Practical Exam "Plant Systematics,
Anatomy & Physiology"

Student Code:

IBO 2018
Tehran, Iran

29th International Biology Olympiad
July 15-22, 2018

Practical Exam

Plant Systematics, Anatomy & Physiology

Total Points: **100**
Duration: **90 minutes**

Please write your student code into the box on the title page.

Use **answer sheet**, which is provided separately to answer all questions.

The answers written in the question paper **will not be evaluated**.

In order to use the flags (the signs on your desk) just put them in the **flag stand** located on the left wall of your desk.

Please ensure that all the materials and equipments are available to you. If anything is missing, put your yellow flag in the flag stand no later that **5 minutes** after beginning of exam. (Any complaints after 5 minutes will not be accepted)

In case of emergencies or questions put your **yellow flag** in the flag stand.

No additional materials will be provided in any case of material loss during experiments.

Please be careful to follow the **safety instructions** while using materials on this task as is noted through protocol.

We suggest you to read the entire protocol before starting the experiments which helps you with time management.

Stop answering and put down your pen **immediately** at the end of exam. Put the entire protocol with the answer sheet in the envelope. Our assistants will collect the envelopes.

Good luck

Write each indicated number in the cell next to it with your own handwriting.

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MATERIALS AND EQUIPMENT (NUMBER OF EACH OBJECT MUST BE NOTICED)

1. Microscope
2. Lab gloves, tissue paper
3. Samplers (100-1000 µL) and blue tips
4. TLC plates (5 plates)
5. Pencil (1)
6. Ruler (1)
7. Tweezers (1)
8. TLC tank with lid (1)
9. Capillary tube (1 box)
10. Transparent tape (1)
11. Safely-taped razor blades (5) **Caution: Do not cut your finger. Take the blades from their yellow-taped site**
12. Band aids (2)
13. Alcohol pad (2)
14. Watch glass (5)
15. Foam (for sectioning)
16. Glass slide and glass slip (5 each)
17. Wash bottle 500 mL (1)
18. Beaker 1000, 50 and 25 mL (1 each)
19. Dropper (1)
20. Pipette filler (1)
21. Pipette 10 mL (1)
22. 1 mL syringe (1)
23. pH- indicator paper
24. Test tube (5)
25. Solution 1 for NO_3 measurement (1) [CAUTION]
26. Solution 2 for NO_3 measurement (1)
27. Color reference strip for NO_3 measurement (1)
28. Solution 1 for $CaCO_3$ measurement (1)
29. Solution 2 for $CaCO_3$ measurement (1)
30- A rack containing:
-One Falcon tube containing TLC solvent (TS: Ethanol: *n*- Propanol; Distilled water, 2:2:1),
- Extracted petal pigments in microtubes labelled as PA to PE,
- Soils washout in falcons labelled as S1-S5
-Leaf extracts in microtubes labelled as L1 to L5
-Sodium hypochlorite solution (cleaning agent)
- Staining solution (Toluidine blue O solution)
- Leaf samples A-E preserved in microtubes
- Microtube containing KOH pellets (1) [CAUTION]
31- Answer sheets (two, 1 for writing the answers and the second one which is labelled as Answer sheet 2 for pasting TLC plates)
32- Green and Yellow flags.

INTRODUCTION

Caryophyllales is the carnation order of dicotyledonous flowering plants. It is a diverse order that includes trees, shrubs, lianas, mangroves, stem or leaf succulents, annuals, and even carnivorous plants. Many members of the order are ecologically specialized to tolerate extreme environments. In the following experiment we are going to investigate ecophysiological features of five members of caryophyllales order in response to different habitats.

Task 1: Betalain and anthocyanin in Caryophyllales

Nearly all flowering plants have coloured petals with red, blue, or purple products of the anthocyanin biosynthetic pathway. Anthocyanin pigments play additional roles in vegetative tissues, providing protection against ultraviolet (UV), herbivores, and pathogens. Moreover, a second group of colourful pigments, the betalains, is found in the plant order Caryophyllales. One of these two types of pigments exists in each family and they are never found together.

The pigments of petals or bracts of five species (plants PA-PE) of Caryophyllales are extracted in microtubes (plants A-E) Each plant belongs to one family. Determine the kind of pigments present in each plant using the protocol 1.

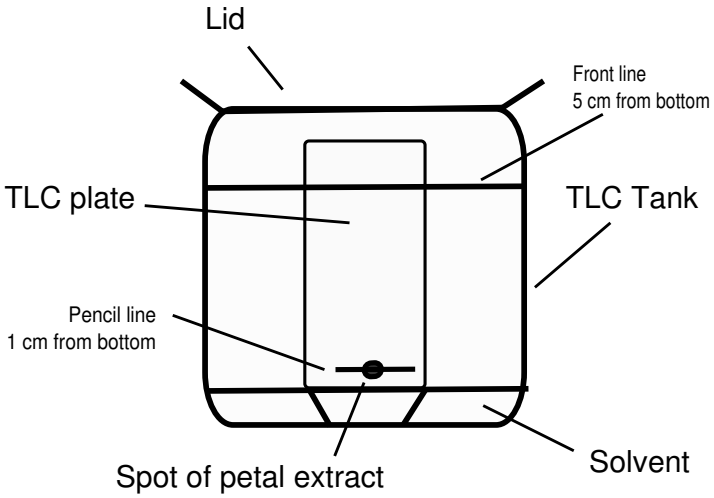
Protocol 1. Anthocyanin and betalain assay:

1.Mark the TLC plates. Draw a straight horizontal line by pencil about 1 cm from the bottom of each plate. (trapez shape. Draw another line 4 cm above the the former one and consider it as the solvent front line).

2. label the TLC plates. Label all the 5 plates from A to E on the top with a pencil (in English alphabet).

3.Take a small amount of solution. Shake the tube before using. Pick an appropriate amount of the pigment extract by using a capillary tube, to spot on the plate.

4.Spotting on plates. As shown in figure 1, gently spot each extract on a separate TLC plate on the horizontal line (Spot radius should be about 0.2 cm). Repeat spotting for each extract to concentrate pigments on TLC plate (about 3 times). It is better to let the TLC plate dry before each spotting.



5. Preparing the tank. Pour the solvent (Falcon TS) to the tank (notice that when you place the plates in the tank, the solvent level should be below the spots). Cover the tank with the lid and wait about **5 minutes** to solvent phase equilibrate with gas phase.

6. Chromatography. When the spots on the TLC plates are dry, gently and immediately (solvent/gas equilibrium must be maintained) place the TLC plates into the solvent tank by tweezers, just in the furrows, with the spotted end of each plate at the bottom.
Note: Put all plates simultaneously in the furrows inside the TLC tank.
Submerge the bottom edge into the solvent. Cover the tank with the lid again. Let the tank be undisturbed. Wait until the solvent reaches the solvent front line.

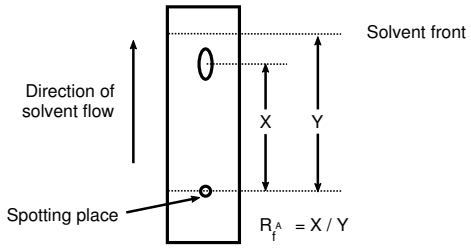
7. Remove the plate. Using tweezers, carefully remove the plate from the tank.

8. Wait for the solvent to evaporate off the plate.

9. Stabilization. Paste transparent tape on your plates to prevent pigment loss and **attach your TLC plates to the answer sheet 2.** Solvent front should be upward.
Note: Once you finished it, put the green flag in the flag stand.

10. Calculate the retardation factor (Rf). Rf is the ratio of the distance a band has moved to the distance the solvent has moved. Use the middle of each band to measure travelled distance. (Figure 2)

Note: Rfs ≤ 0.70 stand for anthocyanin and Rfs > 0.70 for betalain.



Question 1. Indicate presence of anthocyanins or betalians in plants A-E with a "✔" in answer sheet. Also write related Rf-values (Display only two digits of the decimal point and ignore other digits).

To deliver answer sheet 2, put the green flag in the flag stand.

TASK 2: ECOPHYSIOLOGICAL FEATURES OF PLANTS IN ORDER CARYOPHYLLALES

There are five soil washouts indicated as Falcons S1-S5. There are also five leaf extracts (microtubes L1-L5).

Note:The 5 soil washouts (S1-S5) correspond to the 5 leaf extracts (L1-L5), one by one (S1 belongs to L1). But we do not yet know which of these these soil washout and leaf extract pairs belong to which plant (Plants A-E).
By performing protocols 2.1 to 2.3, measure the $CaCO_3$ and Nitrate concentration of soil washout samples and the pH of both soils washouts and plant leaf extracts.

Note: The leaf extracts were prepared at dawn.

Protocol 2.1. Measuring the soil $CaCO_3$ content

To investigate the total concentration of all salts in soil, we measure $CaCO_3$ content of soil washouts by conducting volumetric assay. “Solution 1 for $CaCO_3$ measurement” contains an indicator that turns blue when there is no free Ca^{2+} in the mixture. “Solution 2 for $CaCO_3$ measurement” contains compound X (4 mM) that forms 1:1 complex with Ca^{2+} ions.

1. Add 10 mL of the soil washout to the beaker. (Wash the beaker with distilled water before this step, if necessary).

2. Add 2 drops of the “Solution 1 for $CaCO_3$ measurement” and mix. If the solution is blue, the $CaCO_3$ concentration is ≤1mg/L. If the solution is red, then proceed to next step

3. Using syringe, add “$CaCO_3$ solution 2” to the mixture until the colour turns blue. Shake the beaker while adding the solution. Write the volume of “Solution 2 for $CaCO_3$ measurement”” consumed and Ca^{2+} concentration of soil washouts in **Question 2: Table 1 of the answer sheet** (Ca molar mass = 40 g/mole).

Protocol 2.2: Measuring the Nitrate content

To measure Nitrate content of soil washouts, we use a kit. Instructions of the kit are as follow:

1. Pour 1 mL of the soil washout sample in the 25mL or 50mL beaker.

2. Add 20 drops of the “Solution 1 for NO_3 measurement” c**arefully** and then add distilled water until the total volume reaches 10 mL.

3. **Carefully** and using gloves and tweezers, pick 2 KOH pellets and dissolve them in the above mixture.

4. By adding drops of “Solution 2 for NO_3 measurement”, a yellow colour will appear.

5. Continue to add “Solution 2 for NO_3 measurement” drop-wise and shake until the yellow colour is stable.

6. Transfer about 5 mL of the mixture to a test tube and compare its color with the color reference strip for NO_3 measurement. Record the result in **Question 2: Table 1 of the answer sheet.**

Protocol 2.3: Measuring pH

To measure pH of soil washouts and leaf extracts:

Put one strip of the pH paper into the sample and pull it out after a few seconds. And shake off the paper to remove extra solution.

Determine which colour matches the reference strip and record the corresponding pH in **Question 2: Table 1** in the answer sheet.

According to obtained results answer **Question 2: Table 2 in the answer sheet.**

TASK 3: IDENTIFICATION OF C3, C4 AND CAM PHOTOSYNTHETIC PATHWAY

All three types of photosynthetic mechanisms are known among different families of Caryophyllales. In this task you should determine the photosynthetic pathway of each plant.

You can prepare cross sections with protocol 3.

Notice that **no point** is considered for preparation of cross sections**, but** you may also need your cross sections to answer task 4.

Protocol 3. Preparation of cross sections.

1**. Carefully** prepare free-hand cross-sections from pre-fixed leaves (microtubes A-E) using razor blade and foam pieces. Store the sections in water in the watch glass or on the glass slide.

2. Put hypochlorite (clearing agent) on the sections.

3. Remove the clearing agent after at least **3 minutes**.

4. Wash the sections with water (three times) to get rid of clearing agent.

5. Stain the sections with “Toluidine Blue O” solution. (Dilute the “Toluidine blue O” solution 20 times before using)

6. After **1 minute,** wash the stain away using water.

7. Put the sections on a glass slide. Cover each with a drop of water and then with a glass slip and observe them under light microscope.

8. Based on combined results of task 2 and task 3, indicate the photosynthetic type of each plant with a “✔” **Question 3: Table 1 of the answer sheet.**

TASK 4: CRYSTAL TYPES IN CARYOPHYLLALES

Calcium oxalate crystals are distributed among many members of Caryophyllales. Accumulation of crystals by these plants can be substantial. Major functions of calcium oxalate crystal formation in plants include high-capacity calcium regulation and protection against herbivory. Crystals are formed in specific shapes and sizes. The crystal morphology is species specific and is used traditionally for determination of species and genera in Caryophyllales.

Use the cross-sections of leaves you prepared in the last task in order to identify the crystal type in the plants A-E. The following figure shows the shape of common crystal types.

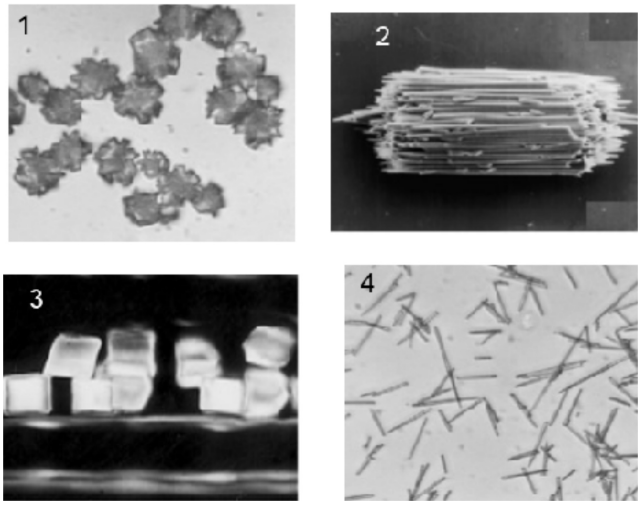


Figure 3. Different types of calcium oxalate crystals, (1) prismatic druse, (2) raphide bundles, (3) tetragonal druse, (4) single raphides.

Question 4:

A: Indicate the presence or absence of each crystal form in plants A-E with with "✔" and "×", respectively. **In the answer sheet.**

B: Given that prismatic druse crystals are abundant in halophytes, soil washout 3 belongs to plant D identify which soil (S1-S5) belongs to which plant (A-E) and fill **the Question 4: Table 2 in the answer sheet.**

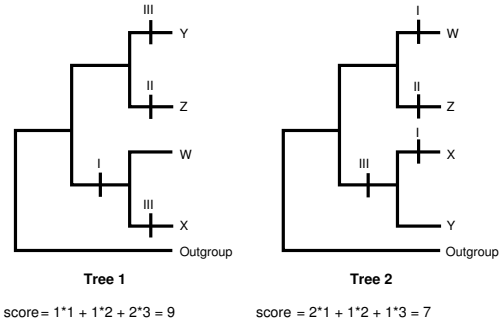
TASK 5: DRAWING A MAXIMUM PARSIMONY TREE

Maximum parsimony is a phylogenetic approach to reconstruct phylogenetic relationships. In this approach, each tree will be evaluated with the number of changes and their weights. The best tree is the one with minimum evolutionary change. Two trees based on the table below for putative taxa W-Z are shown in Figure 4. In this example, 'Tree 2' is better than 'Tree 1' due to its lower score.

taxon \ trait	I	II	III
W	1	0	0
X	1	0	1
Y	0	0	1
Z	0	1	0
weight	1	2	3

$$\text{score} = \sum N_i \cdot w_i$$

N_i is number of changes in trait i in tree and W_i is weight of trait i



The table below shows phylogenetically important traits of putative taxa P-T.

Notice the data in the table does not match with the data you obtained in the previous sections.

Plant \ trait	I (Pigments)	II (druses)	III (raphides)	IV (pollen grain)	V (corolla fusion)	VI (perianth whorls	VII (leaf arrangement
P	0	1	1	1	0	1	0
Q	1	1	0	1	1	0	1
R	0	0	1	1	1	0	1
S	0	0	0	1	0	0	1
T	0	1	1	1	1	1	0
Outgroup	0	0	0	0	0	0	0
Weight	2	2	4	3	2	2	1

The table below lists the states of the traits.

Trait	State 0	State 1
I (pigment)	anthocyanin	betalain
II (druses)	absent	present
III (raphides)	absent	present
IV (pollen grains)	smooth surface	granular surface
V (corolla fusion)	petals free	petals connate
VI (perianth whorls)	1	2 or more
VII (leaf arrangement)	alternate or rosette	opposite

Question 5: Based on the traits in the table above and their weights, draw the maximum parsimonious tree and calculate its score **in the answer sheet**.

Note: Placement of apomorphies on the tree is not necessary and no point is considered for that.

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Practical Exam

Animal Systematics, Anatomy & Physiology

Total Points: **100**
Duration: **90 minutes**

Animal systematics, anatomy and physiology lab

General information

Total points : 100
Task A : 36 points
Task B : 34 points
Task C : 30 points

Exam time : 90 minutes

Please check your student code in the box on the title page.

Use **answer sheet**, which is provided separately to answer all questions.

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Please ensure that all the materials and equipments are available to you. If anything is missing, put your yellow flag in the flag stand no later that **15 minutes** after the beginning of the exam. (Any complaints after 15 minutes will not be accepted)

In case of emergencies put your yellow flag in the flag stand.

The red flag is required for the experiment B, once you have finished the dissection.

No additional materials will be provided in any case of material loss during experiments.

We suggest you to read the entire protocol before starting the experiments which helps you with time management.

Stop answering and put down your pen **immediately** at the end of exam. Put the entire protocol with the answer sheet in the envelope. Our assistants will collect the envelopes.

Good luck

Write each indicated number in the cell next to it with your own handwriting.

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THIS LAB CONSISTS OF THREE PARTS:

A- SYSTEMATICS AND TAXONOMY OF ACARI (25 minutes) (34 points)

B- ANATOMY OF LEECH Hirudo orientalis (20 minutes) (36 points)

C- Physiological changes during exercise (45 minutes) (30 points)

Materials and Equipment

Experiment A.

-A Box containing FOUR Acari specimens on microscopic slides marked by A,B,C,D.
-A compound microscope.

Experiment B:

-Mask (1 piece)
-Set of gloves (2 pairs in each of sizes).
-Tissue papers (1 box)
-Fine dissection scissors (1 pairs)
-Forceps (1 Piece)
-Plastic petri dish (1 piece)
-Leech in tube wrapped in wet tissue (1 specimen).
-Magnifier glass equipped with LED light (1 piece).
-Color-headed pins (6 piece).
-Pins (1 small box)
-Foam dissection board marked with student code (1 piece)
-Cotton wool tampon(5 pieces)
- NaCl drops (2 container)

Experiment C:

Microtubes A-B-C-D (1 each)
Empty microtubes (12 pieces)
Micropipettes and tips (0.5-10, 10-100, 100-1000 microliter)
Falcone containing NaOH
Microtube I (containing phenolphthalein)

EXPERIMENT A

SYSTEMATICS AND TAXONOMY OF ACARI

Acari (or Acarina) is a taxon of arachnids that contains mites and ticks. In most modern treatments, the Acari is considered a subclass of Arachnida and is composed of two superorders: **Acariformes** (or Actinotrichida), **Parasitiformes** (or Anactinotrichida). Acari are arachnids and, as such, evolved from a segmented body with the segments organised into two tagmata: a **gnathosoma** (including chelicerae and palps) and an **idiosoma** (remaining body). Internal transport and exchange of oxygen and carbon dioxide in some acarine taxa usually are mediated by a branched tracheal system that opens externally through spiracular ports or **stigmata**. Stigmata are placed on different parts of body in different orders of Acari and in some orders they are associated with peritremes (See Figs. 1 & 10).

This examination is composed of 2 tasks
You have four specimens of Acari (slides A-B-C-D).

TASK A.1: Based on shape of chelicerae and palps, please suggest the life mode of each specimen with "✔"in table A.1 of **the answer sheet.**

Soil-dwelling predator: Chelicera chelate and narrow (Figure 2). Palp simple, and five-segmented and sometimes with apotele (a thick seta on palpal tarsus with 2–3 distal branches) (Figure 7).

Free-living predator: chelicera blade-like (Figure 4). A large claw placed on palpal tibia distally and palpal tarsus placed on tibia laterally (thumb-claw process, Figure 8).

Parasite: Chelicera without fixed digit and pointed (Figure 5), palp five-segmented.

Saprophagous mite: Chelicera chelate and thick (Figure 3), palp two-segmented.

Phytophagous mite: Chelicera whip-like (stylet) (Figure 6), palp four-segmented.

Specimen letter

Life mode	A	B	C	D
Soil-dwelling predator				
Free-living predator				
Parasite				
Saprophagous mite				
Phytophagous mite				

TASK A.2: Identification of Acari using a dichotomous key.

Use the dichotomous key below to identify the taxon to which each Acari belongs. Indicate your selections in the **answer sheet** by filling in the **most** appropriate boxes for each Acari. Both the figure and the table illustrate the same data.

1a. With 1–4 pairs of dorsolateral or ventrolateral stigmata posterior to coxae II (Figs. 1A, B, C, D) …… Superorder **Parasitiformes** ….....2

lb. Without visible stigmata posterior to coxae II..…… ……...……...….Superorder **Acariformes** ……..……5

2a. Body with 4 pairs of dorsolateral stigmata posterior to level of coxae III (Figure 1A)..…. Order **Opilioacarida**…..…….. Family **Opilioacaridae**

2b. Body with 1 pair of ventrolateral stigmata in region lateral to coxae II-IV or posterior to coxa IV (Figure1 B, C, D) ……..……..……. 3

3a. Stigmata without peritremes (Figure 1D) …Order **Ixodida** .....……..... 6

3b. Stigmata usually with peritremes (Figure 1B, C) ……..…….. 4

4a. Stigmata present between coxae II-III ….. Order **Holothyrida** …… 7

4b. Stigma present between coxae III-IV..... Order **Mesostigmata** ……. 8

5a. Tracheal system with 1 pair of stigmata opening between bases of chelicerae associated with peritremes dorsally on the cheliceral bases (Figure 1E) ……..…. Order **Trombidiformes** …....…. 9

5b. Tracheal system without stigmata, and peritremes never present between cheliceral bases …… Order **Sarcoptiformes** …....…… 10

6a. Paired spiracular plates situated dorsolaterally between coxae III-IV ……...……...… Family **Argasidae**

6b. Paired spiracular plates situated dorsolaterally posterior to coxa IV (Figure 1D)……. Family **Ixodidae**

7a. Corniculus (Figure 7) simple ……...……..…. Family **Allothyridae**

7b. Corniculus toothed …….…… Family **Holothyridae**

8a. Peritremes directly extended to level of anterior edge of coxae I ……...……. Family **Laelapidae**

8b. Peritremes short, looped medially or apically ..... Family **Varroidae**

9a. With 1 pair of stigmata opening between bases of chelicerae, palpal tarsus placed on tibia distally. ……..……..…… Family **Anystidae**

9b. With 1 pair of stigmata associated with peritremes dorsally on the cheliceral bases, palp with thumb-claw process (Figure 8) ……..……...…….. Family **Trombidiidae**

10a. Palps two-segmented, leg tarsi with one claw ……..… Family **Acaridae**

10b. Palps five-segmented, leg tarsi (plural of tarsus) with three claw ……..... Family **Pheroliodidae**

1a	With 1–4 pairs of dorsolateral or ventrolateral stigmata posterior to coxae II (Figs. 1A, B, C, D)	Superorder **Parasitiformes**	2
1b	Without visible stigmata posterior to coxae II	Superorder **Acariformes**	5
2a	Body with 4 pairs of dorsolateral stigmata posterior to level of coxae III (Figure 1A)	Order **Opilioacarida**-Family **Opilioacaridae**	-
2b	Body with 1 pair of ventrolateral stigmata in region lateral to coxae II-IV or posterior to coxa IV (Figure1 B, C, D)	-	3
3a	Stigmata without peritremes (Figure 1D)	Order **Ixodida**	6
3b	Stigmata usually with peritremes (Figure 1B, C)	-	4
4a	Stigmata present between coxae II-III	Order **Holothyrida**	7
4b	Stigma present between coxae III-IV	Order **Mesostigmata**	8
5a	Tracheal system with 1 pair of stigmata opening between bases of chelicerae associated with peritremes dorsally on the cheliceral bases (Figure 1E)	Order **Trombidiformes**	9
5b	Tracheal system without stigmata, and peritremes never present between cheliceral bases	Order **Sarcoptiformes**	10
6a	Paired spiracular plates situated dorsolaterally between coxae III-IV	Family **Argasidae**	-
6b	Paired spiracular plates situated dorsolaterally posterior to coxa IV (Figure 1D)	Family **Ixodidae**	-
7a	Corniculus (Figure 7) simple	Family **Allothyridae**	-
7b	Corniculus toothed	Family **Holothyridae**	-
8a	Peritremes directly extended to level of anterior edge of coxae I	Family **Laelapidae**	-
8b	Peritremes short, looped medially or apically	Family **Varroidae**	-
9a	With 1 pair of stigmata opening between bases of chelicerae, palpal tarsus placed on tibia distally	Family **Anystidae**	-
9b	With 1 pair of stigmata associated with peritremes dorsally on the cheliceral bases, palp with thumb-claw process (Figure 8)	Family **Trombidiidae**	-
10a	Palps two-segmented, leg tarsi with one claw	Family **Acaridae**	-
10b	Palps five-segmented, leg tarsi (plural of tarsus) with three claw	Family **Pheroliodidae**	-

No image info

Show serially your identification pathway **in table A.2 of the answer sheet**. For example, a pathway to the family Holothyridae is as follow:

Step / Specimen	1	2	3	4	5	6
Family Holothyridae	1a	2b	3b	4a	7b	-
Slide “A”						
Slide “B”						
Slide “C”						
Slide “D”						

EXPERIMENT B:

ANATOMY OF LEECH *Hirudo orientalis*

Introduction:

The Persian leech *Hirudo orientalis* Utevsky and Trontelj, 2005 is a clittellate annelid and belongs to family Hirudinidae. This was described from the Caspian region. It has been used for leech therapy (Hirudotherapy) from the time of Zoroastrian in the Persian traditional medicine. Avicenna, the great Persian philosopher and physician, used the leeches for treatment of different diseases. The cure was aided by injection of active compound of its saliva, while sucking blood, into the host bloodstream. Due to wide use of Hirudotherapy in the recent years the wild populations get close to extinction and therefore, School of Biology, University of Tehran has been working on this species life cycle for a decade, firstly to stop the pressure of leech caught from the natural ponds for conservation purposes and secondly to supply clean cultivated ones for leech therapy. The specimen on the table is a cultured one at indoor aquaculture facility at University of Tehran and was fed on pathogen free horse or camel blood.

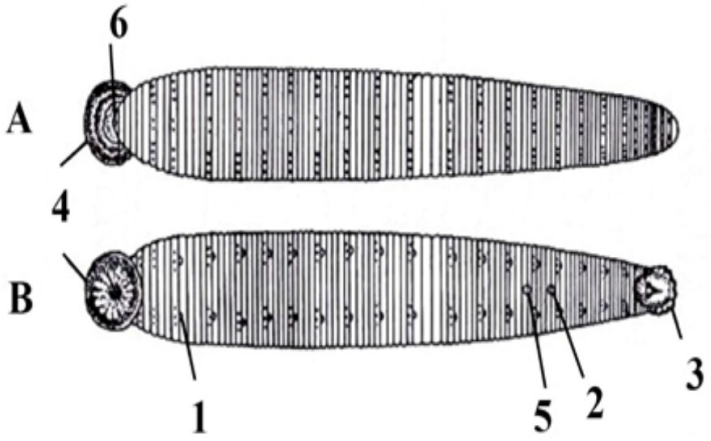
Task B.1 Identify the external structures of *Hirudo orientalis*.
Task B.2 Dissect and identify the internal structures of *Hirudo orientalis*.

Task B.1. Identify the external structure of *Hirudo orientalis*.

Use a hand magnifier glass to observe the anal pore, oral and rear suckers, nephridiopores, male and female genital pores in the provided specimen of *Hirudo orientalis*. Then, answer the following questions in the **ANSWER SHEET.**

Task B 1.1
Indicate If each of the following statements is true or false with a "✓" in the answer sheet.
Q.1 The clitellum is easily seen antero-dorsally on the provided specimen.
Q.2 The crawling of this species requires the use of three types of muscles, namely longitudinal, circular and diagonal.

Task B.1.2
According to the following figure match the correct number with its relevant characters in the following table **in your answer sheet.**



Characters	Oral sucker	Rear sucker	Anal pore	Male genital pore	Female genital pore	Nephridiopore
Number						

Task B.2.2. (18 points)

Dissection and identification of the internal structure of *Hirudo orientalis*.

Put on the gloves, take your alcohol treated leech wrapped in wet tissue from the provided tube using the forceps and place it inside the petri dish. For your convenience its gut blood is mostly ejected thorough a small horizontal cut into the body wall and gut, at the center of the body, after narcotization. But, there is always some blood in the gut. First, take the blue dissecting board (~15×20 cm), place the leech head forward (toward student code) and dorsal up. Fix the leech by inserting the provided ordinary pin obliquely into the anterior and posterior sucker on the foam dissecting board. Then, locate the anus and lift the dorsal cuticle using the forceps, about 2 cm away from the anus, anteriorly. Insert the tip of the scissors into the cuticle and make a small cut. The cut should be deep enough to reach the gut dorsally with the scissors. Continue to cut the dorsal cuticle and dorsal gut wall together toward the oral sucker. Clean the residual gut blood using cotton wool tampon and NaCl drops and throw away the unclean paper tissue to the small rubbish bin on your desk. Take the skin apart and pin it down using the ordinary pin, inserted obliquely. The reproductive, nervous, urinary systems are located ventrally under the gut wall and are easily visible by the naked eye or a magnifying glass.

Note that this species is a hermaphrodite and at any particular time, each individual can act as a potential male or female.

When dissection is finished, **mark the following organs using the provided colored pins.**

Organ	Pin color
Salivary gland	Pink
Vagina	Black
Testis	Yellow
Prostate	Green
Epididymis	White
Segmental ganglion (2 cm below the genital organs)	Blue

In accordance with the internal and external features of the dissected specimen, indicate if each of the following statements is true or false with a "✓" in the **answer sheet**.

Q.1 Gas exchange is cuticular.

Q.2 Potentially, each individual can mate with several potential females.

Q.3 Fertilization is internal.

Q.4 Individuals are capable of self-fertilization.

Q.5 The coelom is highly enlarged to support large amounts of blood storage in the gut.

Q.6 The gut wall bears lateral pouches to increase the intestinal surface.

Q.7 This species bears a proboscis.

Q.8 Analysis of the external rings and internal anatomy of the species shows 50 or more segments.

When the marking is finished, put your **RED** flag in flag stand and a laboratory assistant will take a photo of your dissection. He/she also will take your dissection board away. After this stage take your gloves out and put it in the rubbish bin.

EXPERIMENT C

Physiological changes of exercise.

In this part, we aim to evaluate physiological changes of exercise. To do so, we will go through the following steps:

Measuring QT interval in electrocardiogram (ECG), during exercise.
Indicating significant changes in ECG with statistical analysis.
Measuring post-exercise pH.

3.1. Measuring QT intervals

In this part, you will measure QT intervals in the following ECGs. We have provided four ECGs that belongs to the beginning, 1st, 2nd and 3rd minute of exercise. Based on the guide provided and using the grid, measure the QT interval of each ECG. (Note that in the ECGs, the smallest square equals to 0.04 seconds)

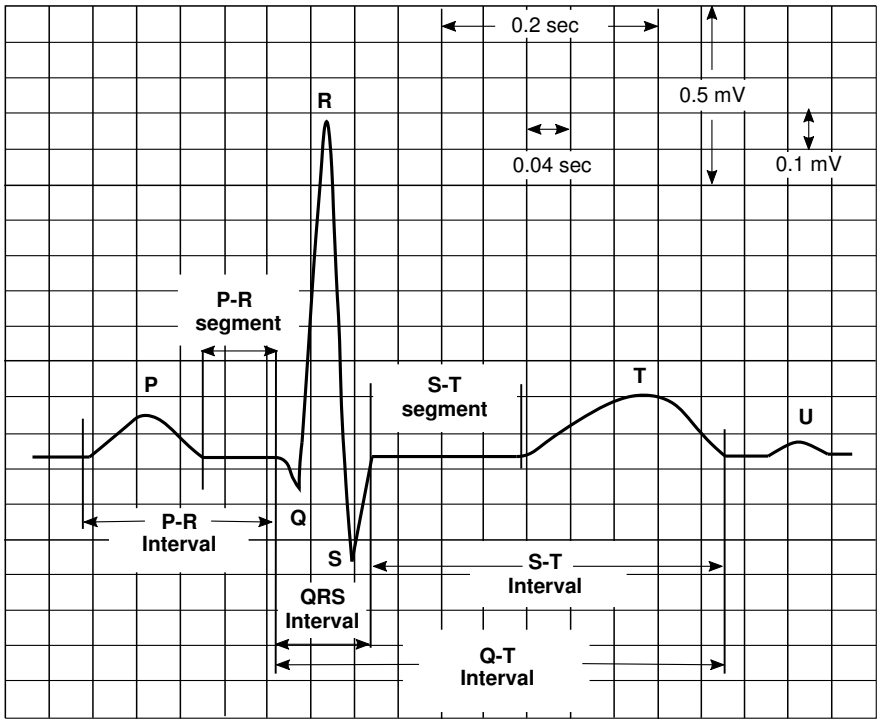


Figure 1. *ECG guide. Note that the QT interval is the exact time beginning at the start of Q wave until the end of the next T wave.*

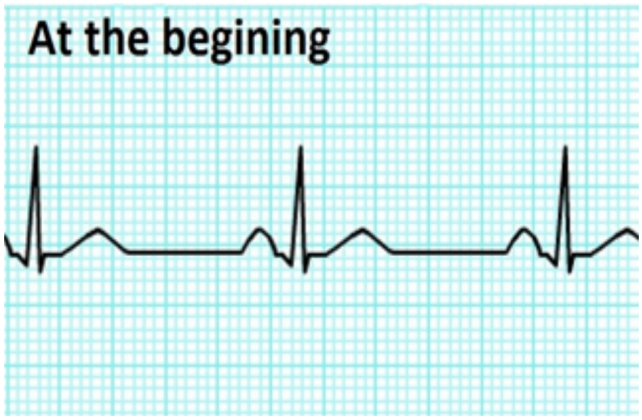


Figure 2. *A part of ECG at the beginning of exercise.*

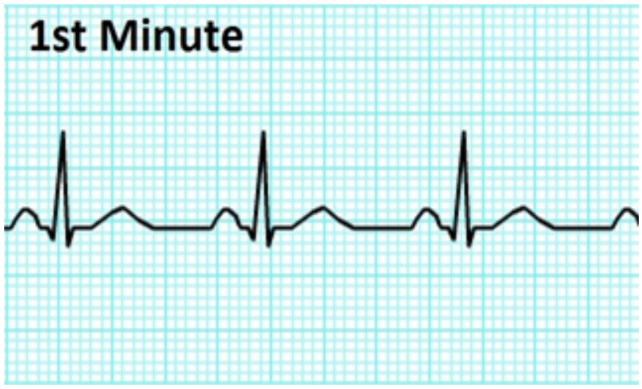


Figure 3. *A part of ECG in the 1st minute of exercise.*

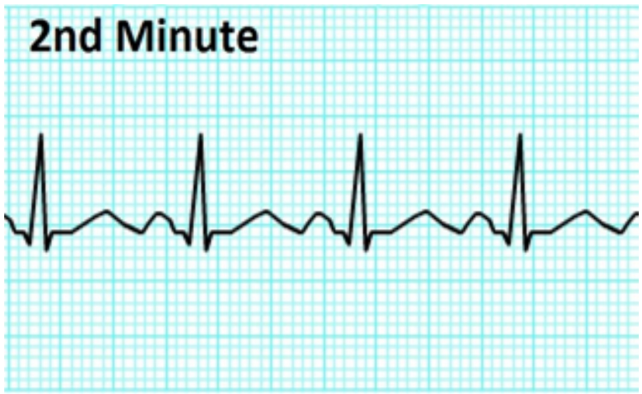


Figure 4. *A part of ECG in the 2nd minute of exercise.*

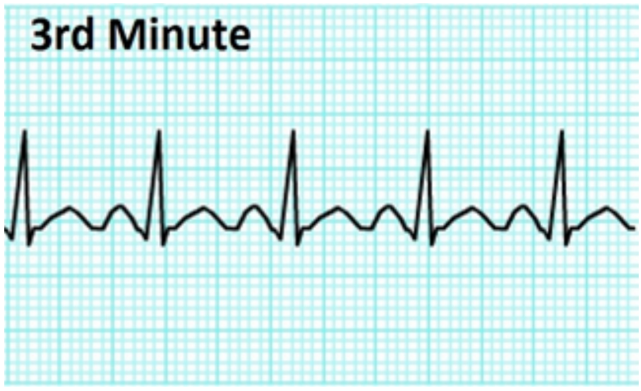


Figure 5. *A part of ECG in the 3rd minute of exercise*

Task 3.1. Write your answers in seconds in the **Answer sheet (rounded to two decimal places)** . (0.5 point each)

	QT interval
At the beginning	
1st minute	
2nd minute	
3rd minute	

3.2. Statistical analysis of QT intervals

We have measured QT intervals in three different subjects during exercise. In **3.2.A.,** you will perform analysis of variance (ANOVA) to test for significant changes of QT interval during exercise in healthy individuals. In **3.2.B.,** the same analysis were performed on healthy individuals during pacing stress testing, instead of measuring QT interval during exercise. Finally, you will interpret your results and make the conclusion.

3.2.A. Conducting ANOVA on QT intervals data.

3.2.A.1. In the following table, we have measured QT intervals (in milliseconds) of three different subjects.

	Subject 1 QT interval	Subject 2 QT interval	Subject 3 QT interval	Mean
1st minute	360	340	347	349
3rd minute	320	312	325	319
10th minute	310	298	307	305
20th minute	298	280	295	291
Mean	322	307.5	318.5	Total mean (M_T) = 316

Task 3.2.A.2. Calculate the "total sum of squares" (**SS_T or SS_{total}**), using the following formula. **Write in Table 3.2.A of the answer sheet (rounded to one decimal place).**

$$SS_T = \sum (X - M_T)^2$$

X: individual value for each subject for each time-point, $\mathbf{M_T}$: total mean,

Task 3.2.A.3 Calculate the "between group sum of squares" ($\mathbf{SS_{between}}$ **or** $\mathbf{SS_B}$), using the following formula. **Write in the Table 3.2.A of the answer sheet (rounded to one decimal place).**

$$SS_B = n \sum (M_G - M_T)^2$$

$\mathbf{M_G}$: mean for each group compared, $\mathbf{M_T}$: total mean, **n**: the number of observations in each group.

Task 3.2.A.4 Based on the following table, fill the blank cells in the summary of ANOVA for QT intervals. **Write in the Table 3.2.A of the answer sheet. (rounded to one decimal place)**

Summary of ANOVA for QT intervals:

Source	Sum of Squares	Degrees of Freedom	Variance Estimate (MS)	F Ratio
Between	SS_B	K - 1	$MS_B = \frac{SS_B}{K - 1}$	$\frac{MS_B}{MS_w}$
Within	$SS_W = SS_T - SS_B$	N - K	$MS_w = \frac{SS_w}{N - K}$	
Total	SS_T	N - 1		

K is the number of time-points and N is the total number of observations.

Task 3.2.A.5. Using the table below, estimate the upper threshold for the P value corresponding to the F ratio you have obtained. **Write in the answer sheet. (1 point)**

F ratio	P value
0.45	0.5
2.92	0.1
5.42	0.025
7.59	0.01
9.60	0.005
15.83	0.001

P value	

3.2.B. In the second experiment, similar data was obtained from three subjects who underwent pacing stress testing during cardiac catheterization. In this experiment, heart rate was elevated gradually during the experiment using a cardiac catheter, instead of the elevated heart rate being observed during exercise, which was done in the previous experiment. The results are presented in the table below.

	Subject 1 QT interval	Subject 2 QT interval	Subject 3 QT interval
1st minute	310	315	310
3rd minute	310	310	310
10th minute	310	305	310
20th minute	310	305	305

Then again, we have performed ANOVA and **F ratio of 2.2 was obtained.** Using the table below, estimate the upper threshold for the P value corresponding to the F ratio given above. **Write in the answer sheet. (1 point)**

F ratio	P value
0.45	0.5
2.92	0.1
5.42	0.025
7.59	0.01
9.60	0.005
15.83	0.001

P value	

Task 3.2.C.1. Considering a P value less than 0.05 to be statistically significant, are the effects of exercise and pacing different? (Indicate the correct answer with "✓" in the answer sheet.) (2 points)

Yes	
No	

Task 3.2.C.2. Based on your physiology knowledge and the results of this study, indicate if each of the following statements is true of false. (Indicate the correct answer with "✓" in the answer sheet.) (1 point each)

statement	**True**	**False**
1. Opening and closure of the aortic valve happen in the QT interval in healthy subjects.		
2. Based on the results from the first study, cardiac output would decrease between 1st and 20th minute of exercise.		
3. Results from the second study indicate that sympathetic activation is probably the main factor behind QT interval changes.		

Task 3.3: Measuring urine and blood plasma pH after exercise.

A group of researchers wanted to measure urine and blood plasma pH 30 minutes after exercise, so they have collected urine and blood plasma samples from three vessels of a monkey with ventricular septum defect (VSD) 30 minutes after exercise. The defect in the ventricular septum allows blood to leak from the left ventricle to the right ventricle.

They used chromatography technique to eliminate the effect of proteins on titration of samples by separating proteins of **"Original samples"**.

In the second step, researchers wanted to remove the effect of other buffers in their sample, so they have added HCl to the collected samples based on their routine protocols.
The "**Treated samples"** from four different sources are provided in four microtubes (A-D).

To find the source from which each of the samples were collected, you have to measure the pH of microtubes (A-D) using the following protocol:

1. Start with choosing one of your samples (A-D) and adding 100 microliters of that sample to an empty microtube.

2. Using your micropipettes, add 10 microliters of phenolphthalein indicator (microtubes “I”) to the micrutube containing 100 microliters of the sample. (The indicator is colorless in the acidic solutions and pink in basic solutions.)

3. Start the primary titration of the solution you have prepared through last two steps by adding 100 microliters of **NaOH (0.01M) solution**.

4. Do the previous step (Adding 100 microliters of NaOH) again until the point that your indicator turns faint pink and the pink color does not disappear by pipetting. (At pH of 7, the indicator turns faint pink and it is the end point of titration.)

5. Now to do a more accurate titration of the chosen sample, throw away the microtube of the titrated solution (solution that turned faint pink in the step 4) and prepare another solution in another empty microtube for the chosen sample based on steps 1 and 2.

6. Add NaOH to the solution of step 5 until you have 100 microliters left to reach the end point volume you have reached at step 4.

7. Add 10 microliters of NaOH to the solution of step 6.

8. Do the previous step (Adding 10 microliters of NaOH) again until the point that your indicator turns faint pink and you reach the end point. Write down the total volume of NaOH you used to reach the end point. The NaOH volume you reached in this step is more accurate than the estimated volume of step 4.

9. Choose another sample (A-D) and do steps 1 to 8 for that sample to find out the NaOH volume you need to reach the end point of titration.

Task 3.3.1. Based on the volume of NaOH (0.01M) solution you have used to reach the end point of titration of each sample, calculate the pH of each **“Treated sample”**. **(rounded to three decimal places).**

sample	A	B	C	D
volume of NaOH added (microliters)				
pH of **“Treated sample”**				

Task 3.3.2. The protocol that researchers used to diminish the buffers of original samples, has provided a formula (find below) to calculate the pH of “Original Sample” based on the results of titration of “Treated Sample”.

$$\text{pH of Original Sample} = 6.37 + \log \frac{10^4 - V}{\alpha}$$

V: Volume of NaOH used to reach the end point of titration of **“Treated Sample” (microliters)**

α: It is an index corresponding to buffer content of the “Original Sample”. The α index of each sample is provided in the table below.

Sample	A	B	C	D
α Index	6420	902	1111	709

Considering the provided formula, the α index and the NaOH volume used to reach the end point of titration, calculate the pH of **"Original Samples"** and write them in the answer sheet. **(rounded to three decimal places).**

Sample	A	B	C	D
pH of **"Original Sample"**				

Task 3.3.3 Based on the calculated pH of the original samples, determine the source of each sample and write its name (A-D) under its source in the answer sheet.

Source	Inferior vena cava	Pulmonary artery	Pulmonary vein	Urine
Sample name (A-D)				

Task 3.4 Determine whether each of the following statements is true or false and check your answer in the answer sheet (indicate your answer with "✓" in the related box).

statement	**True**	**False**
1. Partial pressure of O_2 (PO_2) in right atrium is higher than that of pulmonary vein.		
2. Partial pressure of CO_2 (PCO_2) in pulmonary artery is higher than that of pulmonary vein.		
3. In a healthy subject with hyperventilation that has led to decreased PCO_2 in the blood, the kidney tries to increase the amount of bicarbonate ions in inferior vena cava.		
4. During fasting that leads to increased amount of lactic acid in blood, the kidney tries to decrease the amount of hydrogen ions in urine.		
5. In a subject with normal respiration and without hyperventilation, if the hydrogen ion pumps of the nephrons get inhibited and hydrogen ion secretion into the tubular fluid gets decreased, the amount of bicarbonate ions in pulmonary artery would be less than normal.		

IBO 2018, Tehran, Iran

Practical Exam "Biochemistry & Molecular Biology"

Student Code:

IBO 2018
Tehran, Iran

29th International Biology Olympiad
July 15-22, 2018

Practical Exam

Biochemistry & Molecular Biology

Total Points: **100**

Duration: **90 minutes**

General information

-Protocol instructions and Answer sheet will be provided in your cabins within an envelope.

- Total points: 100

- Exam time: 90 minutes

- Please write your student code into the box on the upper left side of the title page.

-Write all answers on the **answer sheet**, which is provided as separate sheet. Points will not be given to answers written on the question paper**.**

- In order to show a flag, place it in the **flag stand** (=small tube) located on the left wall of your desk.

-Please ensure that all the materials and equipments are present in your cabin. If anything is missing, you must report it **within five minutes** after start whistle by showing your yellow flag in the flag. Report of item(s) missing after the five minutes will not be considered.

-In case of emergency, put your yellow flag in the flag stand.

-Additional materials will not be provided in any case of material loss during the experiments.

-We suggest you familiarize yourself with the experiments before starting by reading the entire protocol before starting.

-Stop answering **immediately** when the stop whistle is blown at the end of the exam. Put the entire protocol along with the answer sheet in the envelope. Lab assistants will collect the envelopes.

-In case you have placed your green flag in the flag stand, but picture of you gel has not been taken by the time that the stop whistle blows, stand up next to your cabin and wait until an assistant comes and takes the picture.

Good luck

Write the indicated number in the tables below.

1	

7	

PRACTICAL EXAM OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

Materials: CHECK AND REPORT ITEMS MISSING WITHIN FIVE MINUTES AFTER WHISTLE

A. Biological

1. 500 µl bacterial lysate (labeled “Bacterial lysate BL”; in box)

2. 500 µl BSA solution, 1 mg/ml (labeled “BSA”; in box)

3. 30 µl plasmid DNA, 250 ng/µl (labeled “plasmid DNA”; in box)

4. 10 µl DNase (labeled “DNase, 0.015 Units/µl”) **on ice**

5. 20 µl DNA size marker (labeled “DNA size marker”; in box)

B. Non-Biological

1. Waste bucket (labeled “Waste bucket”)

2. 1 ml Lysis Buffer (labeled “Lysis buffer LB”; in box)

3. 2 ml Buffer A (distributed in two tubes, each labeled “Buffer A”; in box)

4. 1 ml Buffer B (labeled “Buffer B”; in box)

5. 3 ml phosphate buffered saline (distributed in two tubes, each labeled “PBS”; in box)

6. 8 µl DNase reaction buffer (labeled “DNase buffer”) (**on ice**)

7. 50 µl gel loading dye (labeled “Loading dye”; in box)

8. 26 X 1.5 ml tubes (in box)

9. 4.2 ml Bradford reagent (in 15 ml tube) (labeled “Bradford reagent”)

10. Affinity chromatography column (labeled “Column”; placed on column holder rack)

11. Micropipette Stopper Tip (labeled “MST")

12. Column holder rack (labeled “Column holder rack”)

13. Tube rack for fraction collection (labeled “Tube rack”)

14. 2-20 µl Micropipette

15. 20-200 µl Micropipette

16. 100-1000 µl Micropipette

17. Yellow tips for 2-20 µl and 20-200 µl Micropipettes

18. Blue tips for 100-1000 µl Micropipette

19. 96 well plate (with student’s name)

20. Aluminum foil

21. Agarose gel electrophoresis system with incorporated power supply

22. Agarose gel containing DNA-binding stain (already placed in electrophoresis system)

23. Disposable gloves

24. Goggles

25. Water proof pen marker

26. Three flags, colored red, green, and yellow

27. Flag stand (=small tube) located on the left wall of your desk

28. Your name tag (placed on shelf)

We suggest that you familiarize yourself with the experiment by reading the entire text below before starting the experiment

Introduction

In this experiment, you will test the ability of a protein named Pep (that is positively charged under the experimental conditions) to interact with DNA. You will be supplied with the DNA to be tested, but you must purify the protein Pep from a crude bacterial lysate. The bacteria had previously been transformed with a plasmid expression vector into which the Pep encoding gene with a histidine tag had been cloned. Purification will be done by affinity chromatography. The histidine tag has affinity for and binds to nickel which is attached to the resin in the columns. After binding, the protein can be detached from the resin by changes of buffers used in the chromatography protocol. Eluted fractions will be collected in several tubes. You will determine the protein concentration in two of the fractions by the Bradford method. This is a colorimetric assay in which attachment of Comassie brilliant blue to protein results in increased absorbance at wavelength of 595 nm. By using a standard curve derived by assay of a bovine serum albumin (BSA) protein solution of known concentration, the protein concentration of the fractions can be determined. Subsequently, you will test the ability of the Pep protein in one of the fractions to interact with DNA by performing a gel retardation assay. In this assay, interaction of DNA with protein retards the migration of the DNA on agarose gels during electrophoresis.

PROTOCOL

A. Purification of Pep protein from bacterial lysate by affinity chromatography

1. Take note of the chromatography column that is already placed in the hole of the column holder rack. Also take note that it fits into the hole tightly. Avoid having to remove it from the hole during the course of the experiment. The column is sealed at the bottom and the resin in the column is covered with a small volume of ethanol. Look carefully at the contents of the column to correctly detect the border between resin and the overlying liquid.

2. Open the red cover on top of the column, remove the micropipette stopper tip (MST) at the bottom of the column, and quickly place tube #1 in the tube rack under the column in order to collect the eluting ethanol. Collection should continue just until there is no ethanol left above the resin. Three to four drops will be collected.

3. Quickly, but gently add 500 µl lysis buffer to the column without disrupting the resin within the column in order to equilibrate the column with LB.

4. Collect the eluting drops in tube #2 just until there is no buffer left above the resin. (Approximately 500 µl will be collected.)

5. Quickly, but gently add 500 µl bacterial lysate onto the column and start collecting drops in tube #3. Be sure that all the BL has entered the column. (Approximately 500 µl will be collected.)

6. Quickly, but gently add 500 µl wash buffer A and start collecting drops in tube #4. As the volume of the buffer above the resin decreases to about 100-200 µl, add 300 µl additional buffer A and continue collection of drops in the same tube #4. Continue with addition of 300 µl three more times (i.e. column should be washed with a total volume of 1.7 ml buffer A). Collection can be continued in tube #5. (Approximately 1.7 ml will be collected in total in tubes # 4 and 5.)

7. Quickly, but gently add 500 µl buffer B to start detachment of Pep molecules bound to the resin in the column. Start collecting drops in tubes #6, 7, and 8. Three drops should be collected in each tube. As the volume of the buffer above the resin decreases to about 200 µl, add 200 µl additional buffer B and continue collection of the eluent just until three drops have been collected in tube #8.

8. Quickly seal the bottom of the column with micropipette stopper tip (MST) by inserting the bottom of column into the wide mouth of the tip.

B. BRADFORD PROTEIN ASSAY (THIS TASK HAS TWO PARTS)

Part 1

Prepare BSA (= the standard protein) dilutions as shown in Table 1:

Table 1

Tube #	9	10	11	12	13	14	15
BSA (1 mg/ml)	0 µl	20 µl	40 µl	60 µl	80 µl	100 µl	120 µl
PBS	200 µl	180 µl	160 µl	140 µl	120 µl	100 µl	80 µl

2. Prepare dilutions of tubes #7 and #8 in tubes #16 and #17 as shown in Table 2:

Table 2

Tube #	16	17
Buffer B eluent fractions	40 µl of Tube 7	40 µl of Tube 8
PBS	20 µl	20 µl

3. Mix contents of each of the tubes (tube 9 through tube 17).

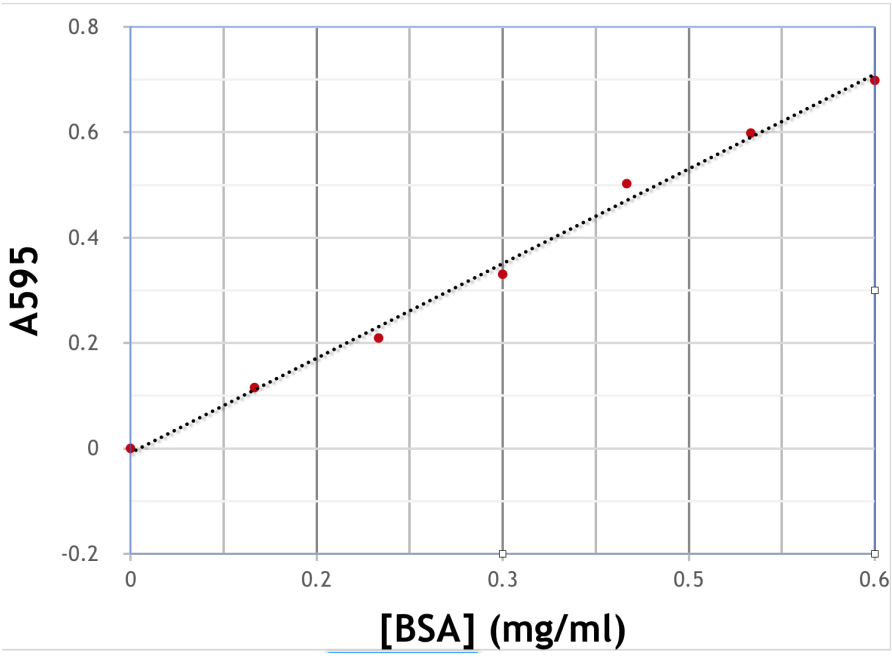
4. Each sample will be assayed in duplicate. For this purpose, add 10 µl of tubes #9-15 into wells of B1-B7, and then again to wells of D1-D7 of your 96 well plate. Subsequently, add 10 µl of tubes #16 into B9 and D9, and 10 µl of tube #17 to wells B11 and D11. Add 190 µl of the Bradford reagent into each of the wells to which samples had been added. Mix gently with micropipette tip and take care not to create bubbles. Bubbles would interfere with absorbance measurements.

5. Place a lid on the 96 well plate and wrap the plate with aluminium foil to prevent exposure to light. Incubation in the dark should continue for 5 minutes. (You may start Part 2 of this task during the 5 minute incubation.)

6. After completion of the 5 minute incubation, placed your red flag in your flag stand. A lab assistant will take your coded plate to a Spectrophotometer station and have absorbance of all your wells read at wavelength of 595 nm. The absorbance readings will be used to score your performance (45 POINTS).

Part 2 NOTE: All data presented in Part 2 of the Bradford assay pertain to experiments performed earlier by an examiner.

1. The Bradford assay described in Part 1 was earlier performed by an examiner, and the absorbance readings were used to draw a standard curve provided below.



2. Using the standard curve and the A_{595} (absorbance at 595 nm) of tubes #16 and #17 obtained by the examiner and shown below, calculate protein concentration of fractions in the examiner's tubes #7 and #8. Write the protein concentrations of tubes #7 and #8 (rounded to two decimal places) **on the answer sheet (5 POINTS)**.

Tube 16:	Well B9: 0.32	Well D9: 0.34
Tube 17:	Well B11: 0.41	Well D11: 0.43

C. GEL RETARDATION ASSAY

This assay will be performed with contents of **your** tube #8 (= tube containing drops you collected in the third tube after addition of buffer B).

1. Dilute contents of tube #8 as follows:
- Mix 10 µl of contents of tube #8 and 40 µl of PBS in tube #18. .

2. Tubes #19-25 should be prepared as described below in Table 3. Add components in said order (from top to bottom):

Table 3

Tube #	**19**	**20**	**21**	**22**	**23**	**24**	**25**
Plasmid DNA	2 µl	2 µl	2 µl	2 µl	2 µl	2 µl	2 µl
Pep, tube #18	0	3 µl	7 µl	0	0	0	0
Pep, tube #8	0	0	0	4 µl	7 µl	0	7 µl
PBS	13 µl	10 µl	6 µl	9 µl	6 µl	9 µl	2 µl
DNase buffer	0	0	0	0	0	2 µl	2 µl
DNase (0.015 U/ µl)	0	0	0	0	0	2 µl	2 µl

3. Three minutes after addition of DNase to tubes 24 and 25, add 3 µl gel loading dye to each of tubes #19-25 and mix.

4. The gel electrophoresis apparatus will be off at this time. Be careful not to press the power button or any other button on the apparatus. Load 15 µl of each of tubes #19-25 consecutively into 7 adjacent wells (from left to right when positive pole is closer to you) of the agarose gel in the electrophoresis apparatus. In the eighth well add 15 µl of DNA size marker which already contains gel loading dye. NOTE: The gel is covered with electrophoresis buffer, therefore add the 15 µl aliquots very gently to the bottom of each well to prevent spill over while loading.

NOTE: One well of the gel has been left empty.

5. After completion of loading, insert the orange colored photo hood onto the apparatus. Press the power button on the lower right surface of the apparatus to start the electrophoresis. Record time of start of electrophoresis. The two buttons on the upper right are for illumination with high level or low level blue lighting. Press the button for high level lighting. This will enable you to visualize migration of the DNA in the gel real time during electrophoresis because the DNA binding stain is in the gel. Migration should be visualized through the hole on top of the photo hood. The photo hood should not be removed.

6. Disconnect the electricity 15 minutes after start of electrophoresis by pressing the power button. Place your green flag in your flag stand to attract attention of lab assistant. He/she will take photo of the gel through the hole of the photo hood (35 POINTS). You may proceed to the theory questions below during the 15 minute interval.

Questions (15 POINTS in total):

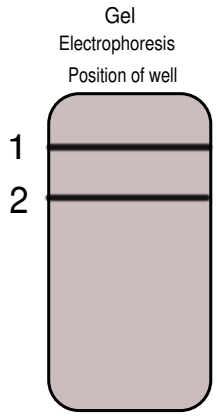
Indicate if each of the following statements is true or false with “X” in the answer sheet.

1. The larger the linearity range of a protein assay, the less one needs to be concerned about the concentration of the sample of interest to be used in the assay (1 **POINT)**.

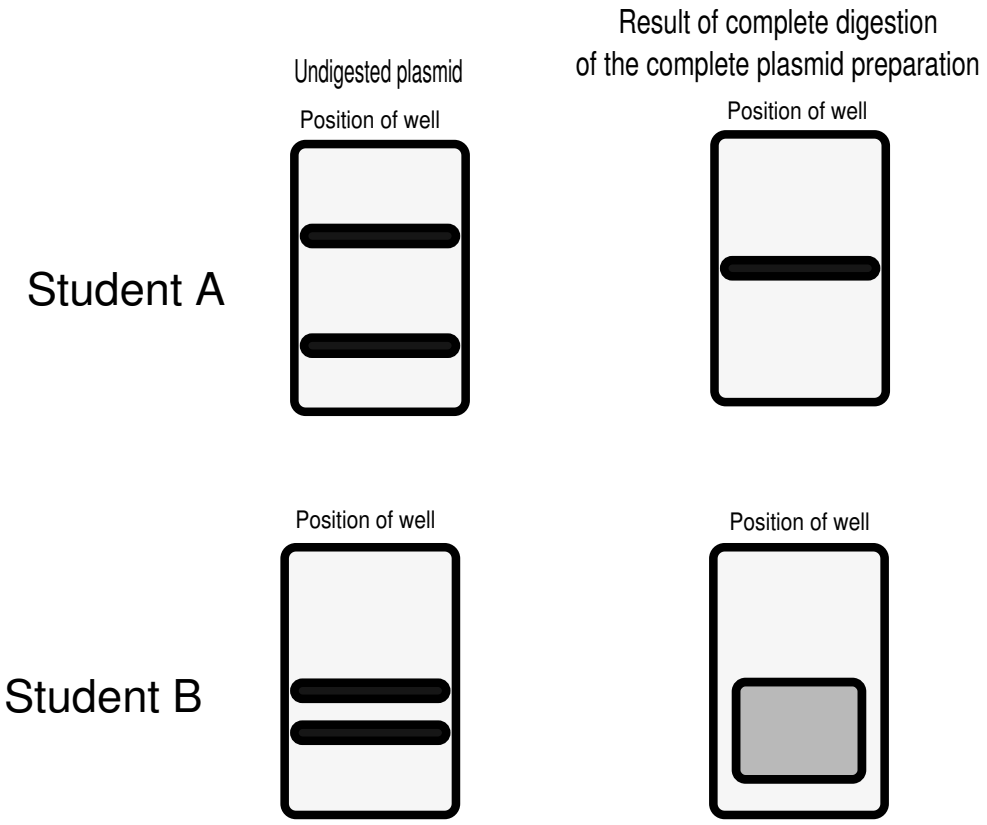
2. The effect of Pep on DNase activity on the plasmid DNA is likely to depend on the sequence of the DNA (1 **POINT)**.

3. Observation of stained DNA in gel regions above the wells would reflect results of interaction of DNA with high concentrations of Pep **(1 POINT)**.

4. Assume the schematic figure shown below represents the electrophoresis pattern of contents of tube 19. The upper band may be circular plasmid DNA in which one phosphodiester bond in one strand has been broken (1 **POINT)**.



5. Student A attempted to purify a plasmid, and student B attempted to purify another plasmid. Neither plasmid had repetitive sequences. After electrophoresis of the plasmids, both students observed two bands as shown below. Based on known size of the plasmids, the upper band was unexpected. Each performed restriction enzyme digestion on the plasmid preparation under conditions of achieving complete digestion, and subsequently electrophoresed the digestion product. Results are shown below. The map of the plasmids showed that each contained only one recognition site for the enzyme used.



Indicate if each of the following statements is true or false **in the answer sheet (4 POINTS)**.

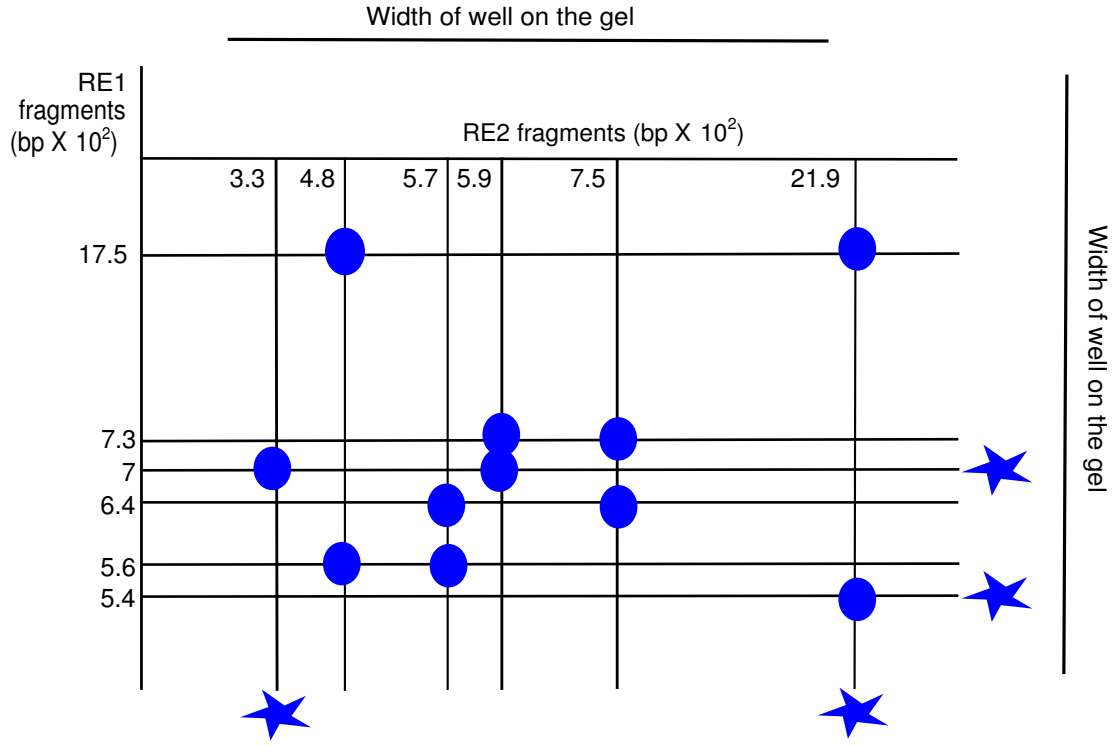
a) The unexpected band of student A may be linearized plasmid DNA.

b) The unexpected band of student A may be circular dimer plasmid DNA.

c) Electrophoresis pattern of partial digestion product of the plasmid preparation of student A with the same restriction enzyme is expected to produce at most three bands.

d) Electrophoresis pattern of partial digestion product of the plasmid preparation of student B with the same restriction enzyme is expected to produce three bands.

6. To determine the restriction enzyme map of two rare restriction enzymes on a linear DNA molecule of interest, RE1 and RE2, the following experiment was performed. First, samples of the DNA molecule were labeled at their 5’ ends and cut separately with RE1 and RE2. The lengths of the fragment products with label are shown in the figure below with **Stars.** Other samples of the DNA in unlabeled form were also cut separately with RE1 and RE2. RE1 digestion products were loaded in a very wide well of a gel and electrophoresed. After electrophoresis, DNA bands on the gel were transferred to a nitrocellulose filter paper by Southern blotting. RE2 digestion products were also loaded in a very wide well of another gel and also electrophoresed. Then DNA bands on this gel were transferred by Southern blotting to the same nitrocellulose filter paper that contained the digestion products of RE1, but transfer was oriented perpendicular relative to the transfer of RE1 restriction fragments. **Circles** show positions of hybridization between RE1 digestion fragments and RE2 digestion fragments.



Indicate if each statement below is true or false (7 **POINTS)**.

a. Complete digestion of the DNA molecule with RE1 and RE2 would produce 11 fragments.

b. The 6.4 $X10^2$ bp and 7.3 $X10^2$ bp DNA fragments produced by digestion with RE1 are adjacent in the undigested DNA molecule.

c. The 5.6 $X10^2$ bp and 4.8 $X10^2$ bp fragments produced, respectively, by digestion with RE1 and RE2 are overlapping in the undigested DNA molecule.

d. The 17.5 $X10^2$ bp and 21.9 $X10^2$ bp fragments produced, respectively, by digestion with RE1 and RE2 overlap in the undigested DNA molecule.

e. No single digestion product of one of the restriction enzymes can overlap with three of the digestion products of the other enzyme.

Practical Exam "Evolution, Ecology & Behavior"

Student Code:

IBO 2018
Tehran, Iran

29th International Biology Olympiad
July 15-22, 2018

Practical Exam

Evolution, Ecology & Behavior

Total Points: **100**
Duration: **90 minutes**

General information

Total points: 100

Exam time: 90 minutes

Please check your student code in the box on the title page.

Use answer sheet, which is provided separately to answer all questions.
The answers written in the question paper **will not** be evaluated.

In order to use the yellow flag (the sign on your desk) just put it in the flag stand located on the left wall of your desk.

Please ensure that all the materials and equipments are available to you. If anything is missing, put your yellow flag in the flag stand no later than 15 minutes after the beginning of the exam. (Any complaints after 15 minutes will not be accepted)

In case of emergencies put your yellow flag in the flag stand.

We suggest you to read the entire protocol before starting the experiments which helps you with time management.

Stop answering and put down your pen immediately at the end of exam. Put the entire protocol with the answer sheet in the envelope. Our assistants will collect the envelopes.

Good luck

Write each indicated number in the cell next to it with your own handwriting.

1	
7	

PART A - INVESTIGATING A MODEL OF BIOLOGICAL DIVERSIFICATION

There are around 10 million different species of eukaryotes on this planet. Including prokaryotes would increase our estimate of the number of extant species on our planet drastically. Understanding why there is so much diversity on Earth has been one of the central questions in evolution and ecology. Rainey and Travisano (1998) attempted to answer certain aspects of this question in the lab. As their model organism, they used a prevalent aerobic bacterium, *Pseudomonas fluorescens.*

Starting from a monomorphic population of *P. fluorescens*, they allowed bacterial cells to grow in a static broth culture, i.e., a beaker that is not shaking. The morphology of the ancestral population could be described as “smooth”, referring to the smooth colonies it would form on a petri dish. It has been shown that two other morphologies are possible in *P. fluorescens*: wrinkly spreader and fuzzy spreader (**Figure 1**).

Figure 1: *The three different morphologies of P. fluorescens: Smooth (SM), wrinkly spreader (WS), and fuzzy spreader (FS).*

A bacterial lineage was established and evolved in the static environment for 7 days (in a beaker with 25ml of broth at 28 ºC). After 7 days, Rainey and Travisano transferred a sample of bacterial cells from the first lineage -i.e., the lineage that evolved for 7 days in the static environment - and established the second lineage. They allowed the first and the second lineage to evolve for another 7 days, in static and shaking environments, respectively. (At the end of each day, a subsample from the current beaker was transferred to a new beaker with fresh media to keep the lineages evolving.) (**Figure 2**).

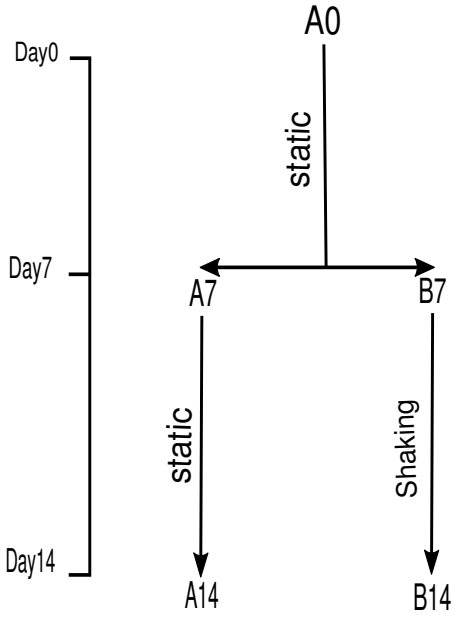


Figure 2: *The schematic representation of the experiment conducted by Rainey and Travisano*

Here, you will play the parts of Rainey and Travisano and try to make sense of the results observed by them. You are given plates from an experiment done in the exact same fashion as the one carried out by Rainey and Travisano. The plates are created by sampling from evolving populations at different stages (as shown in **Figure 2**).

Change in the phenotypic diversity

Count the number of different morphs you see on each plate (Assume each plate is representative of its original population). For each plate, you can calculate the level of heterogeneity (H) using this formula:

$$H = 1 - \sum_{i=0}^{n} (f_i)^2$$

where f_i is the frequency of morph i on the petri dish and n is the number of morphs present on the petri dish.

A-1) Based on the observed results on plates, fill the table 1 in the answer sheet. (Correct to two digits after decimal point).

Plate	SM (Count)	WS (Count)	FS (Count)	SM (Freq)	WS (Freq)	FS (Freq)	H
A0							
A7							
B7							
A14							
B14							

A-2) Based on the data obtained, indicate if each of the following statements is true or false with a "✔" in the answer sheet.

a) It is more likely that diversity is due to phenotypic plasticity rather than mutations.
b) Static environment provides more ecological niches, which increases diversity.
c) The observed diversity results from mutations that existed in the ancestral population.
d) Larger subsamples result in higher heterogeneity.
e) Using bacteria is preferable for conducting this experiment due to short generation time and large population size.

A-3) Assuming that there are only three bacterial morphs, calculate maximum achievable heterogeneity:

PART B

Investigating a model of population evolution

One of the main models for studying population genetics is the Wright-Fisher model (named after two of the founding fathers of the modern evolutionary theory, R. A. Fisher and Sewall Wright).

In its simplest form, the model assumes a population with a fixed number of haploid individuals. Individuals are asexual and simply make copies of themselves to reproduce. In order to create the next generation, one individual is randomly selected from the current generation and contributes one offspring to the next generation. This process is repeated until the next generation reaches the same size as the current one. Note that some individuals can be picked as parents for the next generation more than once by chance alone. After this step, the next generation replaces the current generation. This entire process is repeated to create future generations sequentially one at a time.

If all the individuals in a population have the same fitness, then it is equally likely for each one to be picked as a parent, but if their fitness differ, then sampling process is weighted so that the fitter individuals are more likely to be picked.

On your laptop, there is an application which shows the expected results from a Wright-Fisher model (On your desktop, go to \IBO2018\Task2 and double-click on "WF_model.py" and wait for the application to start). There are 4 variables you can play with. The sliders allow you to change the values for each variable. After you have chosen the desired values for each parameter, simply click on <Simulate> to see how heterogeneity (H) changes over time in the Wright-Fisher model, given the chosen parameters. (See the formal definition of H in **Part A**)

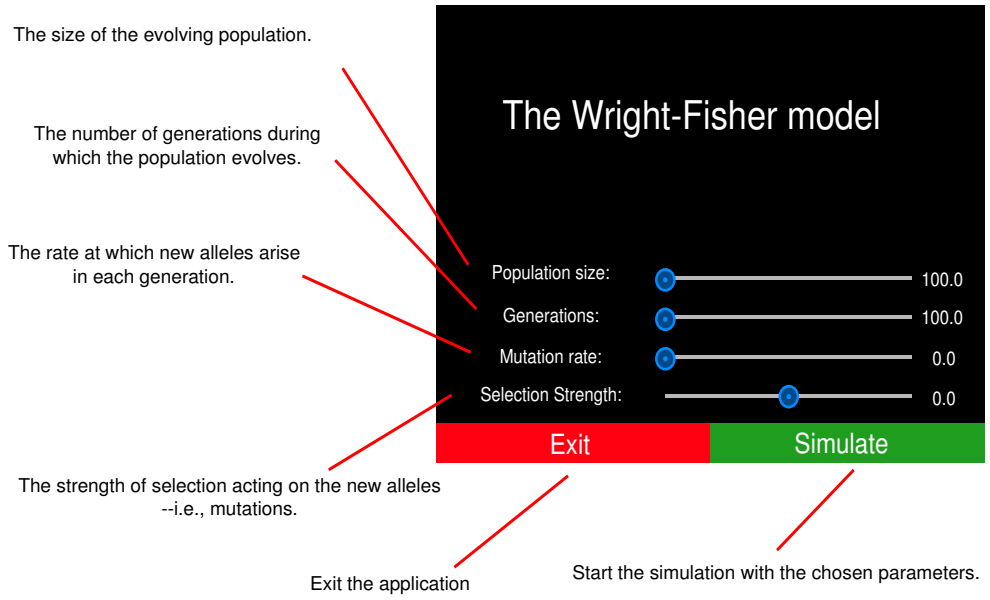


Figure 3: *The interface of the Wright-Fisher model app.*

Using this application, answer the following questions (Keep in mind that the behavior of the model is purely a function of the given parameters).

B-1) indicate if each of the following statements is true or false with a "✔" in the answer sheet.

A) Increasing the number of generations alters heterogeneity at equilibrium.

B) Shrinking population size **does not** increase heterogeneity at equilibrium in any parameter set.

C) Decreasing population size accelerates achieving equilibrium heterogeneity.

D) Increasing mutation rate accelerates achieving equilibrium heterogeneity

E) When there is no mutation, increasing selection strength decreases heterogeneity at equilibrium.

B-2) Indicate with a "✔" in the answer sheet which of the following equations about equilibrium heterogeneity best fit with simulation results. (N is population size and u is mutation rate.)

A) $H=\frac{4Nu}{1+4Nu}$

B) $H=e^{-4Nu}$

C) $H=\frac{2N}{N+4u}$

D) $H=1-\frac{1}{2N}$

E) $H=\frac{1-e^{-2Nu}}{1-e^{-2N}}$

PART C

Investigating the feeding behavior in Drosophila melanogaster larva

There are two forms of fruit flies' larva: active rovers and sedentary sitters. Active rovers maneuver through the medium in search for food, while sedentary sitters do not. It seems that these foraging strategies is genetically determined.
In a series of videos on your laptop (\IBO2018\Task3), you can see the different types of larva in a population for five consecutive generations (Gen1 to Gen5).

C-1) Using the film from the fruit flies larva, complete the table below (Use the same method as part A to calculate *H;* Correct to two digits after decimal point).

Note: For a larva to be counted as a sitter, its **whole body** should **stay in or touch the boundary** of a food patch (two **dark grey circles** in the footage correspond to two food patches) for the entirety of a movie clip.

No. of generation	Sitters (count)	Active rovers (count)	Sitters (freq)	active rovers (freq)	*H*
1					
2					
3					
4					
5					

C-2) Based on the results indicate if each of the following statements is true or false with a "✔" in the answer sheet.

A) The fruit fly larva foraging behavior follows the optimal foraging theory, which means in each generation the best feeding strategy will be chosen according to the environmental factors.

B) The fruit fly larva foraging behavior follows conditional strategy, which is a mechanism that gives individuals the ability to alter their behavior, in this case foraging behavior between sitter and rover.

C) The results are consistent with negative frequency-dependent selection.

D) Assuming negative frequency-dependent selection is acting on the foraging strategies, none of the strategies can go to fixation in a population.

We can predict the change in the size of population over time by either considering the population itself, or taking into the effect of biological interactions, such as competition, on the population dynamics. Below two models are introduced which represent these two approaches when considering the way a population changes over time.

I: discontinuous model of logistic growth:

$$N_{t+1}=N_t+rN_t(1-\frac{N_t}{K})$$

N_t: population size in generation t.
r: intrinsic per capita rate of population growth.
K: carrying capacity.

Note: This model is applicable for both active rovers and sitters.

II: discontinuous model of competition for sitters and active rovers (S, R):

$$S_{t+1}=S_t+r_S S_t(1-\frac{S_t+a_{SR}R_t}{K_S})$$

$$R_{t+1}=R_t+r_R R_t(1-\frac{R_t+a_{RS}S_t}{K_R})$$

S_t: population size of sitters in generation t.
R_t: population size of rovers in generation t.
r_S: intrinsic per capita rate of population growth of sitters.
r_R: intrinsic per capita rate of population growth of rovers.
K_S: carrying capacity for sitters.
K_R: carrying capacity for rovers.
α_{RS}: effect exerted by sitters on rovers through competition on population growth.
α_{SR}: effect exerted by rovers on sitters through competition on population growth.

C-3-1) Complete the table below based on model I and model II. Parameters of model are as follow (Correct to two digits after decimal point):

Model I:

r	3
K	500

Model II:

r_s	3	r_R	2
k_s	500	k_R	500
α_{SR}	0.1	α_{RS}	0.1

	Model I	Model II	Model II
No. of generation	**Sitter (count)**	**Sitter (count)**	**Rover (count)**
0	**184.00**	**184.00**	**262.00**
1			
2	**427.79**	**343.22**	**408.41**
3			
4	**196.94**	**120.45**	**381.17**
5			
6	**371.71**	**539.93**	**368.38**
7			
8	**34.89**	**571.68**	**459.81**
9			
10	**424.04**	**459.64**	**522.19**
11			
12	**182.86**	**516.81**	**497.54**
13			
14	**432.68**	**589.09**	**510.47**
15			

We have conducted a more extensive study to investigate the long-term change in the frequency of feeding strategies in *D. melanogaster* larva. The result of this study are shown in the table below.

C-3-2) Calculate frequencies of feeding strategies predicted by model I and model II and fill the table below (For model I, since we have rovers in the population in addition to the sitters, to calculate the frequency of the sitters divide the number of sitter by 2 × K); Correct to two digits after decimal point).

	Result of our study	Prediction of model I	Prediction of model II	Prediction of model II
No. of generation	**Sitter**	**Sitters**	**Sitter**	**Active rover**
0	**0.41**			
1	**0.54**			
2	**0.61**			
3	**0.62**			
4	**0.91**			
5	**0.32**			
6	**0.68**			
7	**0.63**			
8	**0.46**			
9	**0.27**			
10	**0.84**			
11	**0.67**			
12	**0.47**			
13	**0.39**			
14	**0.42**			
15	**0.54**			

C-3-3) Models I and II need some Initial values in order to make predictions; which of the following initial values, is the best? (Indicate with a "✔" in the answer sheet.)

A) sitter: 289, active rover212

B) sitter: 205, active rover: 295

C) sitter: 523, active rover: 300

D) sitter: 307, active rover: 514

Pearson correlation:
Pearson correlation is a mathematical function to estimate the correlation between two sets of data (x_1, …, x_n and y_1, …, y_n). Correlation coefficient is calculated as follows:

$$\text{correlation coefficient} = \frac{\sum_{i=0}^{n}(x_i-\bar{x})(y_i-\bar{y})}{\sqrt{\sum_{i=0}^{n}(x_i-\bar{x})^2}\sqrt{\sum_{i=0}^{n}(y_i-\bar{y})^2}}$$

n = the sample size
x_i = the individual sample points for sample x
y_i = the individual sample points for sample y

$$\bar{x} = \frac{1}{n}\sum_{i=0}^{n} x_i \qquad \bar{y} = \frac{1}{n}\sum_{i=0}^{n} x_i$$

C-4) Calculate correlation coefficient (r) for the desired correlations and fill in the table below.

Sample x	Observed sitters (frequency)	Observed sitters (frequency)
Sample y	Sitters predicted by model I (frequency)	Sitters predicted by model II (frequency)
Correlation coefficient		

C-5) Indicate each of the following statements is true or false with a "✔" in the answer sheet.

A) The logistic model I explains the observed changes in frequencies given the parameters.

B) Your calculations show that including competition in our model significantly increased the fit between model and our observation.

C) We need to explore the parameter space to establish the ability of these models to explain our observation.

D) The correlation estimates suggest that the change in the frequencies of sitters and rovers is not due to competition.