+	+	Y
Mutant 2	-	-	-	+	X
Mutant 3	-	-	-	+	X
Mutant 4	-	-	-	+	Y

Complementation Tests: Complementation tests were performed by testing for growth of heterokaryons in minimal medium. Results were as follows (+ = growth, - = no growth).

	Mutant 1	Mutant 2	Mutant 3	Mutant 4
Mutant 1	-			
Mutant 2	+	-		
Mutant 3	+	-	-	
Mutant 4	-	-	-	-

Mating Experiment: Mutant strains were mated and % prototrophs among spores of each mating were determined. Results were as follows.

Mating	% prototroph spores
Mutant 1 x Mutant 2	25%
Mutant 1 x Mutant 3	25%
Mutant 1 x Mutant 4	0% (many spores counted)
Mutant 2 x Mutant 3	0.004%
Mutant 2 x Mutant 4	0.001%
Mutant 3 x Mutant 4	0.001%

		True	False
A.	The mutations in the mutated strains were distributed in two genes		
B.	Considering all the mutant strains, at least five different sites of the Neurospora genome were mutated in one or more strains.		
C.	At least two of the mutant strains were double mutants (i.e. > 1 site was mutated in each of these strains).		
D.	The mutation site of Mutant 3 is positioned between the mutation site in Mutant 2 and the mutation site in Mutant 4.		
E.	It is expected that 25% of spores resulting from mating between Mutant 2 and wild type strain will be prototrophs.		

Q. 2 – DNA Barcoding

Taxonomy has traditionally been based on morphology. DNA Barcoding is a new approach that aims to allow accurate and relatively simple species identification based on the nucleotide sequence of a 650-bp fragment of the mitochondrial COI gene. The Kimura-2 parameter (K2P) distance is a widely accepted index that reflects the divergence between two DNA sequences.

In a study on fish inhabiting the Persian Gulf, over 150 individuals were studied that, based on morphology, represented 83 species. The average of intraspecific K2P distances based on COI sequence was calculated to be 1.15%. The average distance reported in various reputable fish studies in the world has been 0.25%–0.45%.

In a study on fish inhabiting the Gulf of Mexico, again over 150 individuals were studied. Based on morphology, these fish represented 76 species, 56 genera, 32 families, 11 orders, and 2 classes. The table below presents K2P distances between specimens at different taxonomic levels.

	No. of comparisons	Min. distance (%)	Mean distance (%)	Max distance (%)	Standard error distance (%)
Within species	185	0	0.18	1.66	0.02
Within genus	76	6.19	12	20.23	0.42
Within family	888	10.88	17.43	24.56	0.08
Within order	9274	14.57	21.51	28.9	0.02
Within class	3439	16.2	22.77	34.41	0.04

- **A.** The evolutionary rate of sequence change in a DNA fragment chosen for DNA barcoding should certainly be more rapid than the evolutionary rate of change in the H4 histone encoding gene.
- **B.** The high average COI intraspecific K2P distance of the Persian gulf study compared to other fish studies could be explained by existence of relatively diverged clusters within one or a few of the nominal species studied (i.e. those based on morphology).
- **C.** The data in table support the proposal that species identification of fish can be made on the basis of COI fragment sequences.
- **D.** Table 1 suggests that COI based barcoding is not appropriate for genus identification.

True	False
IIuv	1 (11)





Q. 3 – Genetic Divergence

Taxonomy has traditionally been based on morphology. DNA Barcoding is a new approach that aims to allow accurate and relatively simple species identification based on the nucleotide sequence of a 650-bp fragment of the mitochondrial COI gene. The Kimura-2 parameter (K2P) distance is a widely accepted index that reflects the divergence between two DNA sequences.

Selected results relating to 16 species from five barcoding studies from different regions of the world are presented below. Three types of divergence values were calculated, global, intraregional, and interregional distances. Global divergence which is commonly used was the average of all pairwise comparisons of sequences belonging to the same species regardless of location of origin.

Intraregional divergences were calculated by averaging the distances of all sequences belonging to the same species from the same location. Finally, interregional distances were calculated by averaging all distance values obtained from comparing each of the sequences from one location with all sequences of the same species in a second location.

Results of three calculations (i, ii, and iii) are given below:

- i. The average standard deviation (SD) of 17 intraregional comparisons pertaining to 10 species was 0.11% (minimum: 0%; maximum: 0.3%). The SD of divergences of an 18th intraregional comparison pertaining to one of these species was 1.26%.
- ii. The intraregional divergence of *Argyrops spinifer* specimens from India was 0.20%, and the interregional divergence of specimens from India and South Africa was 0.13%.
- iii. Global divergence for *Platycephalus indicus* was 9.46%. Interregional divergences for this species were as follows:

India/China 15.78%China/Australia 12.05%India/Australia 10.61%China/ S Africa 16.05%India/S Africa 4.05%Australia/S Africa 10.95%

- **A.** The SD value of the 18th comparison in calculation (i) above is consistent with the suggestion that the fish compared in fact do not all belong to the same species.
- **B.** The K2P divergence values reported in calculation (ii) is consistent with the proposal that A. spinifer populations of India and South Africa arose from a common source population, but that there is greater variation in the ecological niches of India as compared to South Africa.
- **C.** The divergence values in calculation (iii) show that, as compared to interregional divergence values, global divergence values are more informative of extent of divergence that exists for P. indicus in the world.
- **D.** With reference to calculation (iii), the difference between global divergence value (9.46%) and average of interregional divergence values (11.58) can be explained by unequal number of specimens from different regions.



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Q. 4 – Pulse-Chase Experiment

Pulse- chase experiments performed in cells are experiments in which cells are first exposed to labelled precursors of specific molecules for a short period of time (the pulse), unincorporated labelled precursors are then washed away, and presence of label in molecules of interest are followed through time (the chase). In an experiment designed to study gene expression, cells were exposed to labelled UTP during the pulse, and results of the chase part of the experiment are summarized in the figure below:



- **A.** Based on the data presented, most (~90% of mass) of RNA that is synthesized in the nucleus is degraded in the nucleus without ever entering the cytoplasm.
- **B.** Based on data presented, the complexity of RNA (which refers to number of different sequences) in the nucleus is higher than in the cytoplasm.
- **C.** Assuming that introns constitute 60% of primary transcripts, splicing can account for the observed difference in amount of label in the nucleus at start of chase and amount of label in the cytoplasm after eight hours of chase.
- **D.** It is expected that chases of longer than 8 hours would have ultimately shown a much higher amount of label in the cytoplasm than seen at 8 hours.

True False

Q. 5 – Fluorescence Quenching

Enzyme activity usually correlates with its conformational flexibility, such that higher flexibility (lower rigidity) is usually accompanied with higher activity. Tryptophan residues which emit fluorescence are most commonly located within the nonpolar interior environment of the proteins. An excellent way to experimentally determine the exposure of tryptophan residues to solution is by measuring the quenching (decrease) of their fluorescence. The effect of mutations on the accessibility of tryptophan residues can be studied by measuring amount of fluorescence quenching by potassium iodide (KI). Iodide ions selectively quench fluorescence emitted by exposed tryptophan residues.

In the experiment whose results are presented below, fluorescence quenching on equal amounts of three mutated forms of an enzyme (mutant forms 1, 2, and 3) was measured after addition of various concentrations of KI (0–0.6 M). Excitation and emission wavelengths used were specific for tryptophan. Quenching data were analysed in terms of the Stern–Volmer constant, K_{SV} , which can be calculated from the ratio of the unquenched and the quenched fluorescence intensities, F_0/F , using the relationship

$$F_0/F = 1 + K_{\rm SV}[Q]$$



[Q] = the molar concentration of the quencher.

- **A.** Among the three mutated proteins, protein 1 is expected to have the lowest enzymatic activity.
- **B.** Iodide ions have higher accessibility to the tryptophan residues of protein 2 as compared to protein 3.
- C. Protein 3 has the highest KSV.
- **D.** Protein 1 does not have tryptophan residues.





Q. 6 – AntimiRs

MicroRNAs are short non-coding RNAs expressed in various tissues and cell types that suppress the expression of target genes. MicroRNAs involved in cancer are called oncomiRs. Inhibition of oncomiRs using antisense oligomers (that is, antimiRs) is an evolving therapeutic strategy. However, the in vivo efficacy of current antimiR technologies is hindered by physiological and cellular barriers for delivery into targeted cells.

A novel antimiR delivery platform that specifically targets tumour microenvironment makes use of synthetic molecules called peptide nucleic acid (PNA) antimiRs attached to a peptide called pHLIP. PNA antimiRs are antimiRs whose nucleotides are connected by peptide bonds instead of the normal phosphodiester bonds. The structure of pHLIP is pH dependent. At low pH, a transmembrane structure is induced in pHLIP that facilitates transport of attached PNA into tumour cells (Fig. 1). pHLIP mediated transport of antimiR-155 effectively inhibited the miR-155 oncomiR in cultured cells (Fig. 2).



Subcutaneous tumour

Fig. 1: Targeting miR-155 in a mouse lymphoma model using PNA- antimiR-155-pHLIP a: Distribution of pHLIP (yellow and red zones) 36 h after injection into tail. b: Schematic presentation of pHLIP-mediated PNA antimiR delivery.



Fig. 2: pH dependent transport and activity of PNA- antimiR-155-pHLIP. a: Microscopy images of A549 cells incubated with labelled PNA- antimiR-155-pHLIP (red fluorescence) at two pHs; the label for the nucleus is blue. b: Effects of pHLIP-antimiR-155 on cell viability at two different pHs.

		True	False
A.	Based on the data, you can conclude the liver does not function in clearing the pHLIP-antimiRs from the body.		
B.	At pH less than 7, pHLIP inserts into the lipid bilayer and thus facilitates delivery of attached antimiR-155.		
C.	The acidic microenvironment of tumours is responsible for intracellular release of antimiR-155.		
D.	The antimiR cargo will be trapped within endosomes.		
E.	Transition from random coil to helix of pHLIP enhances cell death.		

Q. 7 – Steroid Hormones

Steroid hormones affect expression of primary response genes and secondary response genes in cells as schematically shown in the figure below.



Another primary-response protein turns on secondary-response genes

 A. Inhibition of DNA replication simultaneously with hormone administration
 Image: True False

 B. Inhibition of transcription simultaneously with hormone administration
 Image: Comparison of translation simultaneously with hormone administration

 C. Inhibition of transcription and translation simultaneously with hormone
 Image: Comparison of transcription and translation simultaneously with hormone

administration

Q. 8 – Uncoupling Drugs

Drugs that render the inner mitochondrial membrane permeable to H⁺ are called "uncouplers".

A.	These drugs will increase oxygen consumption.	
B.	These drugs will reduce body carbohydrate catabolism.	
C.	These drugs will decrease body temperature.	
D.	These drugs may cause death upon overdose because of severe weight loss.	
E.	These drugs may cause death upon overdose because of severe ATP loss.	



Q. 9 – Restriction Enzyme Digestion

Some restriction enzymes have different recognition sequences but create the same sticky ends, as shown below for *SalI and XhoI*.



The following gel electrophoresis image shows linearized DNAs obtained after complete restriction enzyme digestion of non-recombinant (empty vector) and recombinant (containing gene X) expression plasmid vectors. The expression vector includes a strong promoter near its cloning site. The insert of the recombinant plasmid was a product of *Sal*I digestion. The insert was ligated into the vector which had been cut with *Xho*I.



* All recombinant vectors gave the same pattern in the gel

- **A.** The data indicate that two copies of the insert were cloned in the expression vector.
- B. Half of the clones are expected to be able to transcribe the mRNA of gene X.
- **C.** An *Xho*I site exists outside of the insert in the recombinant vector of the experiment.
- **D.** The 2 kb fragment seen in the gel can be used as probe to screen for the recombinant vectors
- **E.** If the insert produced by *Sal*I digestion was ligated into a vector that had been cut with both *Xho*I and *Sal*I enzymes and the recombinant plasmid subsequently cut with *Xho*I, a 4 kb product would be obtained.

True	False

Q. 10 – Mating in Yeast

Mating experiments with a yeast species that has 16 chromosomes of equal length were performed. Results of mating between two mutant yeast strains (shown with an unhappy phenotype), each with a mutation in a single gene, are shown below. The mutated allele of each gene is recessive with respect to the wild type allele. Unhappy is polygenic and many genes affect the phenotype. Recombination does not occur in these yeast strains.



- A. If mutations of m1 and m2 were in the same gene, it is possible that all products of meiosis of cross 3 will be unhappy.
- **B.** If 1 happy to 3 unhappy yeasts were produced from meiosis of cross 3, then the mutation of m1 and the mutation of m2 were in different genes.
- **C.** If 1 happy to 1unhappy yeasts were produced from meiosis of cross 3, then the mutation of m1 and the mutation of m2 can suppress each other.
- **D.** If the experiments were performed with various pairs of unhappy mutants, the most frequently observed ratio of happy to unhappy yeasts among meiosis products of cross 3 would be 1:3.

True False

Q. 11 – Thalassemia

Thalassemia, the most common inherited disorder of hemoglobin, is caused by loss or substantial reduction of one of the globin chains. This results in lowered levels of functional hemoglobin and decreased function of red blood cells, which lead to anemia. In α -thalassemia, the α chain of hemoglobin is not produced in sufficient quantity and consequently, hemoglobin tetramers form that contain only the β chain. In β -thalassemia, the β chain of hemoglobin is not produced in sufficient quantity and the moglobin is not produced in sufficient quantity and the α chains form insoluble aggregates that precipitate inside immature red blood cells and prevent differentiation into mature cells.

The normal haploid human genome has one β chain and two α chain coding genes. Presence of four alleles for α chain compared to two alleles for β chain in the cells of normal individuals is expected to results in excess amounts of α chain and production of α aggregates. However, α aggregates do not exist in the cells of normal individuals. One mechanism for maintaining α chains in soluble form was revealed by the discovery of an 11-kDa protein in red blood cells called α -hemoglobin stabilizing protein (AHSP). This protein forms a soluble complex specifically with α chain monomers as they are synthesized. The crystal structure of a complex between AHSP and α -hemoglobin reveals that AHSP binds to the same face of α -globin as does β -globin and ensures the proper folding of α -globin as it is produced. β -globin displaces AHSP when it is expressed.

		True	False
A.	Higher incidence of severe β -thalassemia compared to severe α -thalassemia could be explained by difference in copy numbers of α and β genes.		
B.	α -globin/ β -globin ratio is an appropriate marker for screening of β -thalassemia.		
C.	α -Hemoglobin has a higher affinity for β -hemoglobin than for AHSP.		
D.	AHSP deleterious mutations are expected to mimic β -thalassemia phenotype with respect to α - chain aggregation.		





Reduced glutathione (GSH) acts to neutralize reactive oxygen species (ROSs) in the body. The reaction for GSH generation is shown below:



Individuals with glucose-6-phospho dehydrogenase (G6PD) deficiency have decreased level of NADPH which can cause the favism disorder. Compared to other populations, frequency of G6PD deficiency is higher in Africans, and its prevalence in Africa positively correlates with the prevalence of malaria.

- **A.** The high prevalence of G6PD deficiency in regions with high incidence of malaria can be explained by high susceptibility of Plasmodium falciparum to oxidative agents.
- **B.** Increase in levels of cell division is accompanied by increase in the ratio of fructose-6 phosphate/ ribose-5 phosphate production by the pentose phosphate pathway.
- **C.** G6PD deficiency affects catabolism more than anabolism in individuals with the deficiency.
- **D.** It is expected that the cause of favism in some affected individuals may be glutathione reductase deficiency.









Q. 13 – DNA Polymerase III

DNA polymerase III holoenzyme consists of the pol III* and the β -clamp which hold pol III* and DNA template together. You are investigating whether during lagging strand synthesis, the pol III* synthesizing the lagging strand dissociates from the β -clamp as it finishes one Okazaki fragment and re-associates with another β -clamp to begin making the next Okazaki fragment. You prepared a primed M13phage (a single stranded DNA and a primer) template (M13mp18) as a donor and loaded a β -clamp and pol III* onto it.

As acceptors, you then added two more primed phage DNA templates, one (M13Gori) preloaded with a β -clamp and the other (Φ X174) lacking a β -clamp (Figure A). You incubated the templates together under replication conditions for 90 min – long enough for the donor and acceptors to be replicated – then performed gel electrophoresis (Figure B, lane 1-4). You then repeated the experiment but now loading a β -clamp on Φ X174 instead of M13Gori (Figure B, lane 5-8).



- A. After replication has finished, Pol III* dissociates from its original β -clamp.
- **B.** The data are consistent with the notion that the β -clamp is not absolutely required for replication.
- C. Pol III* prefers an acceptor template preloaded with a β -clamp.
- **D.** Based on the results, one β -clamp is enough to synthesize all Okazaki fragments on the lagging strand of each replication fork.

True False

Q. 14 – Para-nitrophenol

The enzymatic reaction below includes two steps.

para-nitrophenyl-peptide \rightarrow para-nitrophenol + peptide

Para-nitrophenol has maximum absorbance at wavelength 405 nm. The progress curve of the reaction is shown below.



Time

		True	False
A.	The release of para-nitrophenol occurs during the first step of enzymatic mechanism.		
B.	The rate limiting step of this enzymatic mechanism is the second step.		
C.	Enzyme activity can be determined from the slope of region "a" of the curve.		
D.	One of the reaction products activates the enzyme.		

Q. 15 – Amino Acid Metabolism

Amino acids resulting from the degradation of proteins can be further metabolized by conversion to intermediates of the citric acid cycle. The following labelled amino acids are obtained by degradation of a labelled protein.



Indicates radioactively labeled carbon

The Krebs cycle is below. Note that most α -amino acids can be directly converted into their corresponding α -keto acid by transamination reaction as also shown below.



		True	False
A.	Upon introduction of the aspartate into the Krebs cycle, label will first appear in oxaloacetate.		
B.	Upon introduction of the alanine into the Krebs cycle, label will first appear in citrate.		
C.	Labelled alanine will yield 14-CO2 during the first turn of the cycle.		
D.	Labelled glutamate will yield 14-CO2 in the second turn of the cycle.		

Q. 16 – Pain Transmission

In figure a, the pain and temperature sensory pathway is depicted. The first-order neuron enters the spinal cord and forms a synapse with the second-order neuron, which its axon ends in the thalamus. The third-order neuron transmits the information to the brain cortex. Figure b shows the vibration and proprioception (joint position) sensory pathway. The axon terminals of the first-order neurons are located in the brain stem and form synapses with second-order neuron, which crosses the midline and their axons end in the thalamus. finally, the sensory information is conducted to the cortex via the third-order neurons.

Following a spinal injury, the right ventral and left dorsal sides of the white matter in thoracolumbar spinal cord are damaged.



		True	False
A.	The patient has impaired temperature sensation in right hand.		
B.	The patient has impaired vibration sensation in left leg.		
C.	The patient has impaired joint position sensation in left leg and temperature sensation in right leg.		
D.	The patient has impaired pain sensation in left leg.		

Q. 17 – Cardiac Response to Emotional States

Perception of the emotional stimuli is a reciprocal process between the brain and the heart. In an experimental task, pictures depicting facial expressions were quickly and sequentially presented to the subjects at different phases of the cardiac cycle. Participants then rated the emotional intensity of the faces in a scale of 0 to 100. Given the role of the amygdala in the process of fear, the amygdala response was measured in normal individuals and subjects with pure autonomic failure (PAF) at the time of presenting facial expression images (using the fMRI method). The results of the study are as follows. (Consider p < 0.05 indicates significant difference between groups).



D. Increased blood flow to amygdala in the systolic phase compare to the diastole phase *cannot* explain the results of this study.

Q. 18 – Light Refraction

When a light beam enters another medium in an oblique angle, its direction will change. This phenomenon is called light refraction. The amount of this change in beam direction can be calculated by Snell's law, where "n" is the refraction index.



This phenomenon is also the principle of conversion and diversion of beams in lenses. Power of a lens can be calculated by the following formula. Optical power of a lens demonstrates the degree of convergence or divergence of the light beam.

(D: Power of lens, r: radius of curvature)

$$D = (n_1 - n_2)/r$$

The figure below shows the eye structure of *Anableps sp.* which can simultaneously see objects in both aquatic and terrestrial scenes.



A) Anableps sp. B) Eye of Anablebs sp. C) Schematic diagram of Anableps. sp. eye



Q. 19 – Thyroid Hormone Transport

Thyroid hormones are transported in blood by proteins. The TBG (thyroid hormone binding globulin) is the main thyroid hormone transporting protein. Many factors affect TBG concentration such as oestrogen and OCP (oral contraceptive pill), etc. OCP increases TBG concentration. T3RU (T3 resin uptake) assay is a method of quantification of unbound TBG in the blood. It indirectly measures the capacity of patients TBG to bind radioactive labelled T3. The less patients thyroid hormone level is the more radioactive labelled T3 will bound to TBG. Thus, since radioactive T3 is only detectable in unbound form, the T3RU assay result will be lower.

		True	False
A.	In primary hypothyroidism, the thyroid function test would be like below:		
	TSH increased T4 decreased T3RU decreased		
B.	A person who uses OCP and is euthyroid (normal functioning of thyroid) the thyroid function test would be like below:		
	TSH normal T4 decreased T3RU decreased		
C.	In primary hyperthyroidism the thyroid function test would be like below:		
	TSH increased T4 Increased T3RU increased		
D.	In secondary hypothyroidism (pituitary dysfunction) the thyroid function test would be like below:		
	TSH decreased T4 decreased T3RU decreased		
E.	In tertiary hypothyroidism (hypothalamic dysfunction) the thyroid function test would be like below:		
	TSH normal T4 decreased T3RU decreased		

Q. 20 – Sperm Motility

Spermatozoa motility is essential for the fertilization of an oocyte. In most animals, including fish, spermatozoa are immotile in the male reproductive organ (testis or sperm duct). Spermatozoa motility is triggered after being ejaculated into the female reproductive tract (in animals with internal fertilization) or after it is released into the aquatic environment (in animals with external fertilization). Ionic composition and osmolality of freshwater, seawater, and seminal plasma of pike (*Esox lucius*), sturgeon (*Acipenser ruthenus*), and cod (*Gadus morhua*) are shown in the table. Pike and sturgeon spawn in freshwater, and cod spawns in seawater.



A) Effect of osmolality on sperm motility initiation in pike (open markers) and cod (filled markers). NaCl and Sucrose were used to make activation medium for pike sperm. NaCl and artificial sea salt were used to make activation medium for cod sperm.

B) Effect of osmolality on sperm motility initiation in sturgeon. NaCl and Sucrose were used to make activation medium for sturgeon sperm.

C) Effect of potassium ions (K⁺) on sperm motility initiation and sperm velocity in sturgeon. KCl (mM) was added into activation medium made by NaCl or sucrose with osomolality 40 mOsmol/kg.

- A. Sperm motility in pike and cod become triggered in a hypo-osmotic and hyperosmotic environment, respectively.
- **B.** Under physiological condition, osmolality is the main factor that inhibits sperm motility initiation in sturgeon.
- C. Sperm motility in sturgeon become triggered after discharged into a hypoosmotic environment with lower K⁺ ions.
- **D.** Environmental osmolality is a key signal to trigger sperm motility initiation after release from reproductive system into aquatic environment in marine fish

True

False

Q. 21 – Epstein-Barr Virus Screen

Nasopharyngeal carcinoma is closely associated with the Epstein–Barr virus (EBV) infection. In a recent prospective study involving more than 20,000 participants, detection of EBV DNA in plasma was shown to be useful in screening for nasopharyngeal carcinoma. However, 5.4% of participants without nasopharyngeal carcinoma had detectable EBV DNA in plasma at recruitment. Figure below shows the effect of ambient temperature on test results.

- Sensitivity refers to the test's ability to correctly detect affected patients who do have the condition. It is calculated as: number of affected patients with positive tests/total number of affected individuals studied.
- Specificity relates to the test's ability to correctly identified those without disease. It is calculated as: number of healthy individuals with negative tests/total number of healthy individuals studied.



- A. Based on Figure above, performing the test in warmer places will increase the specificity.
- **B.** When a test becomes more sensitive, the specificity of the test will increase.
- C. A 100% specific test will be 100% sensitive.
- **D.** To rule out a disease, it is better to use a test with high sensitivity instead of a test with high specificity.

True	False

Q. 22 – Frog Embryo Morphology

Figures X, Y and Z show the sagittal sections (the plane which divides body into left and right parts) of a frog embryo from the surface to the depth, respectively. Figures a–d are cross sections of the same embryo shown in Figure Z.



- A. Figure "a" is the cross section from region "1".
- **B.** Figure "b" is the cross section from region "3 ".
- C. Figure "c" is the cross section from region "4 ".
- **D.** Figure "d" is the cross section from region "2".





The early embryonic cells pass through two stages of differentiation: specification and determination.

		True	False
A.	These data supports that the fate of the determined cell is not reversible.		
B.	The cell that is specified loses its other differentiation potentials		
C.	If the graft is removed at the late stage and cultured in isolation, it will give rise to the red spinous sphere (shown in the picture)		
D.	If the graft is removed at the late stage and cultured in the presence of the eye inducing factors, the eye-like structure will be developed.		

Q. 24 – Phylogenetic position of Symsagittifera

Uljanin in 1870, discovered a group of marine, soft-bodied, unsegmented hermaphroditic worms without hindgut and coelom. The mouth in these worms opens to a central digestive parenchyma. They moved with their multiciliated epidermis, although, many were 'surprisingly muscular'. Most of the species were free-living and some were ectocommensals. It has now been showed that several species form obligate symbioses with green algae, making them functional photoautotroph organisms. Later, phylogenetic position of these multicellular organisms was studied using the complete mitochondrial genome of a member of this group (*Symsagittifera roscoffensis*) which has been given in the figure below.



	True	False
Based on this tree, Acoelomorpha is a sister group of Deuterostomia.		
Based on the above given description, the identified worm is a triploblastic acoelomate.		
Based on the above given text, the identified worm has incomplete digestive system.		
The provided data is consistent with the hypothesis that the identified worm belongs to the earliest diverging lineage of Bilateria.		

A.

B.

C.

D.

Q. 25 – Phylogenetic Position of Dicyemids

In 1876, Van Beneden, found a group of microscopic ciliated wormlike organisms that inhabited the renal sacs of cephalopods, mainly octopuses and cuttlefishes and named them as Dicyemids. Biologists have been fascinated because of their highly-simplified body organization and complex life cycles of asexual (under normal condition producing vermiform larva in host kidney) and sexual (under crowded condition producing infusoriform larva excreted in host urine). These ciliated animals are composed of approximately 20-30 (or 40) cells arranged in two layers, and they lack coeloms, circulatory systems, and other differentiated tissues and their embryos employ spiral cleavage. Due to their simple body plans, it can be considered as intermediates between the Protozoa and the Metazoa.



The phylogenetic position of dicyemids suggested by previous phylogenetic studies remains controversial.

		True	False
A.	Based on the above explanations, infusiform larva is a mean of dispersal for these animals.		
B.	Based on the above molecular data, these animals are missing link between Metazoa and protozoa.		
C.	Based on the data, these described animals faced a regression evolution during its evolutionary history.		
D.	Based on the given data, it is more likely that Dicyemida is more closely related to Mollusca than to Ecdysozoa.		

Q. 26 – Flight Apparatus of Birds and Bats

The figures 1-4 illustrate the schematic cross-sectional anatomy of bones and flying muscles of a bird and a bat.



		True	False
A.	Figure 1 and 2 are the bat structures.		
B.	The animal of figure 1 and 2 has seven cervical vertebrae.		
C.	In figure 1 and 2 the only mobile joint, which is involved in wing's flapping is scapula-humoral.		
D.	The muscle supracoracoideus in figure 3, is responsible for downstroke movement in flapping cycle.		

Q. 27 – Anthocyanins

Anthocyanins are vacuolar pigments that consist of two phenyl rings and one heterocyclic ring. They are the source of red to blue colours of flowers and fruits. Changes of vacuolar and soil pH, formation of complexes with metal ions, and introduction of –OH groups particularly at 3' and 5' positions and subsequent methylations at these positions all result in production of variations in the colours of the pigments.

For example, increase in the number of hydroxyl groups or complex formation with metal ions shifts absorption of the pigments to longer wavelengths, whereas methoxyl groups (OCH₃) shift absorption to a slightly shorter wavelengths. Given that plargonidin (1) is the most abundant pigment in the variety A of Tropical Water Lily flowers (*Nymphaea spp.*) indicate if each of the following statement is true or false.







- A. Pigment 3 is the most abundant pigment in flower E.
- **B.** In Nymphaea variety C, pigment 4 is more abundant than 5.
- C. Compared to the other flowers, pigment 6 is mostly abundant in flower D.
- **D.** The colour of flower B is explained by presence of higher levels of pigment 2 as compared to pigment 1.



Q. 28 – Boron as Micronutrient

Boron (B) is an essential micronutrient for plants. Its uptake, transport, and function in plants appear to be dependent on the formation of B complexes with sugars, such as sorbitol in phloem sap (Fig. 1) and a specific dimerised form of pectin in growing cell walls. Yokota and Konishi (1999) studied the effect of various exogenously supplied sugars including sucrose, glucose, and fructose on promotion of pollen tube growth by formation of sugar-borate complexes. Pollens were cultivated at different concentrations of B for 20 hours. Effect of sugars on the pH of the media is shown in Table 1, and the length of pollen tubes incubated with the various sugars is shown in Fig. 2.



Figure 1. Boron-Sorbitol complex

Table 1. Changes of the pH values of the pollen culture media containing different sugars after adding boric acid at different concentrations (first row).



- **A.** Growth inhibition of pollen tubes by high concentrations of B is more pronounced in medium containing sucrose as compared to fructose.
- **B.** At low B concentrations (5 mM), growth inhibition of pollen tubes was more pronounced in medium containing glucose as compared to the other sugars.
- C. Inhibitory effects of fructose on pollen tube growth increased with increasing concentrations of B.
- **D.** Based on effects of sugar on pH of the media, relative levels of sugar-B complex formation is as follows: Sucrose < Glucose < Fructose.

True False



Q. 29 – Nitrogen Fixation

Nitrogen is an important nutrient for all plants. While there is an abundance of nitrogen (N₂) in the atmosphere, most plants are unable to convert N2 into a usable form. Fixation of nitrogen gas into ammonia is an ability restricted to nitrogen-fixing bacteria. The figure below shows prerequisite step for nitrogen fixation through symbiosis between these bacteria and some plant species.



		True	False
A.	Between two major inorganic nitrogen forms, NH_4^+ and NO_3^- , the former is mobile in the plant, while the latter is not.		
B.	Immediately after recognition of plant by bacteria and recognition of bacteria by host plant, a calcium spike occurs in the root cells.		
C.	The plant hormone cytokinin is needed for initiation of nodule formation.		
D.	Release of bacterial exopolysaccharides is the necessary and sufficient condition for a functional symbiosis between rhizobia and its appropriate host.		

Q. 30 – Photosynthesis

Arabidopsis thaliana was used in a study on photosynthesis. *A. thaliana* plants of same age, with leaf blades of equal shape and size, were placed under various light sources that emitted different colours. Three plants were exposed to each light source. The groupings of were as follows:

Group a: blue light	Group d: blue and red
Group b: green light	Group e: yellow and green
Group c: red light	

After 5 days of illumination at equal intensity comparable to normal day light, in terms of total amount of photons and duration, the plant's physiological parameters were compared. The compensation point was measured using sunlight.

		True	False
A.	The plants of group "a" showed the highest photosynthesis rate.		
B.	Among the various groups, the light compensation point for CO_2 fixation was the lowest for group "b".		
C.	The biomass of group "d" plants was higher than group "c" plants.		
D.	At light intensities just above the compensation point, the rate of photosynthesis of group "e" is expected to increase linearly with light.		

Q. 31 – Aquatic Flowering Plants

Some flowering plants are aquatic and have adapted to live in aquatic environments. They are divided into 3 types, depending on how much of the plant is normally positioned inside or outside of the water. These groups include emergent, floating, and submerged plants. The organs of submerged aquatic plants grow completely under water.

		True	False
A.	They do not have xylem as they can absorb water from all of their surfaces.		
B.	Given that some species do have stomata, their opening and closing of the stomata is not expected to be driven by circadian rhythm.		
C.	These plants are heterophyllous, with thin and dark green leaves.		
D.	These plants have a well-developed supporting mechanical tissues with thick walls.		

Q. 32 – Speciation and Extinction in Saxifragales

An important question in the evolution of angiosperms is the influence of life form and habit on speciation and extinction. In a phylogenetic study of the morphologically highly diverse plant order Saxifragales, the effect of habit and life history on speciation and extinction was investigated. Figures A–F illustrates estimates of speciation (λ) and extinction (μ) rates in annual, perennial, herbaceous, and woody lineages (Soltis *et al.*, 2013). The results are given as posterior probability density, which reflect the uncertainty associated with parameter estimates. Dashed lines represent the median values of each distribution. Net diversification rate $r = \lambda - \mu$. Posterior probability density is a function that use to specify the probability of the random variable falling within a particular range of values. Range of value, as oppose to taking on any one value.



- A. Among different lineages, woody lineages have higher speciation (λ) and extinction (μ) rates.
- **B.** Results are consistent with the idea that shorter generation time causes higher speciation rate.
- **C.** As compared to the other groups, herbaceous perennial lineages have higher rates of net diversification.
- **D.** Generation time and breeding systems affect rates of diversification.

Q. 33 – Evolution of Annual vs Perennial Life-Histories

Phylogenetic relationship among several species of the genus Nemesia (Scrophulariaceae), accompanied with geological time scale, is shown below. Predicted states for annual/perennial lifehistory have been mapped to the tree. Annual life-history is shown in black and the perennials are shown in blue (from Datson et al., 2008).



D. *Diascia rigescens* is the ancestral species of all other taxa in this phylogenetic tree.

В.

Q. 34 – Polyploidy in Veronica

Correlation between genome size in polyploid and diploid lineages and life history and occupation of alpine habitat for the genus *Veronica* (Plantaginaceae) were investigated (Meudt *et al.*, 2015). The genus primarily originated in northern hemisphere. The phylogenetic tree below includes variables of interest including:

- 1C-value (amount of DNA contained in a haploid genome)
- life history (annual: bar; perennial: no bar)
- habitat (non-alpine: no bar; alpine: bar)
- ploidy $(2 \times \text{ to } 18 \times)$.





Q. 35 – Kleptoplasty

Chloroplast symbiosis or kleptoplasty refers to a naturally occurring process that results in maintenance of chloroplasts of one species in host cells of another species. This process has occurred in sacoglossan sea slugs (Figure 1: Pierce and Curtis, 2012). Cells that line the digestive diverticula of the slugs maintain chloroplasts that originated from algal food. Sea slug species with these chloroplasts are able to tolerate starvation for periods up to one year. It has been shown that some sea slugs are able to retain functional plastids for a year or more, or even to the end of their life.

Several hypotheses were tested to find the mechanisms behind this kind of symbiosis. All earlier attempts to locate algal nuclei in such sea slugs failed. However, recent studies have demonstrated presence of Vaucheria litorea algal nuclear genes in genomic DNA of the larvae of the slug Elysia chlorotica which have never fed on V. litorea. Note that use of animal proteins by chloroplasts seems implausible.



A: E. chlorotica



- A. Horizontal gene transfer might have resulted in transfer of certain genes of the harvested chloroplast into the genome of some slugs, particularly in slugs that have maintained chloroplasts for long periods of time.
- B. Activity of lysosomal enzymes in host cells must be minimal in order to prevent chloroplast ingestion.
- The sea slugs with algal chloroplasts also need algal mitochondria to ensure a С. source of energy during periods of starvation.
- D. Nuclear algal RNA present in ingested chloroplasts could somehow persist for as long as one year in the sea slug.

True	False

Q. 36 – Angiosperm Morphology

The following figure shows two histological sections of an organ from an angiosperm plant. The tissue section on the left was prepared from the medial part of the organ, whereas the section on the right was from the lateral part.



		True	False
A.	The sections were prepared from a monocotyledonous plant.		
B.	The sections represent a modified stem.		
C.	Transpiration occurs in this organ.		
D.	The vascular bundles are of bicolateral type, meaning that phloem is present on both sides of xylem.		

Q. 37 – Trophy Hunting in Bighorn Sheep

Bighorn sheep (*Ovis canadensis*), the males of which are famous for their magnificent curl of horns, live in North America. Their hunting was restricted in 1970. This restriction made trophy rams (males with large and fully-curved horns) extremely valuable, sometime costing over \$100,000 to be hunted. Funds raised in this way were used for preserving bighorn habitats.

Coltman and his colleagues (2003) showed that there is a relationship between year and decrease of mean mass and mean horn length of bighorn sheep in Alberta, Canada where trophy hunting was conducted over 30 years.



- **A.** The observed changes in the mean mass and horn length imply a reduction in the bighorn sheep population.
- **B.** Phenotype-based selective harvest can change population characters if it targets heritable traits.
- **C.** If variation in horn size is mainly due to additive genetic interactions, the heritability (the portion of the phenotypic variance explain by genotype variance) for this trait would decrease over time.
- **D.** By hunting males with longest horns, the variance in the reproductive success of the males increases.
- E. The trends establish genetic correlation between horn length and mass.

Irue	False

Q. 38 – Haldane's rule

J. B. S. Haldane (1892–1964), one of founders of the modern evolutionary biology, stated that if among hybrid offspring one sex is absent, rare, or sterile, that sex is the heterogametic one, i.e., in an X-Y sex-determination system, XY hybrids are preferentially sterile or inviable. Presgraves and Orr (1998) decided to test Haldane's rule on taxa lacking a hemizygous X.

They focused on the mosquitoes from the genera *Aedes* and *Anopheles*. In *Aedes*, males are XY and females are XX, but both X and Y chromosomes carry complete sets of homologous genes, while in *Anopheles* Y chromosomes are degenerate. They compiled the available data on the sterility of the hybrids resulted from crossing pairs of species in these taxa (tables below).

Hint: Assume each character is controlled by many loci.

Α	В	A crossed with B		
Ae. zoosophus	Ae. triseriatus	Only males are sterile		
Ae. triseriatus	Ae. brelandi	Only males are sterile.		
Ae. sollicitans	Ae. taeniorhynch	<i>us</i> Both sexes are sterile.		
Ae. taeniorhynch	us Ae. nigromaculati	<i>us</i> Both sexes are sterile.		
	Data on And	opheles		
Α	В	A crossed with B		
An. albitarsus	An. daeneorum	Only males are sterile.		
An. crucians	An. bradleyi	Only males are sterile.		
An. freeborni	An. occidentalis	Only males are sterile.		
An. freeborni	An. atroparvus	Only males are sterile.		
			True	F

- **A.** If the males in genus *Anopheles* were under strong selection, we would expect the same pattern of hybrid sterility as presented here.
- **B.** The hybrid sterility patterns observed in both genera are consistent with the idea that alleles involved in hybrid sterility are recessive.
- **C.** If the pattern of sterility in *Aedes* were identical to that of *Anopheles*, it would disprove Haldane's rule.
- **D.** The pattern of hybrid sterility in *Aedes* suggest variation in Y chromosome functionality in this genus.

Q. 39 – Selection in Humans

Bustamante *et al.* (2005) analysed the pattern of polymorphism in the human genome by sequencing over 11,624 genes from 39 humans. The table below shows the distributions of synonymous (changes that does not result in amino acid replacement) and non-synonymous (changes that result in amino acid replacement) single *n*ucleotide *polymorphism* (SNPs - variation within species):

	Divergence	SNPs
Synonymous	34,099	15,750
Non-synonymous	20,467	14,311

In the table, divergence refers to the fixed differences between the humans in this study and the chimpanzee genome.

		True	False
A.	Greater ratio of non-synonymous to synonymous SNPs relative to ratio of non- synonymous to synonymous divergent sites suggest the effect of negative selection in human.		
B.	The ratio of synonymous/non-synonymous SNPs is higher in genes involved in chromatin structure.		
C.	Arms races caused by host-specific pathogen would decrease the ratio of synonymous/non-synonymous substitutions in divergent sites between human and chimpanzee.		
D.	SNPs present within chimpanzee population cannot result in any level of reproductive isolation in chimpanzee population.		

Q. 40 – Evolution of Sex

The evolution of sexual reproduction has remained in certain aspects a puzzle which is yet to be fully pieced back together. Over the years many hypotheses have been proposed to explain this phenomenon.

		True	False
A.	The effect of sexual reproduction on the association between alleles is expected to improve the fitness of co-adapted genes.		
B.	Given that the majority of mutations are deleterious, sex is expected to counter the loss of mutation-free individuals in small populations.		
C.	Avoiding inbreeding in sexually-reproducing organisms is expected to be advantageous when deleterious mutations are co-dominant.		
D.	One would expect a greater proportion of sexually reproducing species in stable regions, as compared to unstable regions.		

Q. 41 – Gut Microbiota and Cancer

According to the evolutionary concepts, symbiosis is a general characteristic of living organisms and can help to understand complex features and phenotypes. Gut microbiota and host evolve in symbiosis and developed an integrative circuitry essential for their survival. This complex relationship resulted in a personal evolutionary adapted ecosystem. Recently developed genomics and metagenomics approaches helped to discover the link between the gut microbiota alteration with the host health and disease.

Gagnière and his colleagues (2016) highlighted the unexpected role of human gut microbiota in colorectal cancer. More recently in a study conducted by Flemer and his colleagues (2018) it has been emphasized that the heterogeneity of colorectal cancer might be related to the types of gut microbiota that either predispose or offer resistance to the disease.



Boxplots show the relative abundances of three bacterial groups from individuals with colorectal cancer (CRC), individuals with polyps (the intermediate stage between healthy and cancerous tissue which does not always turning to CRC) and healthy control (HC). An asterisk represents the significant p value which represented as ***p value < 0.001; **p value < 0.01; *p value < 0.05; p value > 0.05.

- A. The microbiota compositional differences between patients with CRC and the control are secondary to the onset of cancer.
- **B.** Changes in the abundance of the bacteria group 2 compared to the control are restricted to polyp.
- **C.** Any bacterial group that populates the gut is in association with an increased risk of CRC.
- **D.** The polyp-associated microbiota can be used as an indicator for individuals with higher risk of developing CRC.

True

False

Q. 42 – Trophic Relationships

One of the basic ways to illustrate trophic relationship in an ecosystem is to use pyramids of energy and biomass, which are the stack of rectangles. Each represents the amount of energy or biomass within one trophic level. We know a proportion of energy and biomass is lost from the ecosystem when transferring from one to the next level. Therefore, the size of each rectangle decreases when we move from one level to the level above it.



Examples of pyramids schemes in different ecosystems. As you can see biomass pyramid can be inverted in some ecosystems.

- A. Inverted biomass pyramids are caused by low density of heterotrophs at any given time.
 B. We can't have regular energy pyramid when biomass pyramid is inverted, thus the energy pyramid for the inverted biomass must be inverted as well.
- **C.** Biomass of autotroph are usually greater than biomass of heterotroph in terrestrial ecosystems.
- **D.** Given that phytoplankton has more rapid turnover, the biomass pyramid is more likely to be inverted.

True

False

Q. 43 – Competitive Exclusion

The competitive exclusion principle states that whenever two or more species compete for same set of limiting resources the superior competitors can drive inferior competitors extinct. On the other hand, some species coexist in naturally nutrient-poor systems while they need same limited resources. For example, pitcher plants (carnivorous plants) are considered inferior competitors. Brewer (2003) investigated an example of interaction between pitcher plants and another species.



Effect of pitchers starving and neighbour removal on production of total dry mass in pitchers' roots. Error bars are ±1 standard error (Pitcher open means the pitcher plant can easily feed. after Brewer, 2003).

		True	False
A.	When using the same resources, inferior competitors fail to coexist with superior competitors.		
B.	Inferior competitors can coexist with superior competitors if periodic disturbance remove or reduce population of superior competitors.		
C.	Intense competition can result in competing species to diversify so they can coexist while reducing the intensity of competitions.		
D.	When pitcher plants are starved, their ability to coexist with their superior competitor is significantly decreased.		

Q. 44 – Species Diversity

Regional species diversity in ecosystems usually increases with primary productivity while local species diversity does not show any defined relation with primary productivity. It suggests that different processes have distinctive influence on species diversity at regional and local scale.

Figure illustrates four hypothesized mechanisms and their influence on α -diversity (mean local diversity) and β -diversity (diversity in species composition between local sites). (HNDD : Interspecific negative population density dependence. CNDD: Intraspecific negative population density dependence.).



- A. Predators or pathogens specific to the dominant species will decrease α -diversity.
- **B.** Increasing CNDD has a homogenizing effect on community composition among sites.
- C. Regions with weak HNDD are expected to have higher degree of community differentiation.
- **D.** Environmental perturbation acts in a fashion similar to effect of increasing CNDD on alpha and beta diversity.

True	False

Q. 45 – Predator Evasion in Adélie penguins

In Adélie penguins when both parent leave the nest to find food, three-weeks-old chicks group together for protection. In this species, social defence behaviour can occur in unusual ways. When adult Adélie penguins reach the coast, they gather in groups before diving into the ocean, where usually their predator (leopard seal) could be lurking. The penguins have two choices, first to dive together, or wait until one of penguins dives so they could make sure if there is a predator or not.

		True	False
A.	This is an example of altruism behaviour as the penguin that jump first could be hunted by possible predators while transferring its genes by helping its relatives.		
B.	According to above example we can say one individual have influence on what other penguins are doing.		
C.	If a new strategy arose which would make a penguin trip another one into the ocean without retaliation from others, everyone in the population would adopt this new strategy in the long run.		
D.	A mutation that results in postponing to jump as long as possible would		

eventually go to fixation.

Q. 46 – Conditional Cooperation

Cooperation is a complicated behaviour that has evolved in animals. Conditional cooperation, such as direct and indirect reciprocity and partner choice, are some of these complicated behaviours. The simple rule which can illustrate how such behaviour evolves is the correlation between the behaviour of interacting individuals (graph below). In fact, such correlation results in cooperative individuals receiving more cooperation, so if there is a cheater, it receives less cooperation, which is a kind of punishment.



Cooperativeness

The relationship between deception and cooperation in non-human primates (After McNally, *et al.*, 2013).

		True	False
A.	Conditional strategies and mechanisms that enforce cooperation make the evolution of deception less likely.		
B.	Although overall net benefit of the cooperative behaviour is higher than the cost of being deceived, the existence of defectors doesn't allow cooperation to persist.		
C.	We can expect deception to evolve as long as it does not significantly increase the cost of cooperation.		
D.	Deceptive behaviour can occur only in novel habitats as there is no time to adapt to the new environmental conditions.		
E.	The relationship between cooperativeness and deception rate in primates can be explained by the relatively large social group size in this taxon.		

Q. 47 – Senescence

Organismal senescence is the aging of the whole organism. It is a widespread phenomenon in nature but the exact etiology is still largely unclear. An evolutionary theory was proposed by George C. Williams which involves the following assumptions:

- 1. Soma (referring to all somatic cells in the body) is essential for reproductive success, but it does not pass on to the offspring.
- 2. Alleles are affected by Natural selection.
- 3. Pleiotropic genes can have opposite effects on fitness at different ages or somatic environments.
- 4. The probability of reproduction decreases with increasing age.

Williams suggested the following example: Perhaps a gene was selected for because it codes for calcium deposition in bones, which promotes juvenile survival, but the same gene promotes calcium deposition in arteries.

A.	Lower adult death rates can be associated with lower rates of senescence,
	according to the theory.

- **B.** Where there is a sex difference, the sex with higher rate of decrease in fecundity after maturation (but with equal death rate to the other sex) should undergo the more rapid senescence, according to the theory.
- **C.** The frequencies of an allele in a gene that behaves similar to the aforementioned calcium deposition gene in a cohort will be smallest at early age and greatest at late age.
- **D.** This model views senescence as an adaptation.

True	False

End