10th International Biology Olympiad

Practical test 1999-07-06

Laboratory 1: Microbiology

Question (1)

Surname _____ First name _____

Country _____ Code number _____

General remarks about the practical tests

In the practical tests you are expected to demonstrate that you know some basic methods, processes and techniques of biology and that you are able to use them for experimental purposes. This will be tested in a series of tests, organized in four different laboratories:

Laboratory 1: Microbiology

Laboratory 2: Genetics and histology

Laboratory 3: Morphology and physiology

Laboratory 4: Behaviour

You have 70 minutes in each laboratory. You can score 26 - 50 points at a maximum in each laboratory, which means a total amount of 150 points for the practical test. If at some task you mark some incorrect alternative(-s) as well as some correct ones, you will get a lower score at that particular task than if you only mark the correct answers.

Introductory remarks to laboratory 1: Microbiology

In this laboratory you will be given two different tasks. First you will examine the contents of the two jars in front of you (jar 1 and jar 2) to find out how they differ from each other, and find an explanation for how these differences may have come about (questions (1) - (5)). Then you will estimate the concentration of a yeast suspension (jar 3), and find out whether this concentration is significantly different from the concentration of another suspension (questions (6) - (9)). You may call for assistance concerning the phase contrast adjustment of the microscopes. You will find information on how to use the calculator, and carry out statistical tests, at the end of this instruction.

Before you start your experiment you should answer question 1. Give your answer to an assistant, who will then give you the remaining questions.

(1) Jar 1 and jar 2 contain mixtures of shredded cabbage (*Brassica oleracea*) and onion (*Allium cepa*), with a little salt (NaCl) added. The two jars have been kept under different conditions, and the differences betweeen them are due to microorganisms. Which of the listed tools are suitable to examine the contents of the jars to find out the microbiological reason why they differ? Mark with an X the most suitable alternatives for an immediate investigation.

(3p)

_____ A. Microscope

_____ B. Autoclave

_____ C. pH-paper

_____ D. Scissors

_____ E. Balance

_____ F. Your own senses

_____ G. Tape measure

_____ H. Scalpel

Now give your answer to question (1) to the assistants, who will hand you the remaining questions, and then you can start your investigation.

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Laboratory 1: Microbiology

Questions (2) - (10)

Surname _____

First name _____

Country _____

Code number _____

(2) Examine the smell and pH of the contents of the two jars. When investigating pH, use a pasteur pipette to extract some liquid and place it on the pH-paper. Mark with X in the appropriate boxes those alternatives which agree with your observations.

(4p)

		Jar 1	Jar 2
smell:	fresh		
	strong or pungent		
	chocolate		
PH:	9		
	7		
	5		

(3) Examine a drop of the liquid from each jar under the microscope, using 10x magnifying oculars and 40x magnifying objectives for a total magnification of 400x. The microscopes are already set up, do not change the objective. You may call for assistance concerning the phase contrast adjustment of the microscopes. In which jar do you find most microorganisms?

(2p)

Most microorganisms are found . . . (mark with an X)

_____ A. . . . in jar 1.

_____ B. . . . in jar 2.

(4) Compare the microorganisms you can see in the liquid from the jars with the pictures attached. Note that the two sheets of pictures show the organisms at different magnifications. Which organisms are abundant in the liquid from the jar with most microorganisms? Mark the abundant organisms with an X.

(6p)

_____ A. *Staphylococcus*

_____ B. *Anabaena*

_____ C. *Lactobacillus*

_____ D. *Saccharomyces*

_____ E. *Streptomyces*

_____ F. *Micrococcus*

_____ G. *Escherichia*

(5) Which explanation(s) for the differences between jar 1 and jar 2 are plausible?

Mark those with an X.

(6p)

_____ A. The vegetables in one of the jars have been contaminated by bacteria.

_____ B. The vegetables in one of the jars are fermented.

_____C. One of the jars has been kept warm (25°C) for about 2 weeks, while the other has been refrigerated at between about +4°C and +8°C.

_____ D. Microorganisms have arisen in one of the jars but not in the other.

_____ E. Microorganisms already present on the vegetables have grown in one of the jars but not in the other.

_____ F. From the beginning there was more water in one of the jars than in the other, and therefore there are more bacteria in it.

Now turn to the yeast suspension in jar 3. You will estimate the concentration of yeast cells with the aid of a counting chamber. The counting chamber is described below.

The counting chamber and its use

Before using the chamber you should clean it, first with water, then with alcohol, and dry it carefully using absorbent paper. Put the cover slip in place. Note that there is a special cover slip to be used with this chamber. Fill the chamber with your liquid sample from the side, as shown in Fig. a (next page), so that the liquid seeps in below the cover slip. Put the chamber underneath the microscope.

In the counting chamber the two areas marked "grid" in Fig. b each contain a 9 mm^2 grid divided in larger and smaller fields. Fig. c shows an A-square (1 mm^2), framed by three lines, and the smaller areas it contains: C, 0.01 mm^2; D, 0.0025 mm^2; E, 0.04 mm^2, and F, 0.005 mm^2. At 400x total magnification, the D-area is most convenient for counting, since its whole area can be seen at one time (Fig. d).

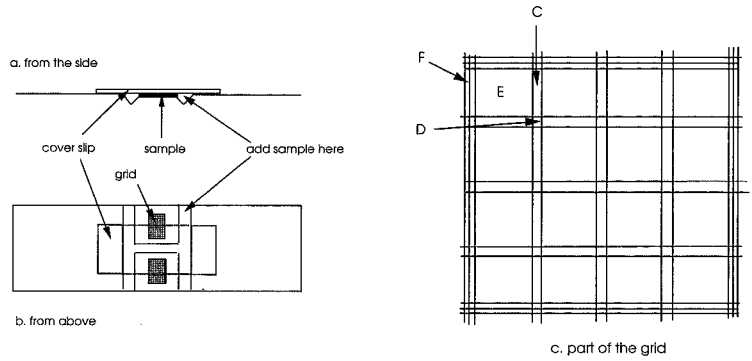


Fig. a. - c. The Bürker counting chamber

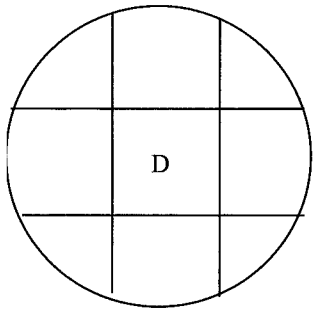


Fig. d. The D area viewed at 400 x total magnification

You will count the number of cells in a number of D-areas (actually, in a number of volumes the basis of which are D-areas). The depth of the chamber is 0.1 mm.

While counting, you have to consider in how many D-areas you need to count the cells to get a reliable measure of the cell concentration.

(6) Count the number of cells in an appropriate number of D-areas. For each D-area in which you have counted the cells, write down the number of cells that you have counted below. Then calculate the average (arithmetic mean) of those values. Write the number of D-areas counted as well as the mean number of cells per D-area on the lines at the bottom of the page.

Number of cells in each D-area counted:

Number of D-areas counted: ..... (2p)

Mean number of cells per D-area:..... (4p)

(7) From the mean number you calculated in question (6), calculate the concentration of the suspension in cells/ml.

(3p)

Concentration: _____ cells/ml

(8) On the following pages you find descriptions of the t-test and the χ^2-test, and of how to use the calculator. Which of these tests is/are appropriate to determine whether the cell concentration you have found is significantly larger or smaller than 2.0×10^7 cells/ml? Mark the correct answer with an X.

(2p)

_____ A. The t-test.

_____ B. The χ^2-test.

_____ C. Both tests are suitable.

(9) Perform the relevant calculations to determine if your estimate is significantly different from 2.0 x 10^7 cells/ml. Write the numerical value of the statistic(-s) you calculate in the appropriete box(-es).

(3p)

t	
χ^2	

(10) Using $\alpha<0.05$ as the level of significance, is your result significantly different from a concentration of 2.0 x 10^7 cells/ml? You will get a score for your answer here only if it agrees with your calculation in response to question 9. Mark the correct answer with an X.

(2p)

_____ A. Yes.

_____ B. No.

10th International Biology Olympiad

Statistics and how to use the calculator

The χ^2-distribution and χ^2-test

$$\chi^2 = \sum_{i=1}^{k} \frac{(O_i - E_i)^2}{E_i}$$

The χ^2-test is useful when you want to compare an observed distribution of frequencies (O_i) with expected frequencies (E_i). O_i and E_i in the formula above are absolute frequencies = numbers of e.g. individuals.

Suppose you want to test the goodness of fit between observed frequencies of different phenotypes in an offspring and the expected frequencies according to your hypothetical, genetic model. The expected absolute frequencies (E_i) are calculated from the relative frequencies according to your hypothesis. The number of classes (k in the formula above) is, in this case, the number of different phenotypes.

The χ^2-distribution, just like the t-distribution described below, is a probability density function. The values of χ^2 range from zero to positive infinity. See figure in the lower right

corner of the attached χ^2-table. Just as for t, the χ^2-distribution varies with the number of degrees of freedom (ν). In the simplest case, the number of degrees of freedom (ν) is one less than the number of classes (k).

$$\nu = k - 1$$

Tabulated χ^2-distributions show the degrees of freedom (ν) down, the probabilities (α) across and their corresponding χ^2-values. See attached table of χ^2-distributions.

As the χ^2-distribution is a probability density function, it follows that α is the probability of χ^2 being as great or greater than the table value, under the null hypothesis (H_0), i.e. if H_0 is true. Example: What is the probability for $\chi^2(\nu = 5) \geq 1.234$? The answer is: 0.90 - 0.975.

The threshold for rejection of the null hypothesis (H_0) is usually set at $\alpha = 0.05$. An investigator is then said to accept the 0.05 (or 5%) level of significance. This means that if the computed χ^2-value exceeds the critical χ^2-value, the hypothesis is rejected.

Suppose that the computed $\chi^2 = 4.666$ and $\nu = 2$. At $\alpha = 0.05$, the critical $\chi^2(\nu = 2) = 5.991$. As the computed χ^2 is less than the critical χ^2, the null hypothesis (H_0) is not rejected.

The *t*-distribution and *t*-test

The *t*-distribution was first described by W.S. Gossett using the pen name "student", and so is often referred to as "student's distribution". The difference between the sample arithmetic

mean (m) and the true mean (μ) divided by the standard error of the sample mean (s_m) follows the t-distribution:

$$t = \frac{m - \mu}{s_m}$$

The standard error of the sample mean (s_m) is defined as

$$s_m = \sqrt{\frac{s^2}{n}}$$

where (s) is the standard deviation and (n) the number of observations.

The shape of the t-distribution depends on the number of degrees of freedom (ν).

$$\nu = n - 1$$

The t-table is arranged with degrees of freedom (ν) going down, and probabilities (α) going across. These probabilities correspond to the areas under the curve of the t-distribution that fall outside the chosen critical values of t - see figure at the lower right of the attached t table. Note that the table shows only the absolute value of t; since the curve is symmetric around 0. Note also that there are two tails each contributing half the value of α.

The value of α is chosen depending on the level of significance you want; the corresponding t-value is called the critical value of t. For $\alpha = 0.05$ and $\nu = 5$, we find $t = 2.571$. The null hypothesis (H_0) is that the sample mean is not significantly different from the true mean. If the absolute value of the observed t-statistic is smaller than the critical t, the null hypothesis is not rejected.

How to obtain the statistical data on your calculator:

To put your calculator into the statistical mode, press the following keys, in this order:

2ndF

on/c

The word STAT will show in the upper right corner of your display, and the designations in red or blue (different on different calculators) apply to the individual keys on the right side of your calculator.

Now enter your values one by one, pressing the key M+ (which now means DATA) after each number entered. As you go through this, the display will indicate the number of values entered, i.e. n. You then obtain the remaining statistics by pressing the appropriate keys:

$\bar{x}$ for the mean (*m*), s for the standard deviation (*s*).

You get out of the statistic mode of your calculator by pressing the on/c key again. You are now ready to perform the rest of the calculations needed to compute the values necessary for comparison with tabulated values.

10th International Biology Olympiad

Practical test 1999-07-06

Laboratory 2: Genetics and histology

Surname _____

First name _____

Country _____

Code number _____

General remarks about the practical tests

In the practical tests you are expected to demonstrate that you know some basic methods, processes and techniques of biology and that you are able to use them for experimental purposes. This will be tested in a series of tests, organized in four different laboratories:

Laboratory 1: Microbiology

Laboratory 2: Genetics and histology

Laboratory 3: Morphology and physiology

Laboratory 4: Behaviour

You have 70 minutes in each laboratory. You can score a maximum of 26 - 50 points in each laboratory, which means a total of 150 points for the practical test. If at some task you mark some incorrect alternative(-s) as well as some correct ones, you will get a lower score at that particular task than if you only mark the correct answers.

Introductory remarks to laboratory 2: Genetics and histology

In this laboratory, the focus is on genes and cells: questions (1) - (10) deal with mendelian inheritance, (11) - (13) are about making chromosome preparations, and in questions (14) - (16) you study microslides of various plant and animal tissues and their cells. You will find information on how to carry out statistical tests and how to use the calculator at the end of this instruction.

Mendelian inheritance:

statistical test of a hypothetical genetic model

In front of you is a box with seedlings of barley (*Hordeum vulgare*). At this stage of development each seedling has only one leaf. The phenotype of a seedling is either green, yellow (*xantha*) or white (*albina*).

Yellow leaf colour shows monohybrid and recessive inheritance to normal green. Thus, the genotype of a yellow (*xantha*) plant can be designated *xx*. Accordingly the genotype of a green plant is *XX* or *Xx*. White leaf colour, also, shows recessive inheritance. Thus, the genotype of a white (*albina*) plant can be designated *aa*. The genotype of a green plant is either *AA* or *Aa*. The *xantha*-locus and the *albina*-locus are not linked.

The yellow and white plants cannot photosynthesise so they will die at the seedling stage. Only green plants survive and produce offspring.

A gardener at the Genetic Centre in Uppsala started this experiment with plants having genotype *Aa Xx* (generation number 0 = G_0). As barley breeds by selfing, the offspring of such a plant is the same as if you make the cross *Aa Xx* x *Aa Xx*. The offspring (offspring generation number 1 = G_1) was grown in a field.

(1) What proportion of the plants in G_1 is expected to survive and produce offspring?

(2 p)

Answer: .....

The box in front of you contains 9 spikes (ears) from 9 different plants in G_1. The seedlings are the offspring generation number 2 = G_2.

The seedlings from one spike belong to one of these four categories:

1) all green, 2) green and yellow, 3) green and white, 4) green, yellow and white.

(2) Write the theoretically expected ratio of spikes producing seedlings that are

(2p)

all green .....

green and yellow .....

green and white .....

green, yellow and white .....

(3) In one of the spikes only 8 seeds germinated and produced 7 green seedlings and 1 white. What is the probability of this outcome, if the parent had the genotype *Aa XX* ?

(2p)

Answer: .....

(4) Now focus your attention on the spikes with seedlings of all three colours: green, yellow and white. (These seedlings give an example of dihybrid segregation.) You need to respond to (4a) and (4b) to solve the remaining questions, but in this question you only score points at (4c).

(4a) Count the total number of seedlings from these spikes with green, yellow and white colour respectively. (The number of such spikes will of course vary by chance from one box to another.)

Spike	Number of seedlings that are		
no	green	yellow	white
1	.....	.....	.....
2	.....	.....	.....
3	.....	.....	.....
4	.....	.....	.....
5	.....	.....	.....
6	.....	.....	.....
Total	.....	.....	.....

(4b) In the introduction no information is given about the phenotype of plants with genotype *aa xx*. What is your hypothesis? Mark with an X.

_____ A. Yellow.

_____ B. White.

_____ C. Do not germinate.

(4c) Which one of the following ratios of the three different phenotypes in generation G_2 is expected according to your hypothesis? Mark the correct answer with an X. (You will get a score only if your answers to 4b and 4c are consistent.)

(2p)

	green	yellow	white
_____ A.	2/4	1/4	1/4
_____ B.	9/15	3/15	3/15
_____ C.	10/16	3/16	3/16
_____ D.	9/16	3/16	4/16
_____ E.	9/16	4/16	3/16

From these hypothetical relative frequencies (i.e. from your null hypothesis = H_0) you can now compute the expected number (E_i) of each of the three phenotypes. At the end of this instruction, you will find information about χ^2.

(5) From your answers in (4a) and (4c), fill in the table below.

(2p)

	Green	yellow	White
O_i			
H_0			
E_i			

(6) Calculate χ^2 from your data.

(3p)

Answer: χ^2 is .....

(7) What is the number of degrees of freedom (ν) in your test?

(2p)

Answer: .....

(8) At the significance level (α) = 0.05, what is the critical value of χ^2?

(2p)

Answer: .....

(9) At the significance level (α) = 0.05, do you have reason to reject your hypothesis?

(2p)

_____ A. Yes.

_____ B. No.

(10) Suppose you calculate χ^2 from the data from another box. If your hypothesis is true, what is the probability of getting a χ^2-value as great or greater than the value you calculated in (6)?

(2p)

Answer: .....

Making and analysing mitotic chromosome squash preparations

On your desk you will find this material:

Roots, some of which have been pre-treated with a drug. All roots have been macerated in a mixture of HAc (acetic acid) and HCl (hydrochloric acid).

Slides, coverslips, acetic-orcein, forceps, mounted needles, plexi-glass rods, blotting paper and plastic gloves are also provided.

Make squash preparations of roots pre-treated with the drug as well as preparations of untreated, normal roots. Analyse your preparations by light microscopy. You have to examine many cells, at different stages of mitosis, in order to solve questions (11) - (13).

(11) Which **five** of the following statements (A-K) are correct? Mark each correct statement with an X. If at this task you mark more than five alternatives, you will get a lower score.

(10p)

_____ A. The drug causes the chromosomes, at the stage corresponding to prometaphase-metaphase, to spread all over the cytoplasm, instead of being gathered with their centromeres in the middle (equator) of the spindle. (This indicates that the drug causes the microtubules to break down.)

_____ B. The drug causes the mitotic spindle to rotate 90° in the cell, so that the metaphase plate can easily be seen in polar view.

_____ C. Centrioles are present in the normal material, but absent from the drug-treated material.

_____ D. The drug causes the chromosomes to become more contracted than normally.

_____ E. The drug causes the chromosomes to become more slender than normally.

_____ F. The drug prevents the sister chromatids from being seen individually.

(11, continued)

_____ G. At prophase-prometaphase the sister chromatids are closely associated (aligned) all along their length. This statement applies to the normal as well as to the drug-treated material.

_____ H. At metaphase, sister chromatids are held together only in the centromere region. A metacentric chromosome then looks like the letter X. This statement applies to the normal as well as to the drug-treated material.

_____ J. In normal material the sister chromatids are closely associated (aligned) all along their length until the onset of anaphase. However, the drug may cause a metacentric chromosome to look like the letter X.

_____ K. The drug prevents normal anaphase movement of daughter chromosomes towards opposite spindle poles.

(12) What is the chromosome number (2n) of the plant?

(2p)

Answer: .....

(13) How many of these 2n chromosomes are acrocentric? (An acrocentric chromosome has one relatively long chromosome arm and, on the other side of the centromere, a relatively very short arm.)

(2p)

Answer: .....

Histological microslides of plant and animal tissues

(14) In microslide 1 you can see a transverse section of a part of a plant. From which part of the plant is this transverse section?

(2p)

_____ A. Root

_____ B. Stem

_____ C. Needle

_____ D. Carpel

(15) The drawing below shows the same transverse section as microslide 1. Five arrows (1-5) point at different types of cell. What is each type of cell? The drawing merely indicates the locations of the cells, so to find out what cell types they are you must observe them in microslide 1. Write the number of each of the arrows on the appropriate line.

(5p)

(fig.)

_____ Fiber

_____ Vessel element

_____ Cambial cell

_____ Endodermis cell

_____ Collenchyma cell

_____ Sieve tube element

_____ Parenchyma cell

(16) In microslides 2 - 5 there are sections of different internal organs and tissues from mammals. What organ or tissue type is represented by each of slides 2-5? Mark the correct alternatives with an X in the appropriate boxes.

(8p)

	Slide 2	Slide 3	Slide 4	Slide 5
Skin epithelium				
Striated muscle				
Smooth muscle				
Heart muscle				
Lung				
Liver				
Testis				
Pancreas				
Ovary				
Bone				
Cartilage				

10th International Biology Olympiad

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10th International Biology Olympiad

Practical test 1999-07-06

Laboratory 3: Morphology and physiology

Surname _____

First name _____

Country _____

Code number _____

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You have 70 minutes in each laboratory. You can score a maximum of 26 - 50 points in each laboratory, which means a total of 150 points for the practical test. If at some task you mark some incorrect alternative(-s) as well as some correct ones, you will get a lower score at that particular task than if you only mark the correct answers.

Introductory remarks to laboratory 3: Morphology and physiology

In this laboratory, you will examine several types of biological material: questions (1) - (2) relate to plants and plant tissues that have been exposed to different treatments, questions (3) - (5) relate to skeletal bones of mammals, while questions (6) and (7) relate to the use and construction of dichotomous identification keys.

(1) T1 - T4 are plants or seeds of four different species, each of which has been grown or **left** to germinate, respectively, in deficiency or absence of some necessary environmental factor. A number of such factors are included in the list below. For comparison there are also control plants and seeds of the same species (numbered C1 - C4) grown under favourable conditions. The maize plants have been grown in a water culture. What factor has been missing during growth / germination of T1 - T4? For each plant, write the letter corresponding to the most probable deficiency / absence on the lines at the bottom of this sheet.

(8p)

Factors:

A. Boron	B. Calcium	C. Chlorine	D. Iodine	E. Lead
F. Light	G. Magnesium	H. Mycorrhiza	I. Oxygen	
K. Phosphorus	L. Sulfur dioxide	M. Water		

Plant T1 (maize, *Zea mays*) has been lacking _____

Plant T2 (garden cress, *Lepidium*) has been lacking _____

Plant T3 (*Coleus blumei*) has been lacking _____

Plant T4 (seeds of wheat, *Triticum*) has been lacking _____

(2) Look at the four pairs of plants or plant tissues that are numbered 5a, 5b - 8a, 8b. In each pair one plant has been treated with a plant hormone or other substance. Your task is to tell which plant in each pair was treated, and with what it was treated. Choose one substance for each treated plant from the list below. In the table below, write the appropriate letter (A - F) in the box corresponding to the treated plant (a or b) in each pair.

(4p)

In pair 6a, 6b there could be some doubt as to which of the plants was treated, and therefore the untreated plant in this pair is marked with an X in the table.

Substances:

A. Auxin B. Gibberellic acid C. Abscisic acid

D. Ethylene E. Cytokinin F. Lactic acid

	a)	b)
5) *Arabidopsis*, dwarf mutant		
6) Cutting of *Pelargonium*		X
7) *Coleus*		
8) Tobacco leaf tissue		

(3) Now examine the two different skulls of mammals in front of you. Skull anatomy provides much information concerning the lifestyle of animals, especially their food habits. What are the food habits of the two mammalian species represented by these skulls?

Mark with an X for each skull what food habit it represents.

(4p)

	Skull 1	Skull 2
carnivorous		
herbivorous		
piscivorous		
omnivorous		

(4) The appendicular skeleton is the skeleton of the limbs. You have in front of you an example of homologous bones, numbered 1 and 2, from two different mammals with different specializations in their appendicular skeletons. From which part of the animals do these bones come? Mark the correct answer with an X.

(2p)

_____ A. The upper forelimb (humerus)

_____ B. Part of the lower forelimb (ulna)

_____ C. The upper hindlimb (femur)

_____ D. Part of the lower hindlimb (tibia)

_____ E. A digit (phalanx)

(5) Look at the shape of the bones 1 and 2. For what kinds of movement are these bones primarily adapted? Mark the correct alternative for each bone with an X.

(4p)

	Bone 1	Bone 2
Digging		
Running		
Swimming		
Flying		

(6) The three beetles (Coleoptera), numbered 1 - 3, belong to three different genera. The task is to identify which beetle belongs to which genus. To identify the beetles, use the identification key on page 8 and the pictures on page 9. Fig. 1 shows what some parts of a beetle are called, and figs. 2-14 are explained in the identification key. To study a beetle under the stereo microscope, lift the beetle by its needle and hold both ends of the needle with your fingers.

Write the number of each beetle (1, 2, 3) in front of the name of its genus.

(9p)

___ *Acanthocinus*	_____ *Agathidium*	_____ *Calathus*
___ *Cassida*	_____ *Dascillus*	_____ *Dryophtorus*
___ *Eledonoprius*	_____ *Hylobius*	_____ *Mycetochara*
___ *Oceoptoma*	_____ *Carabus*	_____ *Tenebrio*
___ *Xylita*		

Identification key:

1a The outermost 3-5 segments of the antennae distinctly thicker than the other segments (fig. 2 & 3) => 2

1b The outermost 3-5 segments of the antennae of approximately the same thickness as the other segments (fig. 4 & 5) => 5

2a The 1st segment (closest to the head) of the antennae several times longer than the other segments => 3

2b The 1st segment (closest to the head) of the antennae of approximately the same length as the other segments => 4

3a Antennae with 11 segments: 1 very long, 7 normal and 3 thick => *Hylobius*

3b Antennae with 8 segments: 1 very long, 4 normal and 3 thick => *Dryophtorus*

4a All feet 5-segmented (fig. 6 & 7) => *Oceoptoma*

4b Hind feet 4-segmented (fig. 8 & 9) => *Agathidium*

5a All feet 5-segmented (fig. 6 & 7) => 6

5b Hind feet 4-segmented (fig. 8 & 9) => 8

6a Pronotum more than twice as broad as long => *Dascillus*

6b Breadth of pronotum less than twice its length =>7

7a Claws with distinct teeth on the inner side (fig. 10) => *Calathus*

7b Claws with inner side smooth (fig. 11) => *Carabus*

8a All feet 4-segmented => 9

8b Fore and middle feet 5-segmented =>10

9a Antennae longer than total body length => *Acanthocinus*

9b Antennae shorter than total body length => *Cassida*

10a Claws with distinct teeth on the inner side (fig. 10) => *Mycetochara*

10b Claws with inner side smooth (fig. 11) => 11

11a Head with protracting edge in front of the eyes, antennae inserted under this edge (fig. 12) => 12

11b Head without protracting edge in front of the eyes => *Xylita*

12a Sides of pronotum toothed (fig. 13) => *Eledonoprius*

12b Sides of pronotum smooth (fig. 14) => *Tenebrio*

(pictures)

(7) Now look at plants 9 - 13. These plants belong to the following species:

9: *Achillea millefolium*

10: *Artemisia vulgaris*

11: *Lamium purpureum*

12: *Potentilla reptans*

13: *Veronica chamaedrys*

On the next page you will find an identification key leading to these five plant species and four others. In the key, nine characters are missing. Choose among the ten characters labelled A - J below. In each place where a character is missing in the key, write one of the letters A - J to indicate which of these characters fits into the key in that place.

(6p)

Characters:

A. Stalk with alternate leaves

B. Stalk with hair in two rows

C. Flowers small, gathered in a capitulum

D. Flowers larger, not gathered in a capitulum

E. Capitula small, in long panicles

F. Two stamens

G. Three stamens

H. Four stamens

I. At least four stamens

J. Two stamens longer and two shorter

Identification key:

1a _____ => 2

1b _____ => 4

2a _____ => *Artemisia vulgaris*

2b Capitula larger, not in long panicles => 3

3a Stalk with opposite leaves => *Bidens tripartita*

3b _____ => *Achillea millefolium*

4a _____ => 5

4b _____ => 6

5a Stalk with hair evenly spread all around => *Veronica montana*

5b _____ => *Veronica chamaedrys*

6a _____ => 7

6b More than four stamens => 8

7a Stamens of equal length => *Brassica rapa*

7b _____ => *Lamium purpureum*

8a Leaves silvery downy underneath => *Potentilla argentea*

8b Leaves not downy underneath => *Potentilla reptans*

10th International Biology Olympiad

Practical test 1999-07-06

Laboratory 4: Behaviour

Questions (1) - (4)

Surname _____

First name _____

Country _____

Code number _____

General remarks about the practical tests

In the practical tests you are expected to demonstrate that you know some basic methods, processes and techniques of biology and that you are able to use them for experimental purposes. This will be tested in a series of tests, organized in four different laboratories:

Laboratory 1: Microbiology

Laboratory 2: Genetics and histology

Laboratory 3: Morphology and physiology

Laboratory 4: Behaviour

You have 70 minutes in each laboratory. You can score a maximum of 26 - 50 points in each laboratory, which means a total of 150 points for the practical test. If at some task you mark some incorrect alternative(-s) as well as some correct ones, you will get a lower score at that particular task than if you only mark the correct answers.

Introductory remarks to laboratory 4: Behaviour

In laboratory 4 you will plan, perform and analyse an experiment with guppies *(Poecilia reticulata)* concerning sexual selection. There are two major types of sexual selection:

	Definition	Common consequence
Intersexual selection	individuals of one sex (usually females) choose their partner non-randomly among individuals of the other sex	may lead to the evolution of conspicuous ornaments in males, which are used to attract females
Intrasexual selection	individuals of one sex (usually males) fight over the opportunity to mate, while the other sex has no choice but to mate with the winner	may lead to the evolution of fighting ability and weapons in males, and also of signals used to scare off competitors

In many varieties of guppies, the males have a large and colourful tail-fin. This trait has been exaggerated through human breeding, but the basis for this breeding is the natural variation among wild guppies. Comparing guppies with extraordinarily large tail-fins with those with smaller tail-fins may help us to understand in what way a large tail-fin may be advantageous in the context of sexual selection.

A large tail-fin could be

- an ornament functioning as a signal to attract females
- a signal of fighting ability used to scare off males

or both, or neither.

The task here is to design, perform and analyse an experiment, which will test the hypothesis that **inter**sexual selection may explain why some males have a large tail-fin.

Waiting for actual copulations to occur would be too time-consuming, but one can use a short-cut often used by behavioural researchers, namely to assume that an individual will spend more time close to an individual with whom it would prefer to mate and less time near a less preferred individual. At your disposal you have female guppies, male guppies with smaller tail-fins, male guppies with larger tail-fins, and an aquarium divided by lines in the bottom into three sections of equal size, each containing one beaker.

In this laboratory, you will answer all questions by marking the correct alternative (or the appropriate boxes) with a cross (X).

You should answer questions (1) - (4) **before** starting with the practical work. When you have answered these questions, give your answers to an assistant. The assistant will then give you the remaining questions, and thereafter you start the experiment.

(1) Which of the following predictions is correct?

(2p)

If **inter**sexual selection makes it advantageous for males to have a large tail-fin, then . . .

_____ A. . . . females will prefer to mate with a male with a small tail-fin.

_____ B. . . . females will prefer to mate with a male with a large tail-fin.

_____ C. . . . females will show no preference for males with either large or small tail-fins.

(2) Which of the experimental designs (A - H) on pages 6-7 would be most appropriate to test the hypothesis that **inter**sexual selection makes it advantageous for males to have a large tail-fin? Each design is described by a sketch of the aquarium from above. To test any one of the designs the behavior of the fish should be recorded after 2,3,4 and 5 minutes.

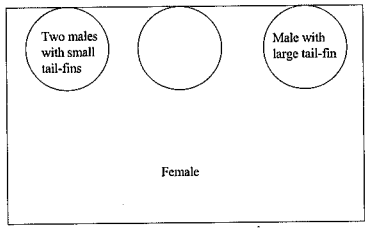
(6p)

_____ Design A _____ Design B _____ Design C

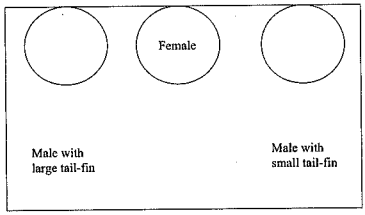
_____ Design D _____ Design E _____ Design F

_____ Design G _____ Design H

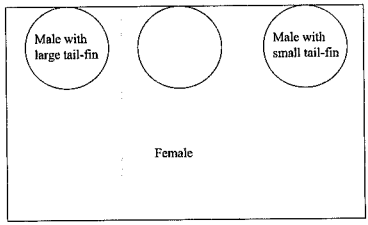
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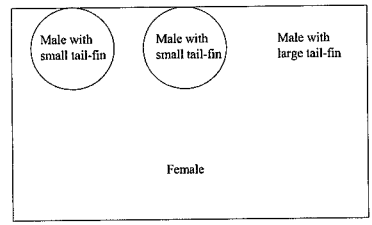
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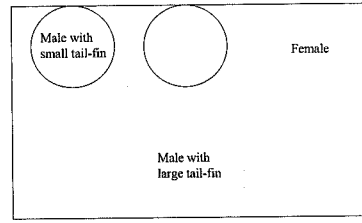
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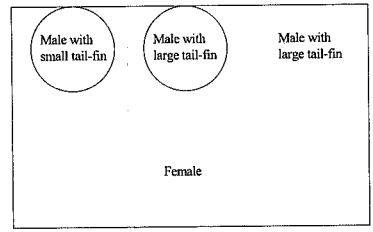
Design D:



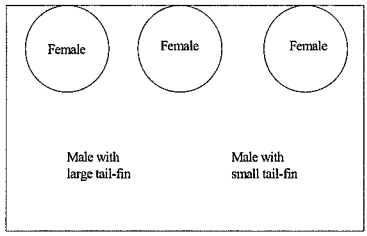
Design E:



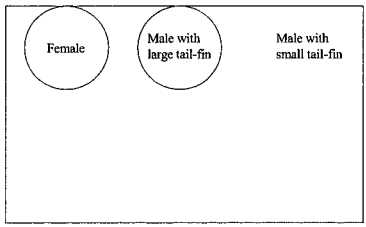
Design F:



Design G:



Design H:



(3) Some previous studies have suggested that female guppies prefer males with much red and orange in their colouration. If this is so, can it affect the interpretation of your results?

(3p)

_____ A. Yes, if males with small tail-fins have more red and orange in their colouration, then it may be difficult to tell whether large tail-fins or red / orange colour is the character that makes the females prefer some males.

_____ B. Yes, if males with large tail-fins have more red and orange in their colouration, then it may be difficult to tell whether large tail-fins or red / orange colour is the character that makes the females prefer some males.

_____ C. Yes, if females have more red and orange in their colouration than males, then it may be difficult to tell whether large tail-fins or red / orange colour is the character that makes the females prefer some males.

_____ D. No, the interpretation of my results regarding the effects of male tail-fin size on female choice cannot be affected by other characters preferred by the females.

(4) When you have finished the experiment with one set of fish, should you put the fish back in their boxes and let them mix with the others, or should you put them in other boxes to keep fish that have been tried already separate from those that have not?

(2p)

_____ A. Put them back and let them mix with the others, so that each replicate is done with a random combination of the fish available.

_____ B. Keep fish that already have been tried separate and use new fish in each replicate, so that each replicate is an independent observation.

_____ C. Put the female back and let her mix with the other females, but keep males that already have been tried separate and use new males in each replicate.

Now, before doing the experiment, give your answers to an assistant, who will then give you the remaining questions.

10th International Biology Olympiad

Practical test 1999-07-06

Laboratory 4: Behaviour

Test protocol and questions (5) - (9)

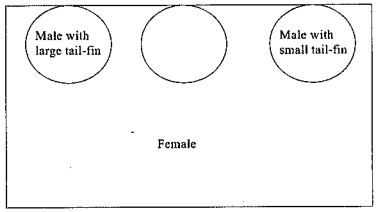
Surname _____

First name _____

Country _____

Code number _____

Now you are ready for the experiment. The experiment should be performed as follows: (Fill in the results in the test protocol on the next page. Mark with a cross (X) in the appropriate boxes)



* Put a female in the test aquarium, a male with a small tail-fin in the beaker on one side, and a male with a large tail-fin in the beaker on the other side. The middle beaker is left empty.
* Watch the fish and after 2, 3, 4 and 5 minutes, record whether the female is in the same section as either of the males or in the middle section with the empty beaker.
* Note also which of the two males has most red and orange in his colouration.
* Put the fish already tested in separate boxes and repeat the experiment with fish that have not been used.
* When you repeat the experiment, alternate having the male with large tail-fin to the left and to the right compartment.
* Because of the limited time, you will only make five trials.
* Fill in the results in the test protocol on the next page. Mark with a cross (X) in the appropriate boxes.

Test protocol:

Replicate nr	Male	Which male has the most red & orange	Placed in Beaker		Position of female (section of aquarium) after 2 minutes			after 3 minutes			after 4 minutes			after 5 minutes		
			left	right	left	mid	right	left	mid	right	left	mid	right	left	mid	right
1	large tail-fin															
	small tail-fin															
2	large tail-fin															
	small tail-fin															
3	large tail-fin															
	small tail-fin															
4	large tail-fin															
	small tail-fin															
5	large tail-fin															
	small tail-fin															

After completing the five trials, proceed with questions (5) - (9).

In this laboratory, instead of calculating statistics, you will interpret what the trend in the results suggests, that is, what would be the conclusion if you had done many more trials and the results were the same. When you have completed five trials, procede with questions (5) - (9).

(5) Did the females prefer males with small or large tail-fins?

(2p)

_____ A. Large tail-fins.

_____ B. No, neither.

_____ C. Small tail-fins.

(6) Did the females prefer the male with more red and orange than the one with less of these colours, or vice versa?

(2p)

_____ A. The females preferred the male with more red / orange.

_____ B. There was no indication of any preference for males with more or less red and orange in their colouration.

_____ C. The females preferred the male with less red / orange.

(7) If the trends you observed were statistically significant, what would your conclusion then be?

(3p)

_____ A. The result would support the hypothesis that a large tail-fin makes a male more attractive.

_____ B. The result would support the hypothesis that red and orange colour makes a male more attractive.

_____ C. The result would support the hypothesis that either tail-fin size or red / orange colour, or both, makes a male attractive, but would not show which of these traits is important.

_____ D. The result would not support any of these hypotheses.

(8) In this experiment you used several males and several females. The number of guppies needed could have been reduced by using the same two males in all trials, and only change the female between trials. How would this have affected your interpretation of the results?

(3p)

_____ A. It is inconceivable that most females would show a preference for one and the same of the two males, so no conclusion could be drawn from such an experiment.

_____ B. It could happen that most females would show a preference for one of the males, but it would be impossible to tell whether this was because of his tail-fin size or because of something else.

_____ C. It could happen that most females would show a preference for the male with the larger tail-fin. This would mean that tail-fin size is an important character when the females choose their partner. However, I would have to give each female much more time to choose before I checked which male she preferred.

_____ D. It would not affect the results or the interpretation of the experiment at all.

(9) As you may remember from the begining of the instruction, **inter**sexual selection is when individuals of one sex choose their partner among individuals of the other sex, and **intra**sexual selection is when individuals of one sex fight or scare off each other to get the opportunity to mate with individuals of the other sex. Today's experiment was designed to test whether tail-fin size is important in **inter**sexual selection. If instead you wanted to make an experiment to test whether **intra**sexual selection may explain why some guppy males have a large tail-fin, which of the following experiment designs would be the most appropriate?

(3p)

_____ A. Put two female guppies in an aquarium. At one short side of the aquarium, let the females see a film of two male guppies, one with a large and the other with a small tail-fin. Check which of the females is closest to the film projection of the males.

_____ B. Put two males with different tail-fin size in an aquarium. At one short side of the aquarium, let the males see a film of a female guppy. Check which of the males is closest to the film projection of the female.

_____ C. Put two female guppies in an aquarium. At one short side of the aquarium, let the females see a film of two male guppies, one with a large and the other with a small tail-fin. Check which of the males on the film projection that the females are closest to.

_____ D. Put a single female guppy in an aquarium. At one short side of the aquarium, let the female see a film of two male guppies, one with a large and the other with a small tail-fin. Check which of the males on the film projection that the female is closest to.