Biology 112

Lab Manual

Spring 2007

Name:_____

Lab Section #:_____

Lab Instructor's Name_____

Syllabus and Contents:

(*) Page numbers in parentheses correspond to page numbers in the Lab Manual as a whole; the other page numbers apply to the individual chapters.

Field Trip I: Harvard Museum of Natural History (HMNH)

Objectives

To observe the diversity of animals. To compare and contrast the various adaptations, body plans, etc. of the animals found at the HMNH.

Introduction

The most casual observation indicates that not all animals look the same. Darwin's theory of evolution through the process of natural selection tells us that the reason animals (or plants) do not look the same is that they have evolved to fit into particular environmental niches and that most differences which we observe reflect some kind of special adaptation to the environment. One of the easiest ways to examine the changes which have occurred during the course of evolution is to visit the Harvard Museum of Natural History at Harvard University. Here, mounted animal specimens from all parts of the world are arranged in groups according to their evolutionary relationships as well as the geographic regions in which they are found. The purpose of this lab is to examine these animals and for you to teach yourself certain principles of animal diversity by using your own observations to answer the questions in these pages.

You should also visit the Glass Flowers exhibit in the same museum. It contains glass models of many important plant types.

You can easily walk from the Harvard Square MBTA station to the HMNH (see map on next page). It is best to go to Harvard Square by subway (red line) or by bus since parking places around the museum are either enormously difficult to find, or they are reserved for the faculty and staff of Harvard (and reserved parking is strictly enforced). The trip from UMass to the HMNH takes about 45 minutes each way.

You will need to pick up a ticket to the HMNH in lecture; this will get you free admission (it is normally $5 for students). You can go to the HMNH anytime that the museum is open. TAs will be at the HMNH during all of the scheduled lab periods during the week listed on the syllabus. The HMNH is open daily 9:00 AM to 5:00 PM. Admission is free (even without a ticket) Sundays from 9 to 12 and Wednesdays from 3 to 5.

YOU SHOULD BRING YOUR COPIES OF *Campbell* and the *Lab Atlas* FOR REFERNCE.

Procedure

VERY IMPORTANT NOTICE: This lab will take you a while to complete, especially if you are unprepared. In order to be able to complete it in 3 hours, you should **be sure to do the following before you go to the HMNH**:

- Read up on classification systems (Purves pp 502 - 504) and familiarize yourself with terms like kingdom, phylum, etc.
- The following phyla can be found at the HMNH; you should go through Purves and make a brief sketch of each phylum so you can recognize it more easily when you are looking for it (each of these is listed in the index):

• chordata	• cnidaria	• anthophyta	• platyhelminthes
• coniferophyta	• arthropoda	• cyanobacteria	• lycophyta
• mollusca			

- Read over **all the questions** and make a plan of how you might go about answering them.

At the HMNH

Be sure to get a map - it will show you where to find various types of organisms.

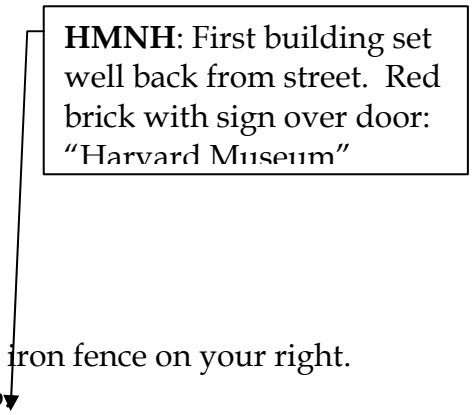
During your visit, you should make notes from which you can answer the questions below. Your lab report will consist of answers to these questions. You need only to answer the questions; it is not necessary to assemble your answers into a larger essay.

Lab report:

- Important note: these questions are difficult & involve some speculation & interpretation on your part. For that reason, we will grade your responses generously. Our purpose is to get you thinking about these issues rather than to emphasize a specific right answer. As long as your answers are reasonable and clearly-explained, you should get full credit.
- Must be typed; handwritten reports will not be accepted. Hand-drawn and labeled drawings are fine.
- Due at the start of the lab session you are currently in during the week listed on the syllabus. This is a firm deadline.
- Although you will perform these activities as a group, each member of the group must turn in an individual lab report. Each person's report must be in his or her own words as much as possible.
- Your lab report must contain answers to the questions on pages 4 through 10.

Getting to the HMNH (not all buildings shown)

26 Oxford St Cambridge, MA 02138



- Exit Harvard station using the "To Harvard yard" exit.
- Go along Massachusetts Ave with the brick and wrought iron fence on your right.
- Go through the first gate you come to; it's near a bus stop.
- Go diagonally across Harvard yard to the gate at the north end (you'll see a big plaza).
- Cross the plaza with the Science Center on your left.
- Cross the street at the corner where Kirkland and Oxford intersect.
- Walk along Oxford with the street on your left until you come to the HMNH.

1) Phyla

Choose three different phyla listed in *Campbell*. For each of the three phyla, find one representative organism at the HMNH or Glass Flowers Exhibit. Be sure to list its genus and species names in addition to its common name (if available). In one brief sentence, describe the organism (size, coloration, feeding, habitat, etc.).

a) Phylum #1_____

organism: Genus_____

Species_____

Common name (if available)_____

Description:

b) Phylum #2_____

organism: Genus_____

Species_____

Common name (if available)_____

Description:

c) Phylum #3_____

organism: Genus_____

Species_____

Common name (if available)_____

Description:

2) Convergent Evolution

Consider the wing bones of the following three flying vertebrates:

- Pterandon – a flying dinosaur. Its skeleton can be found on the wall in the Romer Hall of Vertebrate Paleontology.
- Bird. A drawing of a bird wing can be found in figure 34.28a of *Campbell*.
- Bat – flying mammal. A bat skeleton can be found in the Hall of Mammals in case A2 which is against the wall that separates the Hall of Mammals room from the Holarctic Mammals and Birds room.

a) All three wing structures are based on the same tetrapod vertebrate arm and five-fingered hand structure that is shown in *Campbell* figure 22.14.

Using figure 22.14 as a guide, sketch the wing bones of a bird, a bat, and a pterandon and identify (as best you can) how the bones in each of your sketches correspond to the bones in the human arm and hand. Be sure to label the parts of the wing skeleton that correspond to:

- Humerus (upper arm bone) {shown in gray in figure 22.14}
- Radius & ulna (lower arm or "forearm" bones) {orange and beige}
- Palm & finger bones (carpals, phalanges, & metacarpals) {yellow and brown}

For each wing, give a one-sentence description of its structure. For example, if we had asked about figure 22.14, you would say something like, "The cat's foot is like a human hand, but it walks in its tiptoes."

b) *Campbell* figure 34.29 shows *Archaeopteryx*, the earliest known bird. If you looked at the wing skeleton of this animal, which would you expect it to be most like: bird, bat, or pterodactyl? Explain your reasoning briefly.

3) Common Structures

Virtually all tetrapod vertebrates (see Lab Atlas figure 8.74 for a sample) have the following features (among many others): Numbers in parentheses refer to numbered parts in figure 8.74.

- Two "legs" – appendages near the tail end of the backbone (#23 - #28).
- Two "arms" – appendages near the head end of the backbone (#3 - #8).
- A "tail" – an extension of the backbone beyond the pelvis at the back end of the animal (#22).

These have been extensively modified in certain swimming vertebrates; for example:

- Whales - marine mammals. Several whale skeletons can be found hanging from the ceiling in the Hall of Vertebrates (you can't miss 'em).
- Seals – another group of marine mammals. A seal skeleton can be found by the windows in the Hall of Mammals.

a) To which part(s) (arm, leg, tail) do the front flippers of a whale correspond?

b) How have the leg bones of a "standard tetrapod" been modified in a whale?

c) To which part(s) (arm, leg, tail) does the "tail" (the part(s) of the animal at the back end that are moved up and down for swimming) of a whale correspond?

d) To which part(s) (arm, leg, tail) does the "tail" (the part(s) of the animal at the back end that are moved up and down for swimming) of a seal correspond?

4) Skeletal Morphology and Function

A giraffe skeleton is shown at the right. The arrow indicates the "neural spines" which are bony projections sticking up from the thoracic vertebrae. The thoracic vertebrae are the parts of the backbone to which the ribs are attached; they are indicated by number 16 in figure 8.74 of the *Lab Atlas*.

Muscles connect the neural spines to the bones of the neck; these muscles are used to hold the animal's head up and keep the neck from dropping down. The stronger these muscles have to be, the larger they must be and the larger the neural spines have to be. Thus, a giraffe, which must hold up a very long and heavy neck, has very large neural spines.

For each of the following animals:

a) State whether the neural spines are:

- **Large** - like the giraffe's, which are much larger than the corresponding projections on the lumbar vertebra (see #17 in figure 8.74 of the *Lab Atlas*).
- **Small** - not much larger than the corresponding projections on the lumbar vertebra (see #17 in figure 8.74 of the *Lab Atlas*).

Note that we are interested in the *relative* size of the spines compared to the size of the skeleton of that animal, not their *absolute* size in inches.

b) Provide a plausible explanation for why this is so.

As an example, here is a satisfactory answer for the giraffe skeleton:

*a) The neural spines on the giraffe skeleton are **LARGE**.*

b) This indicates that the muscles attached to the neural spines must be large and therefore strong. This is likely because the giraffe has a long and heavy neck that it must hold up and away from the body.

Answer questions (a) and (b) for the following animals. All of these skeletons can be found in the Hall of Mammals.

- Moose

- Whale

- Human

5) Marine Mammals I: Skeletons and External Anatomy

This is the first part of a three-part exploration of marine mammal anatomy, diversity, and phylogeny. In each of the three parts, you will address the following two questions using evidence collected during the lab:

a) How many major different groups of marine mammals are there? The answer to this lies somewhere between "All marine mammals are so similar that they are really only one big group." and "Each one is so different that there are 20 different groups." How will you resolve this? You look for similarities and differences and decide for yourself if the similarities are enough to put a few organisms into a group or if the differences are compelling enough to split them up.

A full-credit answer to this question consists of three parts:

- The number of groups of marine mammals that you have determined.
- An explanation of why you chose the groups that you chose. We are not interested in the "right" answer here; just a well-reasoned argument based on your observations. What are the key differences between groups? What are the key features that make members of each group similar?
- Which of the marine mammals from the list below belong to each group?

The following marine mammals can be found at the HMNH:

- Amazon Manatee
- Fur Seal
- Harbor Porpoise
- Harbor Seal
- Narwhal
- Right Whale
- River Otter
- Sea Otter
- Sperm Whale

b) Which is the closest living land relative of a seal? Seals evolved from land-dwelling ancestors. Although that ancestor is now extinct, it has modern-day descendants. Based on the evidence you collect, you must decide which order of land mammals this ancestor came from.

A full-credit answer to this question has two parts:

- The order of land mammals that you think is most closely-related to the land ancestor of seals. Choose from the list below.
- An explanation of why you chose that order. Again, we are not interested in the "right" answer; just a well-reasoned argument based on your observations.

These are the major orders of land mammals that can be found in the Hall of Mammals:

- Marsupialia
- Insectivora
- Chiroptera
- Primates
- Rodentia
- Carnivora
- Perissodactyla
- Artiodactyla

In each part, we are not interested in the correct answer; we are interested in the *data* you cite and your *argument* based on that data. The more specific about the data you are and the more clear your argument is, the more credit you will get.

In this part, you will use external and skeletal anatomy to answer these questions. You should look at the whole animals and the skeletons found in the HMNH to collect data to formulate your answer to each question.

Population Genetics

Objectives

To see how the genetics of populations can be modeled using Hardy-Weinberg population genetics. To see the effects of various deviations from the Hardy-Weinberg assumptions on the allele frequencies of a population (micro-evolution).

Introduction

Mendelian genetics (*Campbell*, Ch. 14) deals with inheritance among individuals or small families. It is not useful for dealing with large groups of individuals which are called populations. For example, the genetic disease cystic fibrosis is inherited in an autosomal recessive manner; that is:

allele	contribution to phenotype
D	normal - dominant phenotype
d	cystic fibrosis - recessive phenotype

as a result:

genotype	phenotype
DD, Dd	normal
dd	cystic fibrosis (diseased)

Mendelian genetics could tell you that two carriers (Dd) would have a 1/4 chance of having a diseased child. However, Mendelian genetics cannot help us to find the chance that the parents are carriers in the first place.

In Bio 112 we are also interested in evolution, which has a large genetic component. However, since *populations* evolve, not *individuals*, Mendelian genetics is no help here either.

As a result of these deficiencies in Mendelian genetics, Hardy and Weinberg in 1908 developed a mathematical scheme for modeling the genetics of populations which is based on Mendelian genetics. (See *Campbell* chapter 23 for more details.) In its most general form, Hardy-Weinberg population genetics can model the evolutionary behavior of many genes with many alleles each. However, in order to best illustrate the principles involved, we will consider the simplest case: one gene with two alleles (A and a).

In order for the Hardy-Weinberg model to work , they had to make 5 simplifying assumptions (See *Campbell* p. 458 for details):

(1) Very large population size
(2) Isolation from other populations
(3) No net mutations
(4) Random mating
(5) No natural selection

A population that satisfies these five requirements is said to be at Hardy-Weinberg Equilibrium (HWE) because the allele frequencies will not be changing over time – the population will not be evolving. All of these are **never** true in real life, but the Hardy-Weinberg model is still very useful.

In the case of human diseases, we can make the simplifying assumption that the population is at HWE and then calculate the frequency of carriers, given the frequency of diseased individuals. In the case of evolution, we can compare a population with what we'd expect to see if it was not evolving (that is, at HWE) and see how it is evolving. We can also use Hardy-Weinberg population genetics to model how certain conditions can influence allele frequencies – that is what we'll be doing in this lab.

We will consider the hypothetical creatures known as tribbles. Tribbles come in three colors: blue, green, and yellow. We will simulate the tribbles by beads colored blue, green, and yellow. The color of the tribbles is controlled by one gene with two alleles that are incompletely dominant (*Campbell* p. 260-261):

allele	**contribution to phenotype**
A	blue color - incompletely dominant
a	yellow color - incompletely dominant

as a result:

genotype	**phenotype**
AA	blue
Aa	green
aa	yellow

Procedure

Part I: You will start by simulating a randomly-mating population under conditions that satisfy most of the requirements for HWE. You will randomly select pairs of parent tribbles and each pair will give birth to two tribble offspring. After each pair is selected, it is removed from the population; you will do this until you have mated all the individuals inthe population. Using Mendelian genetics, you will predict the colors of these offspring and see if our findings fit the predictions of HWE. (Notice that population genetics is really just an extension of Mendelian genetics.)

You should work in groups of three.

(1) Each group will start with a population of 40 tribbles with the following colors:
16 blue 16 green 8 yellow

(2) Put the population in a container.

(3) Draw a random pair of tribbles from the population (close your eyes and pick two beads). These are the parent tribbles.

(4) Fill in the sheet on the next page for "Pair #1":
a) Write in the colors of the parents (it doesn't matter which is father or mother)
b) Write in their genotypes based on the table above.
c) Predict and write in the genotypes of their two offspring using mendelian genetics:
i) if the parents are AA and AA, both children will be AA
ii) if the parents are aa and aa, both children will be aa
iii) if the parents are AA and aa, both children will be Aa
iv) if the parents are AA and Aa, the children have a 50/50 chance of being either AA or Aa. Flip a coin for each child:
if Heads, then the child is AA,
if Tails, the child is Aa
v) if the parents are aa and Aa, the children have a 50/50 chance of being either aa or Aa. Flip a coin for each child:
if Heads, then the child is Aa,
if Tails, the child is aa
vi) if the parents are Aa and Aa, the children have
a 1/4 chance of being AA
a 1/2 chance of being Aa
a 1/4 chance of being aa
– so flip a coin twice for each child:
if heads-heads, the the child is AA
if heads-tails (or tails-heads), the child is Aa
if tail-tails, the child is aa

d) Write in the colors of the children using the information above.
(5) Discard the parental beads.
(6) Pick a new pair of tribbles from the population and repeat steps (4) through (6) for pairs #2 through
20.
(7) Total your results and pool with the class. These numbers are the numbers of children of each color
that would be produced, assuming each pair of parents had two offspring.
(8) Discuss the answers to the following questions:
Which of the requirements of HWE does this simulation fit?
Does your data fit the predictions of HWE?

Pair #	Colors of Parents		Genotypes of parents		Resulting offspring		
	Mother	Father	Mother	Father	# Blue (AA)	# Green (Aa)	#Yellow (aa)
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
TOTALS:							

Part II:

You will now simulate and observe the effects of various experimental deviations from the requirements of HWE on the allele frequencies of a population of 100 tribbles. This is an example of micro-evolution. The 4 experiments are as follows:

- Experiment 1: All Blue tribbles die before reproducing.
- Experiment 2: All Yellow tribbles die before reproducing.
- Experiment 3: Every generation, a **random** 95% of the population dies before reproducing.
- Experiment 4: Every generation, a **random** 98% of the population dies before reproducing.
- You will then repeat the selected experimental treatment for 10 generations.

One experiment will be assigned to each lab table; all the groups at that table will do that experiment. Each group will present their results at the end of class.

(1) Count out the beads for the starting population.

40 Blue 40 Green 20 Yellow

(2) Follow the directions on the following pages. At Step (c) in each generation, apply your experimental condition.

Be sure to check that your numbers add up as indicated – it is OK if they are off by a little (0.01 for those that must be 0; 1 for those that must be 100) – but since the next generation is based on the last, a mistake early will make all later results invalid.

You should start off using the beads to simulate the population. Once you get a feel for what's going on, and are sure that you don't need them anymore (check with your TA), you can stop using them.

Keep going for all 10 generations unless one allele goes extinct ($p = 0$ or $q = 0$).

Here are the details of each step and what they correspond to in real life:

Notes:

- you should look at the worksheets on the following pages to see what these steps mean
- the boxes on the worksheets have been assigned arbitrary letters to help identify them in the calculations; you do not need to know these equations for any exam

- Step 0 (start of generation 0): the starting population
- Step 0a: Calculate the alleles contributed to the gene pool from the raw data.
  - – each blue (AA) individual contributes 2 A's. So: d = 2A
  - – each green (Aa) individual contributes 1 A and 1 a. So: e = B and f = B
  - – each yellow (aa) individual contributes 2 a's. So: g = 2C
  - – the total number of A's contributed to the pool (h) = d + e
  - – the total number of a's contributed to the pool (i) = f + g
  - – the total number of alleles in the gene pool (j) = h + i
    (since each individual contributes 2 alleles, this should = 2N)

• Step 0b: Calculate the allele frequencies from the numbers of the alleles. This calculates the fraction of the gametes with each allele produced by the reproductive adults.

$$\text{the frequency of A alleles} = \frac{\#\text{ of A's}}{\text{total }\#\text{ of alleles}} = \frac{h}{j}$$

$$\text{the frequency of a alleles} = \frac{\#\text{ of a's}}{\text{total }\#\text{ of alleles}} = \frac{i}{j}$$

• Step 1a (generation 1): Calculate the fraction of each genotype in the progeny using the allele frequencies of the gametes produced by the previous generation. This assumes that the population satisfies the requirements for HWE. (Actually, in the experiment, it doesn't. But all the requirements are present in this particular step, and we will model the deviations in the next step, so it is OK to use the Hardy-Weinberg model here.) So you can use the modified punnet square:

	gamete: A probability of getting it: p	gamete: a probability of getting it: q
gamete: A probability of getting it: p	genotype: AA probability: p^2	genotype: Aa probability: pq
gamete: a probability of getting it: q	genotype: Aa probability: pq	genotype: aa probability: q^2

So:

Progeny Genotype	**Probability of occurrance**
AA	p^2
Aa	pq + pq = 2pq
aa	q^2

• Step 1b: Calculate raw numbers. This model assumes that, in each generation, all the adults die and give birth to a total of exactly 100 newborn tribbles (in some cases, this will represent an enormous reproduction rate!). The number of each genotype (color) is just 100 times the fraction with that genotype (genotype frequency). So:

A = 100x B = 100y C = 100z

Between steps (b) and (c) is what happens to the tribbles during their lives before they reproduce.

• Step 1c: Simulate your experiment here. The results of this calculation give the tribbles who have survived to reproductive age.

- Experiment 1: Make the number of blue tribbles = 0, keeping all the others the same. Adjust the total number (N) accordingly.
- Experiment 2: Make the number of yellow tribbles = 0, keeping all the others the same. Adjust the total number (N) accordingly.
- Experiment 3: Put 100 beads in a container to represent the newborns. Mix them, close your eyes and pick 5. These are the survivors. N is therefore 5.
- Experiment 4: Put 100 beads in a container to represent the newborns. Mix them, close your eyes and pick 2. These are the survivors. N is therefore 2.

Steps (d) and (e) set up the calculations for the next generation; then calculate the allele frequencies in the gametes (eggs & sperm) produced by the tribbles that made it to reproductive age.

• Step 1d: Calculate the numbers of alleles from the raw numbers (like step 0a)
• Step 1e: Calculate the allele frequencies from the numbers of alleles (like in step 0b).

(3) Graph your results. Draw a graph of p and q as a function of generation number on the overhead transparency your TA will provide (these are the values you calculated: generation 0, use 0b; generation 1, use 1e; generation 2, use 2e, etc.). Be sure to use a blue pen for p and a red pen for q and write which experiment you were performing at the top of the sheet.

(4) Each group will briefly present their data. The class will then discuss the results. The objective of the discussion is to answer the questions required in the lab report (see later).

Lab Report

• Must be typed; hand-drawn graphs are acceptable.
• Due at the start of lab during the week indicated on the syllabus; this is a firm deadline.

Your lab report must include:
(1) A copy of your group's Random Mating Simulation Worksheet.
(2) A copy of the graph of the data (p and q vs. generation) from your group.
(3) A copy of the graph of the data (p and q vs. generation) from another group.
(4) A brief (not more than a page total) discussion of the following questions:

a) In your experiment, briefly describe what happened to the allele frequencies (went up, etc.). Explain how your experimental conditions led to this behavior.
b) Compare your results to those of the other group you included. What are the similarities and differences and how can you explain them in terms of what you know about population genetics?
c) Migration of individuals (also called Gene Flow) can also change allele frequencies (see *Campbell* pp. 462). How would you alter the steps in Part II of this lab to simulate migration? Describe clearly which step(s) you would alter and how you would alter them. There may be more than one correct answer here. Hint: think of migration as a few individuals of specific genotypes entering the population in each generation.

Population Genetics Simulation Experiment#____ Generations 0 &1

Step	Instructions	Raw Numbers: Num. of blu (A)	Num. of grn (B)	Num. of yel (C)	Total Num. (N)	Genotype Frequencies: Freq. of AA (blu) (x)	Freq. of Aa (grn) (y)	Freq. of aa (yel) (z)	Allele Frequencies: Freq. of A (p)	Freq. of a (q)
0	Starting Numbers	40	40	20	100					
0a	Calculate # of each allele contributed. #A's⇒ d = 2A; e = f = B; g = 2C; h = d + e; i = f + g; j = h + i #a's⇒	d	e		h	Total # alleles				
			f	g	i	j				
0b	Calculate allele freq. from allele #s $p = \frac{h}{j}$ $q = \frac{i}{j}$									
*	Check to see that p + q = 1									
1a	Assuming conditions for HWE, calculate genotype frequencies from allele frequencies: $x = p^2$; $y = 2pq$; $z = q^2$									
*	Check to see that x + y + z = 1									
1b	Assuming a population of 100, calculate numbers from genotype frequencies A = 100x B = 100y C = 100z				100					
	Check to see that A + B + C = 100									
1c	Simulate your Experiment HERE (Experiments 1 through 4)									
1d	Calculate # of each allele contributed. #A's⇒ d = 2A; e = f = B; g = 2C; h = d + e; i = f + g; j = h + i #a's⇒	d	e		h	Total # alleles				
			f	g	i	j				
1e	Calculate allele freq. from allele #s $p = \frac{h}{j}$ $q = \frac{i}{j}$									
*	Check to see that p + q = 1									

Experiment#____ Generation 2

Step	Instructions	Raw Numbers: Num. of blu (A)	Num. of grn (B)	Num. of yel (C)	Total Num. (N)	Genotype Frequencies: Freq. of AA (blu) (x)	Freq. of Aa (grn) (y)	Freq. of aa (yel) (z)	Allele Frequencies: Freq. of A (p)	Freq. of a (q)
1e	Copy over allele frequencies from previous page (line 1e)									
2a	Assuming conditions for HWE, calculate genotype frequencies from allele frequencies: $x = p^2$; $y = 2pq$; $z = q^2$									
*	Check to see that $x + y + z = 1$									
2b	Assuming a population of 100, calculate numbers from genotype frequencies A = 100x B = 100y C = 100z				100					
	Check to see that A + B + C = 100									
2c	Simulate your Experiment HERE (Experiments 1 through 4)									
2d	Calculate # of each allele contributed. #A's⇒ d = 2A; e = f = B; g = 2C; h = d + e; i = f + g; j = h + i	d	e		h	Total # alleles				
	#a's⇒		f	g	i	j				
2e	Calculate allele freq. from allele #s $p = \frac{h}{j}$ $q = \frac{i}{j}$									
*	Check to see that p + q = 1									

Experiment#____ Generation 3

Step	Instructions	Raw Numbers: Num. of blu (A)	Num. of grn (B)	Num. of yel (C)	Total Num. (N)	Genotype Frequencies: Freq. of AA (blu) (x)	Freq. of Aa (grn) (y)	Freq. of aa (yel) (z)	Allele Frequencies: Freq. of A (p)	Freq. of a (q)
1e	Copy over allele frequencies from previous page (line 2e)									
2a	Assuming conditions for HWE, calculate genotype frequencies from allele frequencies: $x = p^2$; $y = 2pq$; $z = q^2$									
*	Check to see that $x + y + z = 1$									
2b	Assuming a population of 100, calculate numbers from genotype frequencies A = 100x B = 100y C = 100z				100					
	Check to see that A + B + C = 100									
2c	Simulate your Experiment HERE (Experiments 1 through 4)									
2d	Calculate # of each allele contributed. #A's⇒ d = 2A; e = f = B; g = 2C; h = d + e; i = f + g; j = h + i	d	e		h	Total # alleles				
	#a's⇒		f	g	i	j				
2e	Calculate allele freq. from allele #s $p = \frac{h}{j}$ $q = \frac{i}{j}$									
*	Check to see that p + q = 1									

Experiment#____ Generation 4

Step	Instructions	Raw Numbers: Num. of blu (A)	Num. of grn (B)	Num. of yel (C)	Total Num. (N)	Genotype Frequencies: Freq. of AA (blu) (x)	Freq. of Aa (grn) (y)	Freq. of aa (yel) (z)	Allele Frequencies: Freq. of A (p)	Freq. of a (q)
1e	Copy over allele frequencies from previous page (line 2e)									
2a	Assuming conditions for HWE, calculate genotype frequencies from allele frequencies: $x = p^2$; $y = 2pq$; $z = q^2$									
*	Check to see that $x + y + z = 1$									
2b	Assuming a population of 100, calculate numbers from genotype frequencies A = 100x B = 100y C = 100z				100					
	Check to see that A + B + C = 100									
2c	Simulate your Experiment HERE (Experiments 1 through 4)									
2d	Calculate # of each allele contributed. #A's⇒ d = 2A; e = f = B; g = 2C; h = d + e; i = f + g; j = h + i	d	e		h	Total # alleles				
	#a's⇒		f	g	i	j				
2e	Calculate allele freq. from allele #s $p = \frac{h}{j}$ $q = \frac{i}{j}$									
*	Check to see that p + q = 1									

Experiment#____ Generation 5

Step	Instructions	Raw Numbers: Num. of blu (A)	Num. of grn (B)	Num. of yel (C)	Total Num. (N)	Genotype Frequencies: Freq. of AA (blu) (x)	Freq. of Aa (grn) (y)	Freq. of aa (yel) (z)	Allele Frequencies: Freq. of A (p)	Freq. of a (q)
1e	Copy over allele frequencies from previous page (line 2e)									
2a	Assuming conditions for HWE, calculate genotype frequencies from allele frequencies: $x = p^2$; $y = 2pq$; $z = q^2$									
*	Check to see that $x + y + z = 1$									
2b	Assuming a population of 100, calculate numbers from genotype frequencies A = 100x B = 100y C = 100z				100					
	Check to see that A + B + C = 100									
2c	Simulate your Experiment HERE (Experiments 1 through 4)									
2d	Calculate # of each allele contributed. #A's⇒ d = 2A; e = f = B; g = 2C; h = d + e; i = f + g; j = h + i	d	e		h	Total # alleles				
	#a's⇒		f	g	i	j				
2e	Calculate allele freq. from allele #s $p = \frac{h}{j}$ $q = \frac{i}{j}$									
*	Check to see that p + q = 1									

Experiment#____ Generation 6

Step	Instructions	Raw Numbers: Num. of blu (A)	Num. of grn (B)	Num. of yel (C)	Total Num. (N)	Genotype Frequencies: Freq. of AA (blu) (x)	Freq. of Aa (grn) (y)	Freq. of aa (yel) (z)	Allele Frequencies: Freq. of A (p)	Freq. of a (q)
1e	Copy over allele frequencies from previous page (line 2e)									
2a	Assuming conditions for HWE, calculate genotype frequencies from allele frequencies: $x = p^2$; $y = 2pq$; $z = q^2$									
*	Check to see that $x + y + z = 1$									
2b	Assuming a population of 100, calculate numbers from genotype frequencies A = 100x B = 100y C = 100z				100					
	Check to see that A + B + C = 100									
2c	Simulate your Experiment HERE (Experiments 1 through 4)									
2d	Calculate # of each allele contributed. #A's⇒ d = 2A; e = f = B; g = 2C; h = d + e; i = f + g; j = h + i	d	e		h	Total # alleles				
	#a's⇒		f	g	i	j				
2e	Calculate allele freq. from allele #s $p = \frac{h}{j}$ $q = \frac{i}{j}$									
*	Check to see that p + q = 1									

Experiment#____ Generation 7

Step	Instructions	Raw Numbers: Num. of blu (A)	Num. of grn (B)	Num. of yel (C)	Total Num. (N)	Genotype Frequencies: Freq. of AA (blu) (x)	Freq. of Aa (grn) (y)	Freq. of aa (yel) (z)	Allele Frequencies: Freq. of A (p)	Freq. of a (q)
1e	Copy over allele frequencies from previous page (line 2e)									
2a	Assuming conditions for HWE, calculate genotype frequencies from allele frequencies: $x = p^2$; $y = 2pq$; $z = q^2$									
*	Check to see that $x + y + z = 1$									
2b	Assuming a population of 100, calculate numbers from genotype frequencies A = 100x B = 100y C = 100z				100					
	Check to see that A + B + C = 100									
2c	Simulate your Experiment HERE (Experiments 1 through 4)									
2d	Calculate # of each allele contributed. #A's⇒ d = 2A; e = f = B; g = 2C; h = d + e; i = f + g; j = h + i	d	e		h	Total # alleles				
	#a's⇒		f	g	i	j				
2e	Calculate allele freq. from allele #s $p = \frac{h}{j}$ $q = \frac{i}{j}$									
*	Check to see that p + q = 1									

Experiment#____ Generation 8

Step	Instructions	Raw Numbers: Num. of blu (A)	Num. of grn (B)	Num. of yel (C)	Total Num. (N)	Genotype Frequencies: Freq. of AA (blu) (x)	Freq. of Aa (grn) (y)	Freq. of aa (yel) (z)	Allele Frequencies: Freq. of A (p)	Freq. of a (q)
1e	Copy over allele frequencies from previous page (line 2e)									
2a	Assuming conditions for HWE, calculate genotype frequencies from allele frequencies: $x = p^2$; $y = 2pq$; $z = q^2$									
*	Check to see that $x + y + z = 1$									
2b	Assuming a population of 100, calculate numbers from genotype frequencies A = 100x B = 100y C = 100z				100					
	Check to see that A + B + C = 100									
2c	Simulate your Experiment HERE (Experiments 1 through 4)									
2d	Calculate # of each allele contributed. #A's⇒ d = 2A; e = f = B; g = 2C; h = d + e; i = f + g; j = h + i	d	e		h	Total # alleles				
	#a's⇒		f	g	i	j				
2e	Calculate allele freq. from allele #s $p = \frac{h}{j}$ $q = \frac{i}{j}$									
*	Check to see that p + q = 1									

Experiment#____ Generation 9

Step	Instructions	Raw Numbers: Num. of blu (A)	Num. of grn (B)	Num. of yel (C)	Total Num. (N)	Genotype Frequencies: Freq. of AA (blu) (x)	Freq. of Aa (grn) (y)	Freq. of aa (yel) (z)	Allele Frequencies: Freq. of A (p)	Freq. of a (q)
1e	Copy over allele frequencies from previous page (line 2e)									
2a	Assuming conditions for HWE, calculate genotype frequencies from allele frequencies: $x = p^2$; $y = 2pq$; $z = q^2$									
*	Check to see that $x + y + z = 1$									
2b	Assuming a population of 100, calculate numbers from genotype frequencies A = 100x B = 100y C = 100z				100					
	Check to see that A + B + C = 100									
2c	Simulate your Experiment HERE (Experiments 1 through 4)									
2d	Calculate # of each allele contributed. #A's⇒ d = 2A; e = f = B; g = 2C; h = d + e; i = f + g; j = h + i	d	e		h	Total # alleles				
	#a's⇒		f	g	i	j				
2e	Calculate allele freq. from allele #s $p = \frac{h}{j}$ $q = \frac{i}{j}$									
*	Check to see that p + q = 1									

Experiment#____ Generation 10

Step	Instructions	Raw Numbers: Num. of blu (A)	Num. of grn (B)	Num. of yel (C)	Total Num. (N)	Genotype Frequencies: Freq. of AA (blu) (x)	Freq. of Aa (grn) (y)	Freq. of aa (yel) (z)	Allele Frequencies: Freq. of A (p)	Freq. of a (q)
1e	Copy over allele frequencies from previous page (line 2e)									
2a	Assuming conditions for HWE, calculate genotype frequencies from allele frequencies: $x = p^2$; $y = 2pq$; $z = q^2$									
*	Check to see that $x + y + z = 1$									
2b	Assuming a population of 100, calculate numbers from genotype frequencies A = 100x B = 100y C = 100z				100					
	Check to see that A + B + C = 100									
2c	Simulate your Experiment HERE (Experiments 1 through 4)									
2d	Calculate # of each allele contributed. #A's⇒ d = 2A; e = f = B; g = 2C; h = d + e; i = f + g; j = h + i #a's⇒	d	e		h	Total # alleles				
			f	g	i	j				
2e	Calculate allele freq. from allele #s $p = \frac{h}{j}$ $q = \frac{i}{j}$									
*	Check to see that p + q = 1									

Bio 112 Population Genetics Practice Problems

These are intended as practice for the exams; you should do them & write out answers before looking at the solutions. I will hand out solutions in a week or so.

1) In Shorthorn cattle, the genotype C^RC^R is phenotypically red, C^RC^W is roan (a mixture of red and white), and C^WC^W is white.

a) Given that 108 red, 48 white and 144 roan animals were found in the central valley of California, calculate the frequencies of the C^R allele and the C^W allele in the gene pool of the population.

b) If all 5 assumptions for Hardy-Weinberg Equilibrium hold for this population, what is the expected frequency of each genotype in the next generation? Is the population represented in part (a) in Hardy-Weinberg equilibrium?

c) The rancher has observed that white Shorthorn cattle are sterile (unable to reproduce). What are the frequencies of the C^R and C^W alleles in the part of the population that is capable of reproducing? (this is harder than you'd likely find on an exam)

d) Taking into account the sterility of the white cattle, and assuming that the 5 assumptions of Hardy-Weinberg equilibrium hold for the breeding population, what are the expected frequencies of genotypes in the next generation? Would you expect this next generation to be at Hardy-Weinberg equilibrium? Why/why not?

2) Populophobia is a dreaded (but hypothetical) autosomal recessive condition in humans that causes genetics students to go into convulsions whenever they see the Hardy-Weinberg formula. That is: D is the normal allele; d is the populophobia allele (recessive phenotype), so

DD and Dd are normal
dd are populophobic - go into convulsions when they see the H-W formula

In a class of 200 genetics students, 32 had convulsions during their first population genetics lecture. Assume that students are a representative sample of a population at Hardy-Weinberg equilibrium.

a) What is the frequency of the populophobia (d) allele in this population? How many students in this class are heterozygous (Dd) for this condition?

b) During the second population genetics lecture, the professor decided to derive the Hardy-Weinberg formula for a population having 10 alleles at a given locus. The trauma induced by the derivation caused 75% of the students with populophobia to spontaneously combust...

POOF!

What is the frequency of each allele in the surviving population of genetics students? What are the frequencies of each genotype and phenotype?

c) The devious professor planned to rid the world of populophobia by forcing all young science students to derive the Hardy-Weinberg formula. Why was he a poor geneticist (besides being insane)?

3) You are studying an obnoxious weed in your backyard. These weeds are sexually-reproducing, freely-interbreeding, diploid organisms. They are either tall, medium, or short; the height is determined by one gene with two codominant alleles:

Genotype	**Phenotype**
HH	tall
Hh	medium
hh	short

a) Last year, you counted all the weeds in your yard and got the following results:

Height	**Number**
tall	16
medium	48
short	36

i) What are the frequencies of the two alleles (H and h) in this population? Show your work.
ii) Is this population at Hardy-Weinberg equilibrium? Justify your answer.

b) This year, after a particularly harsh winter, you count all the weeds in your yard again and get the following results:

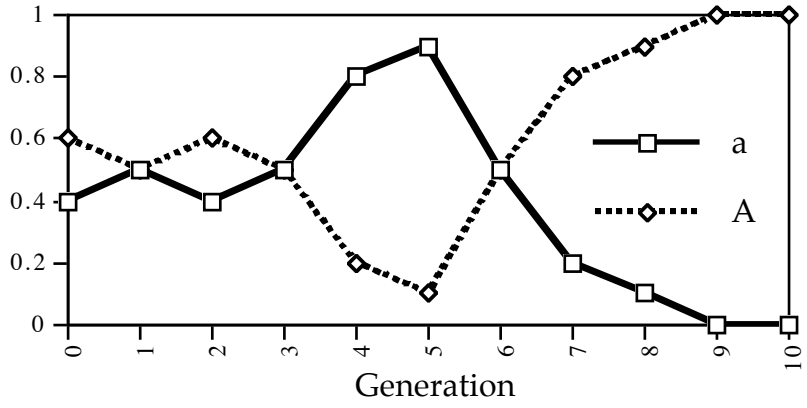
Height	**Number**
tall	2
medium	57
short	41

i) What are the frequencies of the two alleles (H and h) in this population? Show your work.
ii) Is this population at Hardy-Weinberg equilibrium? Justify your answer.

c) Offer a reasonable hypothesis to explain the changes in the genetic structure of the population from last year to this year assuming that the changes are **due to natural selection**.

d) Offer a reasonable hypothesis to explain the changes in the genetic structure of the population from last year to this year assuming that the changes are due to **some factor other than natural selection**.

4) Shown below is a graph similar to what you constructed in lab. It is the result of randomly killing 95% of the population in each generation.



Notice that the A allele takes over the population completely and the a allele goes extinct in generation 9. Is this the result of the A allele increasing the fitness of the organisms? Explain your reasoning briefly.

5) **Microevolution**
You are a time-traveling evolutionary biologist studying a particular species of snake on an island off the coast of Massachusetts. You travel 100,000 years into the past and observe that the snakes are a variety of colors (red, blue, yellow, green) and that most of the snakes are blue. This same species of snake and the same mix of colors are found on the mainland.

a) Assuming that all the snakes are descended from an ancestral blue snake, where did the other colors come from?

At the present time, the snakes are still the same variety of colors, but most of the snakes are green.

b) Explain this change in color frequency (micro-evolution) as though it were based solely on each of the following processes:

i) Bottleneck effect.

ii) Founder effect.

iii) Migration.

iv) Non-random mating.

v) Natural selection.

Bio 112 Solving Population Genetics Problems

Reminder:
Genotype frequencies are the frequencies of the three possible genotypes (AA, Aa, aa).
Allele frequencies are the frequencies of the two alleles (A with frequency p; a with frequency q)

In general:
Solving population genetics problems usually follows this pattern:

(1) Find the allele frequencies; there are 2 methods to use depending on what you know:
  (1a) if you know all the genotype frequencies
  (1b) if you know at least one of the genotype frequencies **and** can assume that the population is at HWE. You can then find the other genotype frequencies.
(2) Predict the genotype frequencies in the next generation.
(3) See if the population is at HWE.

The particular steps:
(1a) Find the allele frequencies given all three genotype frequencies. This is the most general way to get allele frequencies; it works under **all** conditions (at HWE and not at HWE). You do it by calculating the number of alleles contributed by each genotype to the gene pool.

- since each individual has two alleles to contribute, the size of the gene pool is the number of individuals times 2.
- each AA individual contributes 2 A's to the pool
- each Aa individual contributes one A and one a to the pool
- each aa individual contributes 2 a's to the pool

For example: given this population

genotype	number
AA	30
Aa	20
aa	50

you calculate the total # of individuals = 30 + 20 + 50 = 100; this lets you calculate the genotype frequencies:

genotype	number	genotype frequency (number divided by total)
AA	30	30/100 = 0.3
Aa	20	20/100 = 0.2
aa	50	50/100 = 0.5

Now, you can get the allele frequencies in two ways:

⇒ either calculate the contributions using the numbers of each genotype (easy):

		contributions to gene pool		
genotype	number	total alleles	A alleles	a alleles
AA	30	60	60	0
Aa	20	40	20	20
aa	50	100	0	100

total # of alleles = 100 x 2 = 200 (or 60 + 40 + 100 = 200)
total # of A's = 60 + 20 = 80 frequency of A = p = 80/200 = 0.4
total # of a's = 20 + 100 = 120 frequency of a = q = 120/200 = 0.6

⇒ or calculate using the frequencies of each genotype (more advanced):

frequency of A = p = (frequency of AA) + (frequency of Aa)/2
= 0.3 + (0.2)/2 = 0.3 + 0.1 = 0.4
frequency of a = q = (frequency of aa) + (frequency of Aa)/2
= 0.5 + (0.2)/2 = 0.5 + 0.1 = 0.6

(1b) Find the allele frequencies given at least one genotype frequency **and** assuming the population is at HWE. This **only** works if you can assume that the population is at HWE; this will be given in the problem. To do this, you need to find one of the genotype frequencies and then use the following relationships which only hold at HWE:

frequency of AA = p^2
frequency of Aa = 2pq
frequency of aa = q^2

Once you've found either p or q, you know that p + q = 1 always, so you can get the other. For example: "Sickle-cell anemia is an autosomal recessive genetic disease:

allele	**contribution to phenotype**
A	normal (dominant)
a	sickle-cell anemia (recessive)

In a particular population, 99% (frequency = 0.99) are normal (AA and Aa) and 1% (frequency = 0.01) are sickle-cell (aa). Assuming that this population is at HWE, find the frequencies of each allele."

If we call the frequency of the normal allele (A) p, and the disease allele (a) q:
From above, we know that $0.99 = p^2 + 2pq$ (a difficult equation to solve)
but, we also know that $0.01 = q^2$ (this is much easier to solve)

take the square root of both sides: $\sqrt{0.01} = \sqrt{q^2}$ so: $\sqrt{0.01} = q$ so $0.1 = q$

since we know that p + q = 1, p = 1 - q so p = 1 - 0.01 so p = 0.9

Now we have both allele frequencies. We can then go on to find the frequencies of the other genotypes using the relationships above:

frequency of homozygous normal (AA) = $p^2 = (0.9)^2 = 0.81$ 81% AA
frequency of carriers (Aa) = 2pq = 2(0.1)(0.9) = 0.18 18% Aa

(note that 81% + 18% = 99%, the number of phenotypically normal individuals).

Note that the sum of the allele frequencies (p + q) is **always** = 1 whether at HWE or not.

(2) Predict the genotype frequencies in the next generation. To do this, you must assume that the 5 conditions for HWE hold for the population. In the case where they do not hold (like in lab where there was selection), you can assume that they hold for the breeding population (the part of the population that can reproduce). As long as you are using the allele frequencies (p & q) for the breeding population, you can use the relationships below:

frequency of AA = p^2
frequency of Aa = 2pq
frequency of aa = q^2

(3) See if the population is at HWE. If the conditions of HWE hold, the next generation calculated using (2) will be at HWE. You can use this as a test to see if a population is at HWE.

- calculate the allele frequencies using (1a). You cannot use (1b) because (1b) **assumes** that you are at HWE already!
- predict what the genotype frequencies will be at HWE using (2)
- compare the predicted genotype frequencies with the actual ones. If they're equal, then the population is at HWE.

For example: the population in 1a has p = 0.4 and q = 0.6. If it were at HWE, the frequency of AA would be $p^2 = 0.16$; the frequency of Aa would be 2pq = 0.48; and the frequency of aa would be $q^2 = 0.36$ – since the actual frequencies are 0.3, 0.2 and 0.5, respectively, the population is **not** at HWE.

Note that $p^2 + 2pq + q^2$ **always** = 1, whether at HWE or not; so testing to see if $p^2 + 2pq + q^2 = 1$ does **not** test to see if the population is at HWE.

Hints for solving the practice problems: (numbers in [brackets] are appropriate steps as above)

#1a = [1a] #1b = [2] #1c = [not above] #1d = [2]
#2a = [1b] #2b = [1a] #2c = [not above]
#3ai = [1a] #3aii = [3] #3bi = [1a] #3bii = [3]

You should try the problems before using these hints.

Solutions to: Population Genetics Practice Problems

1) a)

	C^R	C^W
108 red cows C^RC^R	216	
144 roan cows C^RC^W	144	144
48 white cows C^WC^W		96
	360/600	240/600

frequency of C^R allele = p
p = 360/600 = 0.6

frequency of C^W allele = q
q = 240/600 = 0.4

b) Assuming that the population satisfies the 5 requirements for HWE, we can predict that:
frequency of $C^RC^R = p^2 = (0.6)^2 = 0.36 = 36\%$ red cows
frequency of $C^RC^W = 2pq = 2(0.6)(0.4) = 0.48 = 48\%$ roan cows
frequency of $C^WC^W = q^2 = (0.4)^2 = 0.16 = 16\%$ white cows

If the assumptions required for Hardy-Weinberg equilibrium are met, then any population will reach equilibrium in one generation. Therefore, we know that the frequencies above are equilibrium frequencies. To find if the population in part (a) is in equilibrium, take these frequencies, multiply by the population size, and compare the numbers to those given in part a.

number of red cows = 0.36(300) = 108
number of roan cows = 0.48(300) = 144
number of white cows = 0.16(300) = 48

Since these are the same as those in part (a), then the population in part (a) was already in equilibrium.

c) To answer this, one recalculates p and q in the same way as in part a, but does not include the 48 sterile (unable to reproduce, infertile) white cows. It is important to realize that the breeding population is reduced to 252 cows, or a total of 504 alleles.

	C^R	C^W
108 red cows C^RC^R	216	
144 roan cows C^RC^W	144	144
	360/504	144/504

frequency of C^R allele = p
p = 360/504 = 0.71

frequency of C^W allele = q
q = 144/504 = 0.29

d) Assuming that the conditions for HWE hold for the breeding population, we can calculate:
frequency of $C^RC^R = p^2 = (0.71)^2 = 0.504 = 50.4\%$ red cows
frequency of $C^RC^W = 2pq = 2(0.71)(0.29) = 412 = 41.2\%$ roan cows
frequency of $C^WC^W = q^2 = (0.29)^2 = 0.084 = 8.4\%$ white cows

This population is not in equilibrium due to the reproductive selection against the C^W allele resulting from the sterility of white (C^WC^W) cows. Realize that $p^2 + 2pq + q^2 = 1$ **at all times**, even when not in equilibrium. That is, the frequencies will always add to 100% of the population. (50.4% + 41.2% + 8.4% = 1 but is not in equilibrium.)

2) a) You need to use the Hardy-Weinberg equilibrium formula for 2 alleles to solve this problem. If we define q as the frequency of the populophobia allele (d) and p as the frequency of the normal allele (D):

$$1 = p^2 + 2pq + q^2$$

where, q^2 represents the fraction of individuals who are homozygous recessive (dd - populophobic). Thus,

$$q = \sqrt{\frac{32}{200}} = \sqrt{0.16} = 0.4$$

since p + q = 1, p = 1 - q or: p = 1 - 0.4 = 0.6
The frequency of heterozygotes (Dd) is:
2pq = 2(0.6)(0.4) = 0.48
(0.48)(200) = 96 individuals are heterozygous.
That leaves: 200 - (96 +32) = 72 who are homozygous normal
b) If 75% of the individuals with populophobia spontaneously combust, then:
(0.75)(32) = 24 dd individuals lost from the population
32-24 = 8 individuals remaining who are homozygous recessive (dd)
Thus, the surviving population is:

72 DD
96 Dd
8 dd
total: 176

To calculate the new allele frequencies, you must determine the total number of p and q alleles remaining in the population. This is equal to the number of heterozygotes plus *twice* the number of homozygotes.
total D alleles = 2(72) + 96 = 240
total d alleles = 96 + 2(8) = 112
total alleles in population = 240 + 112 = 352

The frequency of each allele in the new population is:
frequency of D = p = 240/352 = 0.68
frequency of d =q = 112/352 = 0.32

The frequencies of each *genotype* are:
frequency of homozygous normal = 72/176 = 0.41
frequency of heterozygotes = 96/176 = 0.545
frequency of homozygous populophobia = 8/176 = 0.045

The frequencies of each *phenotype* are:
frequency of normal = 0.41 + 0.545 = 0.955
frequency with populophobia = 0.045

c) Most recessive alleles are masked in heterozygotes and are unaffected by selection pressures. Even if there was complete selection against populophobia (100% spontaneously combust), it would take almost 100 generations to reduce the populophobia allele from 40% to 1%.

3) a) i)

height	Number	Genotype	#H's contributed	#h's contributed
tall	16	HH	32	0
medium	48	Hh	48	48
short	36	hh	0	72

total H's = 80 total h's = 120 total alleles = 200

frequency of H = p = 80/200 = 0.4

frequency of h = q = 120/200 = 0.6

ii) If it were at HWE, then:

frequency of HH = $p^2 = (0.4)^2 = 0.16$ so in 100 plants 0.16 x 100 = 16 plants

frequency of Hh = $2pq = 2(0.4)(0.6) = 0.48$ so in 100 plants 0.48 x 100 = 48 plants

frequency of hh = $q^2 = (0.6)^2 = 0.36$ so in 100 plants 0.36 x 100 = 36 plants

These are the same numbers as observed, so the population is at HWE.

b) i)

height	Number	Genotype	#H's contributed	#h's contributed
tall	2	HH	4	0
medium	57	Hh	57	57
short	41	hh	0	82

total H's = 61 total h's = 139 total alleles = 200

frequency of H = p = 61/200 = 0.305

frequency of h = q = 139/200 = 0.695

ii) If it were at HWE, then:

frequency of HH = $p^2 = (0.305)^2 = 0.093$ so in 100 plants 0.093 x 100 = 9 plants

frequency of Hh = $2pq = 2(0.305)(0.695) = 0.424$ so in 100 plants 0.424 x 100 = 42 plants

frequency of hh = $q^2 = (0.695)^2 = 0.483$ so in 100 plants 0.483 x 100 = 48 plants

These are not the same numbers as observed, so the population is not at HWE. One of the 5 assumptions must be violated. Note that there are much fewer tall plants than expected.

c) The number of tall plants must have dropped because the tall plants were at a disadvantage because of their height. Perhaps they poked out of the snow more than the other plants and were frozen by the cold winds and died. The shorter plants were protected by the snow.

d) There are several possiblities, here are some:

- genetic drift: all the tall plants happened to be near a tree that fell down and crushed most of them.
- migration: your neighbor has mostly short and medium plants and seeds from them blew onto your yard.

4) No. Survivors are randomly selected (not on the basis of genotype) so frequencies are not related to fitness. Because of this, the results would likely have been different if the experiment were run again.

5) a) Mutation. Mutations in the pigment-producing genes of the snake could have resulted in progeny of different colors.

b) i) It happened that most of the snakes on the island were killed by a flood that did not discriminate on the basis of color. Although there was no selective advantage to green, it just so happened (chance events) that most of the surviving snakes happened to be green (the survivors were not a representative sample of the original population); resulting in a mostly green population.

ii) Although there was no selective advantage to green, it just so happened that a catastrophe killed all the snakes on the island. It then happened that the island was colonized by a group of snakes from the mainland of different colors, most of which were green, resulting in a large population of green snakes on the island.

iii) A large number of green snakes migrated to the island from the mainland; the mainland population happens to be mostly green. This increased the frequency of the green allele in the island population, resulting in mostly green snakes.

iv) For some reason (a genetic influence on behavior), green snakes become the "mate of choice" among the snakes on the island, leading to an increase in the frequency of the green allele in the population.

v) Some selective force acts against the non-green snakes, reducing their number or reproductive output, resulting in a relative increase in green snakes. For example, a predator could come to the island which is unable to see the green snakes against the green foliage and therefore eats only the other snakes.

Molecular Phylogeny

Purpose

• to show how data about molecules can be used to find evolutionary relationships.

Introduction

Since all living things descended from a common ancestor, their cellular components (DNA, RNA, protein, etc.) share a common origin. Originally, there was only one species of life on earth. However, mutations occurred in its DNA, resulting in the production of different proteins in different individuals of that organism and their descendants. Once some of these descendants became different enough to be reproductively isolated from the parent a new species was formed. The resulting two species are then subject to further mutation and evolution.

In this lab, we will use the amino acid sequence of the protein cytochrome c as a 'molecular clock'. Cytochrome c is an essential part of cellular respiration and was presumably present in the first air-breathing ancestor of all modern animals and plants. As a result of this, all modern air-breathing plants and animals have cytochrome c's which are evolutionary descendants of the original cytochrome c. Since much time has passed since the ancestor existed, there have been many mutations in the cytochrome c gene and thus many changes in the amino acid sequence of cytochrome c.

Two organisms of the same species should have identical cytochrome c molecules. The longer the time since two organisms had a common ancestor, the more different the cytochrome c molecules will be. We will compare the amino acid sequences of cytochrome c from various organisms to determine their degree of evolutionary relatedness.

There are two main methods for comparing protein sequences from different organisms in order to determine their phylogenetic relationships:

- **Sequence Divergence** This compares the sequences and counts the number of differences between them. The longer since their common ancestor, the more differences expected. This is the simplest method. You will do this 'by hand' to see how it works and then let the computer do the hard work. This method is best for finding approximately how long it has been since two species had a common ancestor. It works fairly well for finding out which creatures are related to which. In studies of cytochrome c from many organisms, it has been found that (very approximately) one amino acid change occurs every 21 million years. The rates of change of other proteins are different.
- **Parsimony** This is a more sophisticated method that also takes into account the particular differences between the sequences. It is described in detail in *Campbell* pages 501-504. Although it can not tell you how long ago two organisms had a common ancestor, it is much better at telling which creatures are most closely related to which than the Sequence Divergence method.

In this lab, you will use both methods to see their strengths and weaknesses. You should remember that the software generates the most likely tree, but not necessarily the way the organisms actually evolved.

You will need your copy of *Campbell* for this lab.

Procedure

You will work in groups of three per computer in this lab.

The instructions in the manual are for the Macintosh computers; you can also access all of the resources for this lab from any computer with www access – no special plug-ins are required.

Phylogenetic Trees

For the purposes of some pre-labs, etc., you will be asked to draw a partial phylogentic tree showing the relationships between various organisms. Here is a hypothetical example to show you what we are looking for.

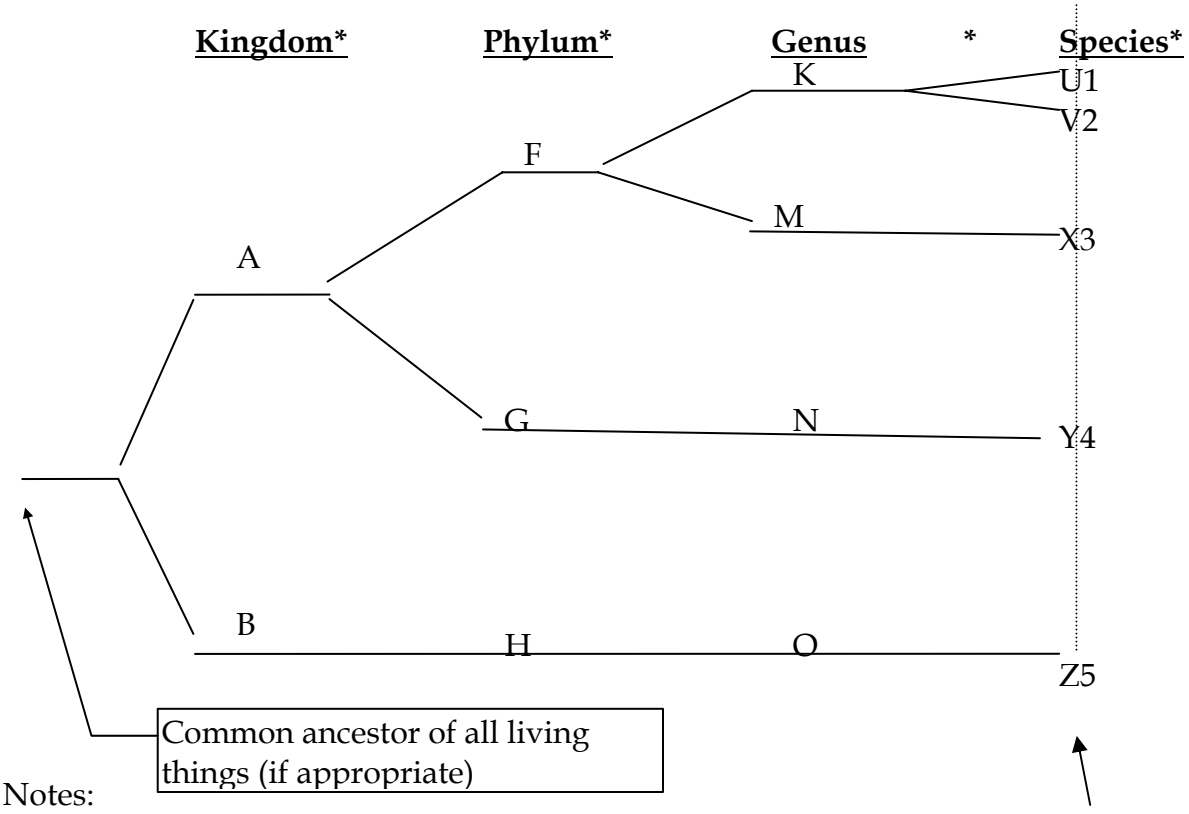
Given organisms 1 through 5 with the following classifications:

Organism	Kingdom	Phylum	Genus	Species
1	A	F	K	U
2	A	F	K	V
3	A	F	M	X
4	A	G	N	Y
5	B	H	O	Z

Thus:

- the difference between 1and 5 is at the kingdom level - they are extremely different.
- the difference between 3 and 4 is at the phylum level - they are in the same kingdom but still very different.
- the difference between 2 and 3 is at the genus level - they are in the same kingdom and phylum, but still are rather different.
- 1 and 2 differ at only the species level - they are different.

This is shown in the diagram below: (this is what we will want on pre-labs, etc.)



Notes:

- since all species listed are currently alive, they must line up vertically, like this:
- the only distances that matter are the horizontal ones; vertical positions don't matter
- since all the species listed are currently alive, the distance from any one to the common ancestor must be the same.

* If given.

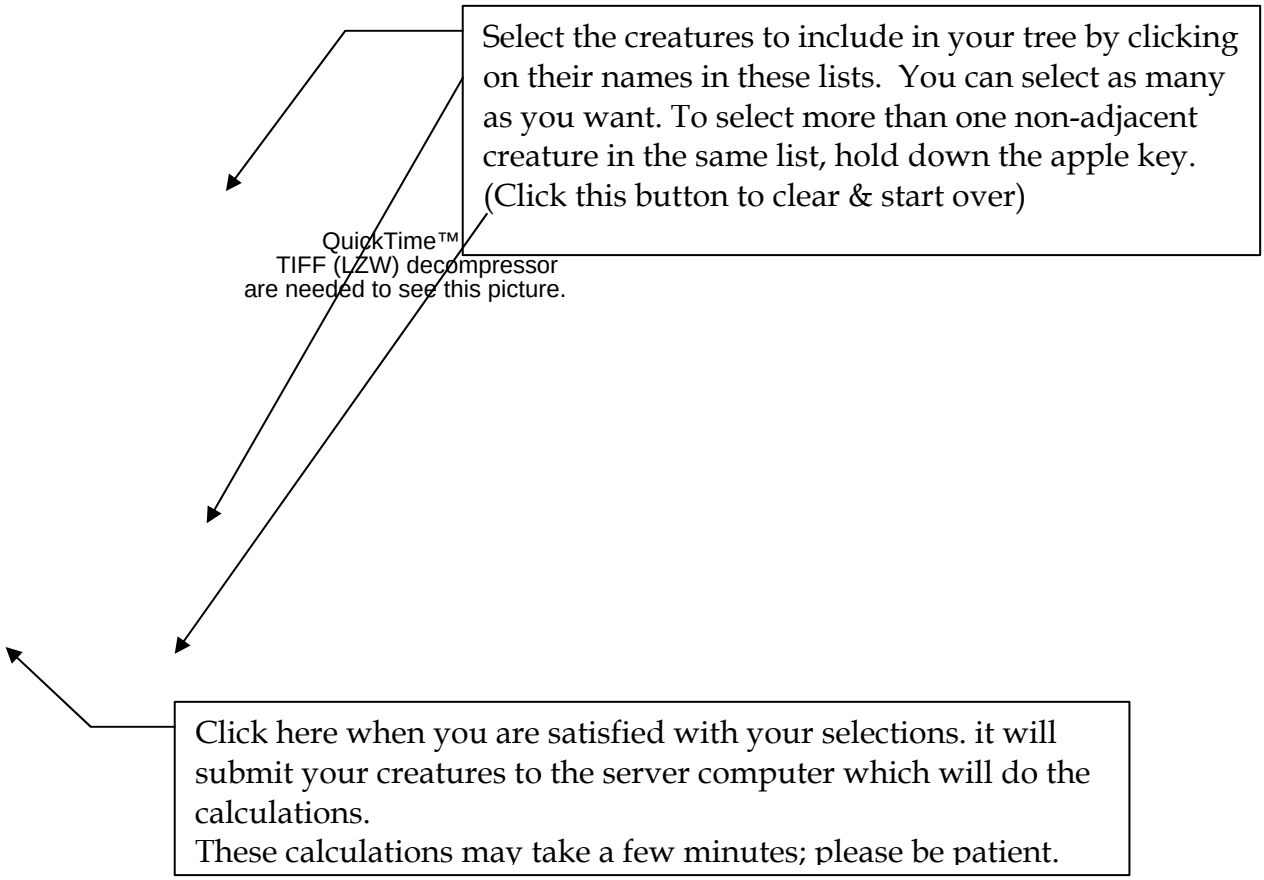
Part I: Sequence Divergence "The hard way" (you do half of the work)

In this part, you will use the software to show you the number of differences between two protein sequences - this will help you to understand how this information is generated. You will then use this information to construct a simple tree manually.

1) To access the "Tree Constructor", start Safari from the Dock.

2) Click on the link to the OLLM and then the link for the "New Phylogenetic Tree Constructor".

3) You will see a page that looks like this:



4) For this first exercise, we will use the program in a slightly unusual way. Choose two organisms that you think are closely-related. Select one in the "Main Tree Organisms" and one in "Outgroup Organism". You have to select one in each set or the program will complain. In this example, I have chosen "cow" and "donkey". You should choose two other organisms that are closely-related. The screen should look something like this (except your

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

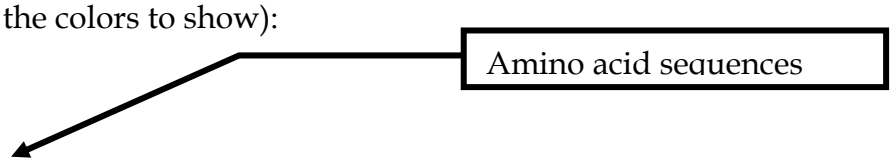
organisms are selected:

5) Click "Calculate Tree" and wait a little while and you should see this:

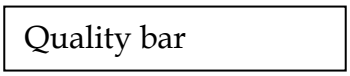
QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

The creatures you selected should be listed here. If not, go back to step (4) using Safari's "Back" button.

6) Click the "JalView" button and wait 20-60 seconds and you should see this (you may have to wait a little for all the colors to show):



This shows the amino acid sequence of cytochrome c from the cow (top line) aligned with the amino acid sequence of cytochrome c from the donkey (bottom line). There are several important features of this display:



- The amino acid sequences are listed left to right from amino to carboxyl ends.
- The length of the protein sequences is listed at the left end of the colored bands: "cow/1-104" means that the sequence is 104 amino acids long. This will be important later.
- The amino acid sequence is listed using the single letter amino acid code. That is, one letter per amino acid. For example, the amino-terminal amino acid in both cytochrome c's is glutamic acid, which we would have abbreviated "glu" in Bio 111; here it is "E". The next amino acid is lysine ("lys" in Bio 111), abbreviated "K".
- The amino acids are color coded by functional category. For example, aspartic acid (D) and glutamic acid (E) both have (-) charged side chains and are both colored purple.
- The computer program has done its best to match up identical amino acids. Any places where there are differences are shown by white spaces in the purple "Quality" bar under the amino acid sequences. In this case, there are two differences between cytochrome c from cow and donkey:
  - Amino acid #60 in cow cytochrome c is G (glycine); amino acid #60 in donkey cytochrome c is K (lysine).
  - Amino acid #89 in cow cytochrome c is G (glycine); amino acid #89 in donkey cytochrome c is T (threonine).

From this, we can conclude that there are two amino acid differences between the cytochrome c's of cow and donkey. We would then say "cow and donkey differ by 2 substitutions".

7) Using this technique, find the number of substitutions between your two closely-related organisms. Save this number for later.

8) Choose a third, more distantly-related organism and find the number of substitutions between it and your two original organisms. This will take two separate runs of the program.

I chose corn as my distantly-related organism. Here are the results I got:

• corn vs. cow:

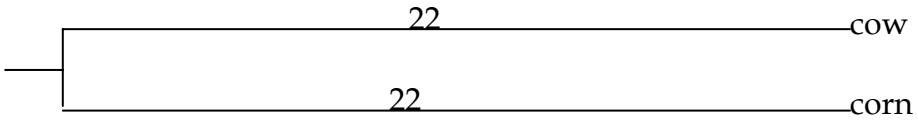
Counting all the places where the sequences don't match (anyplace where the "Quality" bar isn't at its full height), there are 44 substitutions out of 112 amino acids.

• corn vs. donkey:

Counting all the places where the sequences don't match (anyplace where the "Quality" bar isn't at its full height), there are 40 substitutions out of 112 amino acids.

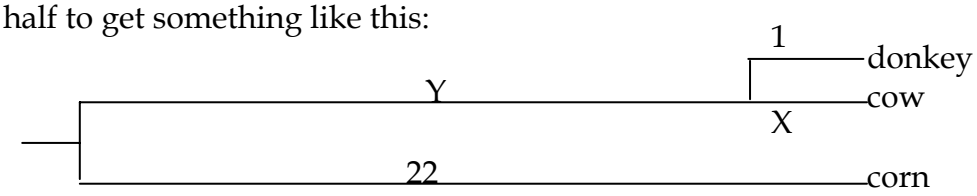
9) Make a phylogenetic tree of your three organisms based on the substitution data. Here is a simple way:

i) Take the most distantly-related organisms, in this case cow and corn. Make a tree with 2 branches, each 1/2 the number of substitutions long, in this case 44/2 or 22 each.

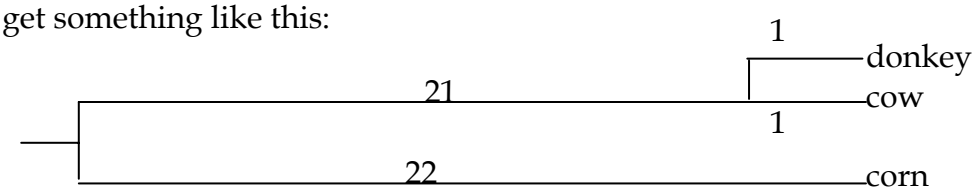


Note that the <u>total distance</u> between cow and corn is 22 + 22 = 44.

ii) Now take the more closely-related organism and add it as a branch off of its closely-related partner. In this case, donkey & cow differ by 2. Again, split the difference in half to get something like this:

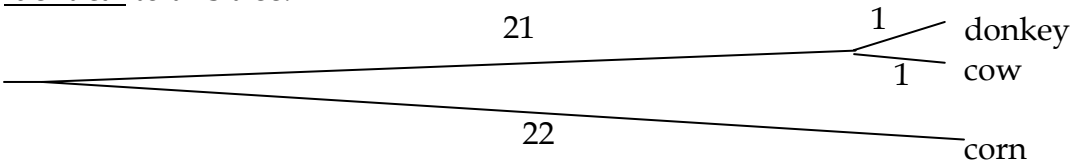


iii) But what about the "X" and "Y"? Since the distance between cow and donkey must be 2, X + 1 must = 2. Therefore X = 1. Since the total length from the branch at the left to cow must equal 22 and X = 1, Y = 22 –X or 22 – 1, or 21. This gives the final tree: get something like this:



There are a couple of things to notice about this tree:

• The lengths of the vertical lines are <u>not</u> counted in the branch lengths. Therefore it is <u>identical</u> to this tree:



• It is <u>approximate</u>! The distance from donkey to corn should be 44 substitutions (as measured from the sequences) but the tree shows it as 41. Sometimes, it comes out like this and sometimes the numbers add up properly. This is what we call "close enough for government work".

10) Check the tree you made by having the program calculate it for you.

a) Go back to the "Tree constructor" page.
b) Select your three creatures and click "Calculate Tree".
c) Click "JalView".
d) When the window appears,

e) From JalView's "Calculate" menu, select "Calculate Average Distance Tree using PID". Again, be patient. Set the "Font Size" to 12 and check "Show Distances" (these controls are near the bottom of the window)You will get a tree like the one on the next page:

these are JalView's menus

Note that this number (112 in this case **- this number may be different in different alignments**) is the total number of amino acids in the protein under consideration. This is a very

You can roughly check the numbers using the following calculations. The numbers are % difference = 100% x (the number of differences)/(the # of amino acids = 112*) .

- the top branch = 19.64% = 0.196. The number of substitutions would be 0.194 x 112 = 22 (which is close to the 20.5 in my tree)
- the bottom fork = 0.89% = 0.0089. The number of substitutions would be 0.0089 x 112 = 1 (which exactly matches my tree)

> Note that the numbers here are percentages, not number of substitutions.

* This number may be different in different alignments.

Now that you have seen what the computer does 'behind the scenes', you can leave the hard and boring work to the computer for the rest of the lab.

Part II: Draw a phylogenetic tree for 5 organisms of your choice and use the rough rule "1 change per 21 million years" to put approximate dates (in Ma) on your tree.

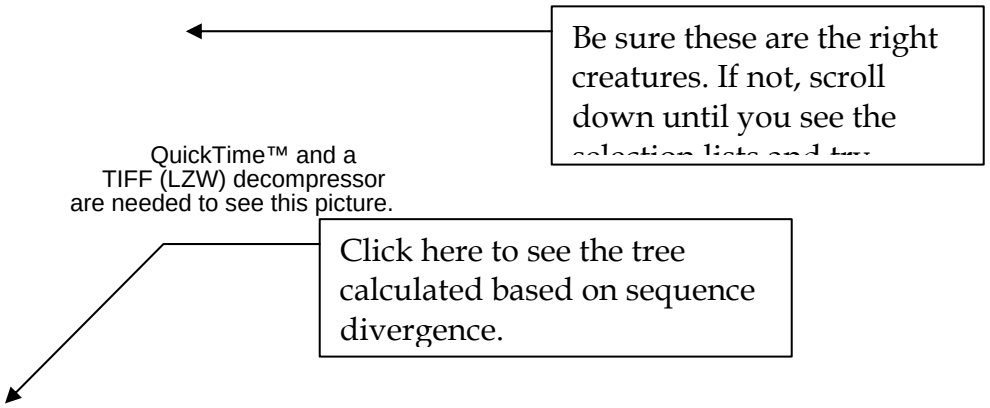
a) Look in the list at the end of this section of the lab manual. Find 5 organisms of your choice. Choose 4 that are relatively similar and one rather different one as an "Outgroup organism". Having a distantly-related outgroup organism makes it more likely that the program will give a meaningful tree (the reasons why this is so are beyond the scope of Bio 112).

b) Select the 4 "Main Tree Organisms" as you did previously. Use shift-click to select more than one organism at a time. If you want to select non-adjacent organisms in a list, use apple-click. Once you have made your selections, click the "Calculate Tree" button.

In the example below, I selected:

Main tree:	carp
	chicken
	Chimpanzee
	Cow
	(all of these are vertebrates)
Outgroup:	Corn
	(this is very different from a vetebrate!)

c) After a few minutes, you will get a screen like this:



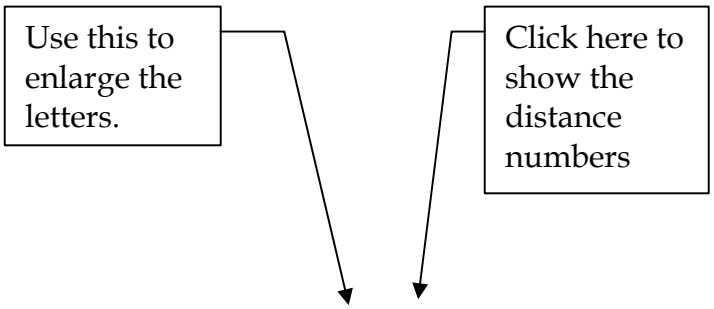
[The only time you should really worry is if you get a message like "server not responding" at this point. In this case, contact Brian White ASAP.]

d) Click the "JalView" button to see the tree calculated based on sequence divergences. (Note that if this is the first time that you have made a tree since Safari was started, it will take a while to load and start the JalView part of the program. You will see messages in the bottom of Safari's window like "starting Java" and "loading..." please be patient.) You will get a screen like this:

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

e) From JalView's "Calculate" menu, select "Calculate Average Distance Tree using PID". Again, be patient. Set the "Font Size" to 12 and check "Show Distances" (these controls are near the bottom of the window). You will get a tree like the one on the next page:

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.



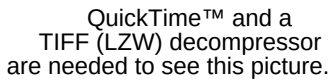
The tree gives the numbers needed to find the approximate date of the last common ancestor of these creatures. You should put approximate dates on your tree using the calculations described on page MolPhyl-14.

f) Unfortunately, you cannot print this out; you will have to copy it down by hand. Do not have the program mail it to you, that feature does not work.

g) Close the JalView windows by clicking the box in the upper left of each JalView window. This should return you to the window shown in step (c).

Part III: Compare trees generated using Sequence Divergence with those generated using Parsimony

a) Now, scroll down to the green area where it says, "Tree Constructed using Parsimony Analysis". It should look something like this:



This shows a slightly different result than the one generated using Sequence divergences. Here, the chimp is the most distantly-related vertebrate rather than the carp.

So, which is the "right" tree?

In an ideal world, both methods would give the same answer, more or less. In the real world, things are more complicated. If you were doing this 'for real' (as a scientist trying to draw conclusions about things based solely on this evidence), you would look at more data. Typically, this involves comparing the protein or DNA sequences from many genes in each of the organisms you are interested in. Using only one gene can lead to problems. For example, the cytochrome c sequences of human and chimp are *identical*. Based only on this data, you would conclude that humans and chimps were indistinguishable. This is why we ask that in your lab report, you cite data from more than just this program when drawing conclusions.

In the end, with the limited data and tools available in this lab, the method you choose depends on what you want to know. In general, we would hope that both methods would give similar results. If they don't, then use the following general rule:

- Use Sequence Divergence to find approximately when organisms had a common ancestor.
- Use Parsimony to find out which organisms are more closely-related to each other.

b) To build another tree, click Safari's "back" button twice to return to the "Construct a Tree" screen.

Part IV: Phylogenetic Questions

4) Using these techniques, answer one of the following questions; you should choose the method (Sequence Divergence or Parsimony) that best suits the question you are asking.

a) Is a Schreibers's long-fingered bat a bird or a mammal?
b) Is a california grey whale a fish or a mammal?
c) We will talk about monocots and dicots later in the course; are they distinct groups of plants?
d) What is a cuckoopint? (What kingdom is it in?)
e) What is a ramtil? (What kingdom is it in?)
f) What is a love-in-the-mist? (What kingdom is it in?)
g) A question that you have thought up (it must be approved by your TA in advance). For example:
    1. When was the last common ancestor of X and Y?
    2. Which of these organisms are more closely-related?

Note that you will have to compare your results with what is known about these organisms in your lab report (see below), so choose your questions wisely.

Hint: To answer these questions properly, you should use at least two members of each group you are testing and one distantly-related 'outgroup'. The example shown is the correct way to answer the question "is a human a mammal or a plant". The analysis included 6 organisms:

- the organism being examined: human
- two organisms from one test group = 2 mammals = rat & horse
- two organisms from the other test group = 2 plants = corn & cauliflower
- one distantly-related organism: fungus Asperg niger

Your analyses must use this form, when appropriate. Check with your TA if you have questions.

Part V: Marine Mammals II

You will answer the two questions from HMNH-8, this time using molecular phylogenetic data. See under "Lab Report" for details. You should do this at home.

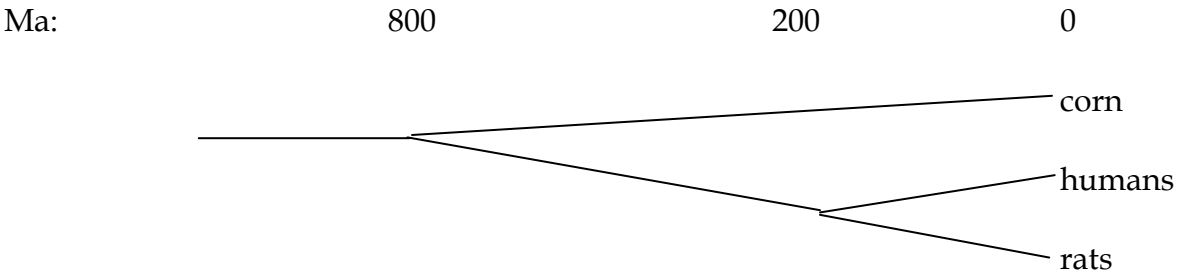
Lab report:

- Must be typed; handwritten reports will not be accepted. Hand-drawn and labeled drawings are fine.
- Due at the start of the lab session you are currently in during the week specified on the syllabus. This is a firm deadline.
- Although you will perform these activities as a group, each member of the group must turn in an individual lab report. Each person's report must be in his or her own words as much as possible.
- Your lab report must contain answers to the following 5 questions:

Part II: Draw a phylogenetic tree for 5 organisms of your choice

1) The organisms you chose and their kingdom and phylum.

2) The tree you constructed using Sequence Divergences with the approximate dates shown. For example, from the data given previously (you tree would have 2 more organisms):



3) Compare your tree with the actual values from *Campbell* as best you can. You should compare **both** the actual numbers (which will probably be off at least a little) and the general relationships (which is closer to which). Also compare this tree to the one generated using Parsimony.
Cite your sources. For the above example: "*Campbell* figure 26.10 puts the plant/animal split at about 800Ma - this is pretty close to the 800Ma we found. *Campbell*, p. 694 says 'mammal-like synapsids emerged... 200 Ma' We put humans and rats as having a last common ancestor about then (when most major groups of mammals may have diverged), which is probably pretty close." In your lab report, you would expand on this slightly to make a more coherent answer.

Part IV: Phylogenetic Questions

4) Answer one of the questions listed. Explain your reasoning and your choice of method (Sequence Divergence or Parsimony). Your answer must include a phylogenetic tree and your conclusions based on that tree. You should also mention if the two methods gave different trees. Your conclusions should hopefully be right but do not have to be right; they must be based on your data, though. You **must also** compare your findings to what is known about the organisms from other sources; you **must also** cite your sources. For example, if you chose

question 4b, you should state your results along with something like: "According to *Campbell* (page 699), whales are members of the mammalian order cetacea and are therefore considered to be mammals. This agrees (or does not agree) with our findings which showed that....."

There is one more question on the next page!!

Part V: Marine Mammals II

5) You answered these two questions in the HMNH lab based on skeletal and morphological data. In this part, you will use molecular data to look at the same questions.

You should use the link on the On-Line Lab Manual to the "Mammalian Tree Constructor"
http://www.securebio.umb.edu/cgi-bin/COIITreeConstructor.pl

This will provide you with a large set of protein sequences from different marine and terrestrial mammals. In this case, the protein is Cytochrome Oxidase II from the mitochondria of the different animals. This protein evolves more rapidly than Cytochrome c, so it is more useful for resolving more recent evolutionary events like the divergence of mammalian groups.

Answer the two questions below using data you gather from the "Mammalian Tree Constructor". Since the "molecular clock" for cytochrome oxidase II is not well-calibrated, you should use **only parsimony analysis** when answering these questions.

You may need to consult *Campbell* or other sources to determine the mammalian orders to which various animals belong.

a) How many major different groups of marine mammals are there? A full-credit answer to this question consists of three parts:
   - The number of groups of marine mammals that you have determined.
   - An explanation of why you chose the groups that you chose. We are not interested in the "right" answer here; just a well-reasoned argument based on your data. Show a parsimony tree that supports your conclusion and explain your reasoning briefly.
   - Which of the marine mammals belong to each group? Your answer should include at least two members of each group.

b) Which is the closest living land relative of a seal? Seals evolved from land-dwelling ancestors. Although that ancestor is now extinct, it has modern-day descendants. Based on a phylogenetic tree that you construct, you must decide which order of land mammals this ancestor came from.
   A full-credit answer to this question has two parts:
   - The order of land mammals that you think is most closely-related to the land ancestor of seals. Choose from the list below.
   - An explanation of why you chose that order. Again, we are not interested in the "right" answer; just an informative tree (one that shows seals' close relatives and some groups that are not closely-related) and a well-reasoned argument based on your tree.

   All of the orders of land mammals can be found in the "Mamalian Tree Constructor" **except**:
   - Xenarthra
   - Hyracoidea

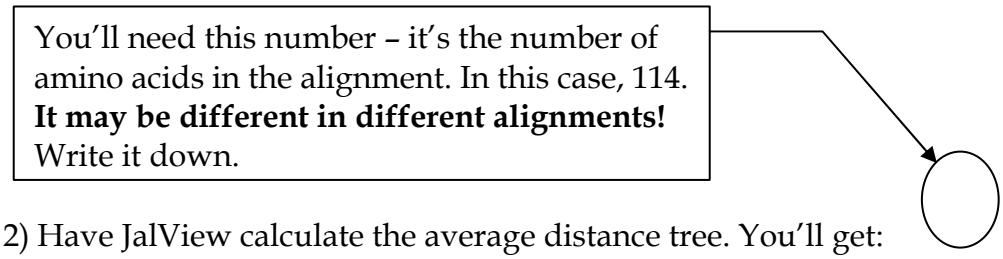
In each part, we are not interested in the correct answer; we are interested in the *data* you cite and your *argument* based on that data. The more specific about the data you are and the more clear your argument is, the more credit you will get.

Calculating time since last common ancestor based on JalView's data.

Note that JalView gives the % divergence between two sequences, **not** the number of substitutions. Here is how to use the numbers that JalView puts on a tree to put dates on a phylogeny.

Example: tree of Human, American Alligator, Ailanthus silkmoth, and corn.

1) Construct the tree & click the JalView button. You'll get:



2) Have JalView calculate the average distance tree. You'll get:

(I have labeled the splits A, B, and C)

• Find the date of the last common ancestor of corn and the ailanthus silkmoth (split A).

a) The distance from ailanthus & corn is the sum of the lengths of all the branches between them.

12.94 + 6.36 + 19.30 = 38.6; this is the % divergence.

b) Convert the % divergence to number of substitutions. Multiply % divergence by # of amino acids

(from step 2). # substitutions = 0.386 x 114 = 44.0 substitutions.

c) Correct the number of substitutions by the formula:

$$\textit{corrected}\ \text{subs} = -100\left(\ln\left(1-\frac{\textit{raw substitutions}}{100}\right)\right)$$

$$\text{in this case: corrected subs} = -100\left(\ln\left(1-\tfrac{44}{100}\right)\right) = -100(\ln(0.56))$$

$$= -100(-0.5798) = 57.98 \text{ which rounds to } 58.0$$

d) Convert the corrected divergences to Ma. Use the rule that 1 substitution = 21Ma.

In this case: 58.0 x 21 = 1218 Ma ; round this to 1200Ma.

For example:

- Find the date of the last common ancestor of humans and the ailanthus silkmoth (split B).
    a) distance = 8.77 + 4.17 + 12.94 = 25.88;
    b) # of substitutions = 0.2588 x 114 = 29.5
    c) corrected # of substitutions = 34.95;
    d) 34.95 x 21 = 733.95 or roughly 750 Ma

- Find the date of the last common ancestor of humans and the alligators (split C).
    a) distance = 8.77 + 8.77 = 17.54;
    b) # of substitutions = 0.1754 x 114 = 20.0
    c) corrected # of substitutions = 22.31;
    d) 22.31 x 21 = 468.51 or roughly 450 Ma

⇒ Thus, the correctly-labeled tree would be:

1200 Ma 750Ma 450Ma now

Tables of Organisms:

• Sorted by **kingdom, phylum, etc.**

kingdom	**phylum**	**subgroup**	**name**
animal	annelida		earthworm
animal	chordata	?	California gray whale
animal	chordata	?	Schreibers's long-fingered bat
animal	chordata	mammals	Arabian camel
animal	chordata	mammals	chimpanzee
animal	chordata	mammals	cow
animal	chordata	mammals	dog
animal	chordata	mammals	donkey
animal	chordata	mammals	eastern gray kangaroo
animal	chordata	mammals	guinea pig
animal	chordata	mammals	hippopotamus
animal	chordata	mammals	horse
animal	chordata	mammals	human
animal	chordata	mammals	llama
animal	chordata	mammals	mouse
animal	chordata	mammals	pig
animal	chordata	mammals	rabbit
animal	chordata	mammals	rat
animal	chordata	mammals	rhesus macaque
animal	chordata	mammals	sheep
animal	chordata	mammals	southern elephant seal
animal	chordata	mammals	spider monkey
animal	chordata	mammals	zebra
animal	chordata		American alligator
animal	chordata		bullfrog
animal	chordata		carp
animal	chordata		chicken
animal	chordata		duck
animal	chordata		eastern diamondback rattlesnake
animal	chordata		emu (bird)
animal	chordata		king penguin
animal	chordata		ostrich
animal	chordata		Pacific lamprey
animal	chordata		pigeon
animal	chordata		Puget Sound dogfish
animal	chordata		skipjack tuna
animal	chordata		snapping turtle
animal	chordata		turkey
animal	chordata		western rattlesnake
animal	crustacea		monsoon river-prawn
animal	echinodermata		starfish
animal	hexapoda		ailanthus silkmoth
animal	hexapoda		fruit fly (*D. melanogaster*)

• Sorted by **kingdom, phylum, etc.** continued

kingdom	phylum	subgroup	name
animal	hexapoda		house fly (*Musca domestica*)
animal	hexapoda		desert locust
animal	hexapoda		flesh fly
animal	hexapoda		greenbottle fly
animal	hexapoda		honeybee
animal	hexapoda		horn fly
animal	hexapoda		Mediterranean fruit fly
animal	hexapoda		tobacco hornworm
animal	mollusca		brown garden snail
animal	nematoda		Nematode
bacteria	proteobacteria		Bacterium: Desulfovibrio vulgaris
fungi	ascomycota		Aspergillus nidulans
fungi	ascomycota		Aspergillus niger
fungi	ascomycota		Neurospora crassa
fungi	basidiomycota		smut fungus
plantae	angiospermae	dicot	castor bean
plantae	angiospermae	dicot	cauliflower
plantae	angiospermae	dicot	China jute
plantae	angiospermae	dicot	hemp
plantae	angiospermae	dicot	mung bean
plantae	angiospermae	dicot	nasturtium
plantae	angiospermae	dicot	oriental sesame
plantae	angiospermae	dicot	parsnip
plantae	angiospermae	dicot	potato
plantae	angiospermae	dicot	pumpkin
plantae	angiospermae	dicot	rape
plantae	angiospermae	dicot	sea-island cotton
plantae	angiospermae	dicot	spinach
plantae	angiospermae	dicot	sunflower
plantae	angiospermae	dicot	tomato
plantae	angiospermae	monocot	buckwheat
plantae	angiospermae	monocot	corn
plantae	angiospermae	monocot	leek
plantae	angiospermae	monocot	rice
plantae	angiospermae	monocot	wheat
plantae	Ginkgophyta		ginkgo
protoctista	chlorophyta		green alga
protoctista	ciliophora		Tetrahymena pyriformis
protoctista	discomitochondria		Crithidia fasciculata
protoctista	discomitochondria		Crithidia oncopelti
protoctista	discomitochondria		Euglena gracilis
protoctista	discomitochondria		Euglena viridis
?	?	?	love-in-a-mist
?	?	?	ramtil
?	?		cuckoopint

• Sorted by **name**:

kingdom	phylum	subgroup	name
animal	hexapoda		ailanthus silkmoth
animal	chordata		American alligator
animal	chordata	mammals	Arabian camel
fungi	ascomycota		Aspergillus nidulans
fungi	ascomycota		Aspergillus niger
bacteria	proteobacteria		Desulfovibrio vulgaris
animal	mollusca		brown garden snail
plantae	angiospermae	monocot	buckwheat
animal	chordata		bullfrog
animal	chordata	?	California gray whale
animal	chordata		carp
plantae	angiospermae	dicot	castor bean
plantae	angiospermae	dicot	cauliflower
animal	chordata		chicken
animal	chordata	mammals	chimpanzee
plantae	angiospermae	dicot	China jute
plantae	angiospermae	monocot	corn
animal	chordata	mammals	cow
protoctista	discomitochondria		Crithidia fasciculata
protoctista	discomitochondria		Crithidia oncopelti
?	?		cuckoopint
animal	hexapoda		desert locust
animal	chordata	mammals	dog
animal	chordata	mammals	donkey
animal	chordata		duck
animal	annelida		earthworm
animal	chordata		eastern diamondback rattlesnake
animal	chordata	mammals	eastern gray kangaroo
animal	chordata		emu (bird)
protoctista	discomitochondria		Euglena gracilis
protoctista	discomitochondria		Euglena viridis
animal	hexapoda		flesh fly
animal	hexapoda		fruit fly
plantae	Ginkgophyta		ginkgo
protoctista	chlorophyta		green alga
animal	hexapoda		greenbottle fly
animal	chordata	mammals	guinea pig
plantae	angiospermae	dicot	hemp
animal	chordata	mammals	hippopotamus
animal	hexapoda		honeybee
animal	hexapoda		horn fly
animal	chordata	mammals	horse
animal	hexapoda		housefly
animal	chordata	mammals	human
animal	chordata		king penguin

• Sorted by **name** continued:

kingdom	phylum	subgroup	name
plantae	angiospermae	monocot	leek
animal	chordata	mammals	llama
?	?	?	love-in-a-mist
animal	hexapoda		Mediterranean fruit fly
animal	crustacea		monsoon river-prawn
animal	chordata	mammals	mouse
plantae	angiospermae	dicot	mung bean
plantae	angiospermae	dicot	nasturtium
animal	nematoda		Nematode
fungi	ascomycota		Neurospora crassa
plantae	angiospermae	dicot	oriental sesame
animal	chordata		ostrich
animal	chordata		Pacific lamprey
plantae	angiospermae	dicot	parsnip
animal	chordata	mammals	pig
animal	chordata		pigeon
plantae	angiospermae	dicot	potato
animal	chordata		Puget Sound dogfish
plantae	angiospermae	dicot	pumpkin
animal	chordata	mammals	rabbit
?	?	?	ramtil
plantae	angiospermae	dicot	rape
animal	chordata	mammals	rat
animal	chordata	mammals	rhesus macaque
plantae	angiospermae	monocot	rice
animal	chordata	?	Schreibers's long-fingered bat
plantae	angiospermae	dicot	sea-island cotton
animal	chordata	mammals	sheep
animal	chordata		skipjack tuna
fungi	basidiomycota		smut fungus
animal	chordata		snapping turtle
animal	chordata	mammals	southern elephant seal
animal	chordata	mammals	spider monkey
plantae	angiospermae	dicot	spinach
animal	echinodermata		starfish
plantae	angiospermae	dicot	sunflower
protoctista	ciliophora		Tetrahymena pyriformis
animal	hexapoda		tobacco hornworm
plantae	angiospermae	dicot	tomato
animal	chordata		turkey
animal	chordata		western rattlesnake
plantae	angiospermae	monocot	wheat
animal	chordata	mammals	zebra

Name _____

Pre-Lab: Molecular Phylogeny

1) You will be looking at variation in the amino acid sequence of the protein cytochrome c. Look up cytochrome c in *Campbell*. In which biochemical pathway does cytochrome c participate? Briefly describe the role of cytochrome c in this pathway.

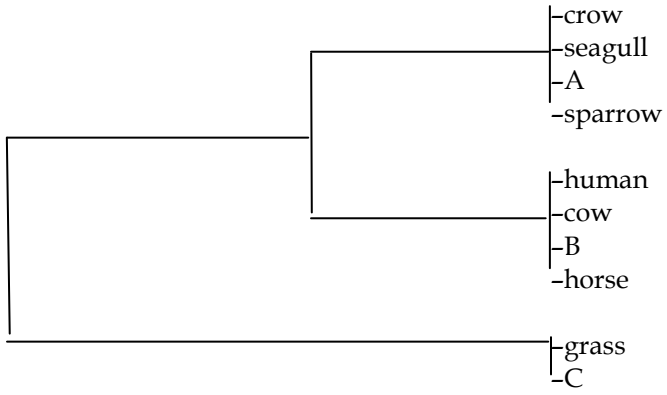
2) Given the following data for organisms alive today:

Pair	**# of differences**
A–B	20
B–C	20
A–C	4

Draw a phylogenetic tree relating organisms A, B, and C. Show the relative distances between organisms.

3) A flying squirrel is a mammal, not a bird. Based on this, where would you expect to find the flying squirrel on the following phylogenetic tree? Circle your answer.

position A position B position C



Phylogenetic Collection Lab

Objectives

- to connect the diversity of organisms described in class with the real world.
- to connect particular phyla of organisms with their characteristic habitats.
- to compare & contrast organisms within the different phyla.
- to show that most of "nature" that you usually see belongs to only a few phyla.
- to have you look at the world in a different way.

Assignment

Between now and your lab meeting during the week listed on the syllabus, each lab group (1 student minimum; 3 students **maximum**) must collect representatives of 12 different phyla. Groups may not share specimens.

Note that you must have collected your specimens **before** this lab meeting!!!

Specifically:

1. Because different sources disagree on the definitions of several phyla, we have created a set of web pages with the "Official Bio 112 Phylum List". Links to these pages can be found in the section of the On-Line Lab Manual for this lab. You can click on the name of each phylum to "Google" the name of that phylum; this will give you a set of links that can help you find samples of that phylum.

2. In lab during in the week of the lab, each group will present and discuss their collection.

3. In order to get credit for a particular phylum, you must bring in something that is clearly recognizable as a member of this phylum to show to your TA. It can be a whole organism or a piece of an organism, but it must be clearly recognizable as a member of that phylum. For example, a dog hair is clearly from a mammal (the only animals with hair) and since mammals are craniates, this is clearly a member of the phylum chordata. You can use a microscope to show your TA any microscopic samples.

4. **You** are responsible for defending the classification for your organism. If you have any doubt, check with your TA **in advance**. Bring any necessary supporting materials.

5. You can obtain samples from any source, including the supermarket, bait shop, florist, woods, etc.

6. In order to get credit, you must also specify where each of your samples came from. You must specify both geography (part of the world) and habitat. Note that, if you get your sample from other than its natural habitat (greenhouse, supermarket, etc.), you must specify where this organism originally came from. For example, if you include atlantic salmon that you got at Star Market, you'd have to say that it came from the north atlantic (geography) and from the open ocean (habitat).

7. Points for your collection will be given as follows (to a maximum of 60 points):
   – to count as a member of a phylum:
   - your TA must be able to recognize it as a member of that phylum
   - you must specify where it came from (geography & habitat)
   - you must have a name for it (common or genus/species)

Number of phyla	**Points**
1 – 4	4 points each
5 – 8	5 points each
9 – 12	6 points each

This is a group effort for a group grade. All group members will receive the same grade.

You must be prepared to *defend* your selections. That is, it is up to you to prove to your TA that a particular organism is what you say it is and that it belongs in the phylum you say it is.

Procedure

- You can get samples from anywhere. Some suggestions:
  – marshes near UMB – ethnic markets – supermarket
  – fish store – in your house – in your neighborhood
  – bait shop – off of the docks near UMB
  – the links on the course website for this lab can give other hints
  – a greenhouse (not the one at UMB, though)
- You can consult any sources you need (you will need to consult outside sources).
  – the library – your TA – Brian White
- the WWW (I have put relevant links in the OLLM for this lab)

- You will need to preserve some of your specimens. You can try freezing, drying, or putting them in a mixture of 2 parts rubbing alcohol (isopropanol) to 1 part water (keep this in a tightly closed container!) and storing at room temperature.

THIS WILL TAKE A LONG TIME; DON'T WAIT UNTIL THE LAST MINUTE!

In lab during the week listed on the syllabus:

- Each group should bring in their collection with the completed list as described below.
- Your TA will check off the various organisms and collect your lab reports for grading.
- Your TA will then go phylum by phylum and ask "does anyone have an …".
- The class will then discuss what they have found, where they found it, etc.

Phylogenetic Collection List:

- The list of your collection will be due in lab during the week listed on the syllabus in your regular lab section.
- It must conform to the following format **exactly**:

1) At the top, you should list your TA's name & section, and the names of all the group members.

2) A table, in the following format, with your organisms listed. You may use the one on the following pages:

						Where it lives	
TA checkoff	**Sample #**	**Phylum**	**Page**	**Name**	**Where you found it**	**Geography**	**Habitat**
leave blank	same as on sample container or label	From On-line Lab Manual website	page in *Campbell* that describes this phylum (if listed in *Campbell*)	common name or genus, species name	(beach, market, etc.	where on *earth* it lives	what kind of environ-ment it lives in

Important Note:

Not all of the categories of living things listed in *Campbell* are phyla. For example, on page 624 and 625, *Campbell* lists "cnidarians" - this is a phylum - and "anthozoans" - not a phylum. Therefore, if you brought in an anthozoan and a hydrozoan, they would only count as *one* phylum since they are both members of the *same phylum* (cnidaria).

Lab Report

Lab reports are due to your TA during the week listed on the syllabus at your regular lab time. Your lab report will be worth 40 points. You should choose one phylum of organisms that was represented in your classmates' collections (it need not be a phylum that you brought in, but it must have been brought in by somebody). In a report of no more than 1-1.5 double-spaced pages, answer the following questions about the specimens of your phyla that you and/or your classmates brought in. Your report may only deal with organisms that were presented by you or your classmates.

1. Which organisms are you talking about in your report (a minimum of 3) and to which phylum do they belong?
2. What is similar about these organisms? Give six similarities. Be specific about body plan, habitat, etc.
3. What is different about these organisms and how do differences in their habitat, food source, 'life style', etc. explain these differences? Give 3 differences.

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						Where it lives	
TA checkoff	**Sample #**	**Phylum**	**Page**	**Name**	**Where you found it**	**Geography**	**Habitat**
	1						
	2						
	3						
	4						
	5						
	6						

						Where it lives	
TA checkoff	Sample #	Phylum	Page	Name	Where you found it	Geography	Habitat
	7						
	8						
	9						
	10						
	11						
	12						

Microscope Warm-up

Objectives:

- To familiarize you with the use of the microscope in preparation for next week's lab.

Procedure

You should work in pairs.

Always treat the microscope with great care. Make certain that you do not touch any part of the lens system with anything abrasive (such as a slide or dirty water) or greasy (such as even the cleanest fingers). Never clean a lens with anything except clean lens paper! If the view gets foggy (as it probably will sometime during the semester), and lens paper will not clean it, call your laboratory instructor.

(1) Structure of the Compound Microscope

It is very important that you familiarize yourself with the parts of the microscope and their function. Your first task is to locate all of the parts named in the diagram on the next page. Place the microscope so that it is at right angles to you.

In addition to the stand (arm & base) and a movable stage by which the object can be positioned and focused for viewing, the microscope consists of the following sub-units:

A. The system involved in illuminating the object to be viewed, i.e., light, diaphragm and condenser.

B. The lens system - eyepiece, body tube and objective lens which magnify the object.

A. The System of Illumination. Keeping the microscope in the same position: (l) plug it in, (2) turn on the light and (3) move the diaphragm lever as far to the left as possible. Place a clean slide on the stage over the condenser and put a piece of white paper about 25 mm square on top of the slide. Now slide the condenser knob and move the condenser up and down while observing the light on the piece of paper (do not look through the microscope but continue to look at the paper with your naked eye). Note that you see a fairly intense small circle of light when the condenser is at its uppermost position and that this circle gets larger and more diffuse as one lowers the condenser. For most work with the l0X and 40X objectives it is best to have the condenser near the top of its travel.

Put your eye at table level and look up at the bottom of the condenser. Now move the diaphragm lever and observe what happens. This is an iris diaphragm. Why do you suppose it is called this? Look at the piece of paper again while opening and closing the diaphragm. The diaphragm serves to regulate the amount of light passing through the condenser. It also serves to cut down stray light. Later when you look through the microscope you will see that the diaphragm can be kept partly closed without cutting down on the light passing through the lens (i.e., only light beyond the field of the lens is being blocked). Further closing of the diaphragm will cause less light to enter the lens and decrease the resolving power of the lens while increasing contrast in the viewed object (Resolving power is how well specimen detail is preserved. Contrast is the ability to see particular detail against its background.) Control of the light entering the microscope is very important.

B. The Lens System. Light passes through the condenser, through the object which is placed on the slide and into the lens system. The lens system consists of: (l) an objective lens - the revolving nosepiece of your microscope has at least two of these, (2) a body tube - in your microscope the body tube has prisms in it to allow the tube to be inclined and (3) the eyepiece lens. Basically, the objective lens magnifies the object and forms an image in the tube which is further magnified by the eyepiece lens. (If you are skeptical about this, ask your instructor to demonstrate the image in the tube.) The objective lens is the most important (and most expensive) part of the microscope and the quality of a microscope is largely a question of the quality of its objective lens. The ones in your microscope are very good indeed and deserve care. The l0X objective (low power) has a working distance (the distance from lens to object when the object is in focus) of about 4 mm. The 40X objective (high power) has a working distance of about l mm.

While still looking at the microscope from the side, move the stage down well clear of the objective lenses by turning the coarse adjustment knob. Now rotate the nosepiece and notice that each lens clicks into the proper position. Move the l0X objective into position. Next move the stage up until the lens is about 4 mm from the slide. Notice while doing so that the knob you are turning is both a coarse and fine adjustment (most microscopes have separate knobs for these) and that extreme movement of the knob moves the stage rapidly, but immediately after you reverse the direction of movement, the stage moves almost imperceptibly for a short distance. This fine adjustment allows precise focusing.

II. Principles of Microscopy

A. Magnification Total magnification is roughly the product of the magnification of each lens (objective x eyepiece). With l0X objective and l0X eyepiece the magnification should be l00X. A microscope with a straight body tube is designed to project the image to the level of the bench surface. Your microscope, since it has an inclined tube, projects the image at an angle to the bench. The magnification refers to the increase in size of this apparent image over the object on the slide. Put the microscope on low power (3.5 X or l0X objective).

B. Depth of Field The compound microscope has a very limited depth of field. It is necessary to continually focus up and down to get an impression of depth. Make a slide using colored threads which cross over each other, i.e., put a red thread on the slide and a blue cross over it to form an X. Add water and a cover slip and observe. Notice that you can focus clearly on only one of the threads at the point where they cross and must focus up or down to get the other clearly in view. This is especially noticeable under high power. To get an impression of depth with the microscope, one continually focuses up and down and takes optical sections through the object.

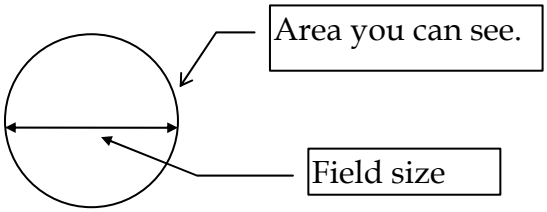
Now turn the microscope around so that you can look through the eyepiece. We are now ready to look at something. You should practice with one of the prepared slides in the lab.

POINTS TO REMEMBER: The following are worth remembering as you use the microscope.

1. Be sure that you are using the condenser and diaphragm correctly.
2. Do all preliminary focusing under low power.
3. Do not move the stage upward when first getting the object in focus (i.e. beware of smashing slide and lens together).
4. Try to use the microscope with both eyes open - it will seem hard at first, but is easier in the long run.
5. Use the fine adjustment constantly to keep things in focus.
6. Use lens paper to clean the lenses occasionally, you will find that the microscope works best when clean.

How big is it?

You can use the microscope to measure the approximate size of the objects you are looking at. Given the magnification, the table below gives the diameter of the field of view. See diagram:



Once you know that, you can estimate the size of what you're seeing. If the field size is 450µm and the thing you're looking at it half as wide as the field, then it's about 220µm wide. For the microscopes we use:

Magnification shown on objective lens	Actual magnification		Field size (millimeters)	Field size (microns (µm))
3.5x	35x	⇒	5.1	5100

10x	100x	$\Rightarrow$	1.8	1800
40x	400x	$\Rightarrow$	0.45	450

Part II: c-Ferns part I

Objectives

To observe the phenomenon of alternation of generations and its genetic concequences. To follow the life cycle of plant through a complete cycle. Today, we will observe gametophytes and sperm release. In two weeks, we will observe the resulting sporophytes and do a genetic analysis.

Introduction

Variations on the pattern of alternation of generations are an important part of the diversity among living things. It varies extensively – see figure 13.6 in *Campbell* for a general description; figure 29.5 shows it for a generalized plant. The life-cycle of a c-fern can be found on page PlantDiv-8.

You will be following the development of a fern *Ceratopteris richardii* – "c-fern" for short. It is a tropical homosporous (*Campbell* page 586) fern. Its life cycle is like that of most ferns (figure 29.12) with one exception: figure 29.20 shows a hermaphroditic gametophyte – the gametophytes have both male (antheridia that make sperm) and female (archaegonia that make eggs) parts; c-ferns have hermaphroditic gametophytes which are "heart-shaped" as in fig. 29.12 as well as male gametophytes (which have only antheridia).

The strain of c-ferns you will be observing also has a mutation:

Allele	**Contribution to phenotype**
normal: D	normal distribution of chloroplasts – dominant phenotype
mutant: d	chloroplasts clumped ("polka-dot") – recessive phenotype

Some pictures are show below:

Polka-dot:
notice the
spotty green
color

Normal:
notice the even
green color.

This phenotype is visible in both haploid and diploid forms of the fern (except the spores, eggs, and sperm). As a result:

For haploids:	**Genotype**	**Phenotype**
(gametophytes)	D	normal
	d	polka-dot

For diploids:	**Genotype**	**Phenotype**
(sporophytes)	DD	normal
	Dd	normal
	dd	polka-dot

Two weeks ago, we sowed spores produced by a Dd sporophyte onto the petri dishes you have. The gametophytes have grown up by now and are ripe for fertilization (not in the sense of fertilizer, but in the sense of egg and sperm). When you add water, the sperm will be released from the antheridia and will swim towards the archaegonia to fertilize the eggs there. At this stage, it *may* be possible to tell normal from polka-dot, but in a further two weeks the differences will be more plain.

Procedure

(1) Get a plate of gametophytes from your TA. Label it (on the lid) with your lab section, TA and names.

(2) Using a toothpick, scoop up 2 or 3 gametophytes; put each on a separate slide with a coverslip. Try to get a gametophyte of each type – the males are smaller than the hermaphrodites.

(3) Draw one gametophyte of each type. Label the diagrams with their approximate sizes as well as hermaphrodite vs. male. Also label the rhizoids.

(4) One at a time, add water to one of the male and one of the hermaphrodite gametophytes and observe the release of sperm.

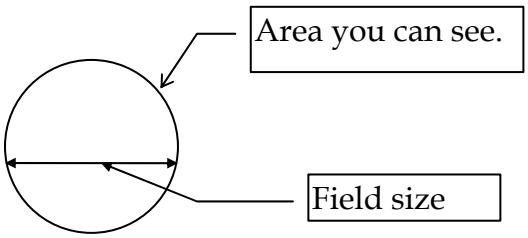
(5) Try to tell if the hermaphrodite gametophytes are normal or polka-dot. Mark the bottom of the plate under the gametophyte with a + for normal and a p for polka-dot.

(6) Flood the plate with about 1ml of water and swirl it so that the entire surface is wet. Give it to your TA and he or she will incubate it for another two weeks when we will look at it again.

Be sure to get drawings of male and hermaphrodite gametophytes with sizes - you will need this for your plant diversity lab report.

Magnification and Size

This handout should help you to figure out the sizes of the things you're looking at. For each level of magnification, it gives the "field size" - that is, the width of the are you can see. See below:



Once you know that, you can estimate the size of what you're seeing. If the field size is 1 inch and the thing you're looking at it half as wide as the field, then it's 1/2 inch wide. See back for an example.

There are two kinds of microscopes used in the lab:

(1) DISSECTING MICROSCOPE - the ones with two eyepieces made by Leica (Leica Zoom 2000). These are good for looking at big things. You adjust the magnification by turning the knob on the part you look into.

Magnification shown on knob	Actual magnification		Field size (inches)	Field size (millimeters)
10.5x	10.5x	⇒	0.83	21
20x	20x	⇒	0.43	11
30x	30x	⇒	0.28	7
40x	40x	⇒	0.22	5.5

(2) COMPOUND MICROSCOPE - the black ones with one eyepiece made by Leitz. These are good for looking at small things. You adjust the magnification by changing the objective lens. Be sure it clicks solidly when changing magnification or you won't see anything.

Magnification shown on objective lens	Actual magnification		Field size (millimeters)	Field size (microns (μm))
3.5x	35x	⇒	5.1	5100
10x	100x	⇒	1.8	1800
40x	400x	⇒	0.45	450

The following tips were prepared by Erin Williams in the Spring of 1999 as a make-up for missing this lab.

Tips to Get the Best Use of Your Microscope

1. While obtaining a microscope, carry with one hand around the arm and the other hand under the base.
2. Plug in the microscope and turn on power using the power switch located on the light source.
3. Make sure the lamp is burning.
4. With lens paper clean the eyepiece and objectives. Turn the objective until it clicks to the lowest power.
5. Obtain a clean slide and coverslip.
6. Pipet 2 to 3 drops of liquid specimen or use 1cm^2 of dry specimen onto the slide. •When attempting to obtain fast-moving specimens: On a clean slide make a ring about 1/2 inch in diameter of "Proto-slow". Drop a drop of the specimen in the middle of the ring. Put a coverslip over the specimen.

   •How to drop the coverslip: 1) _____•_____; 2) _____•_____; 3) Let the coverslip drop slowly, it will result in fewer bubbles under the coverslip.
7. Place the slide onto the stage.
8. Secure the slide with the stage clips.
9. Look through the eyepiece with both eyes open.
10. Move the slide back and forth in Low power until your specimen goes by the center.
11. Use the Image Focusing knob to bring the specimen into focus, while in Low power. Always focus so that the stage moves down.
12. Then go to High power.
13. Use the Condenser Diaphragm Adjustment lever to control the light flow and improve the contrast.
14. When done viewing lower the stage downward with the Image Focusing knob completely and put objectives into lowest power.
15. Remove the slide and dispose of the specimen properly, and clean the slide.
16. Clean the lens with the lens paper.
17. Turn power off; unplug the microscope and return to proper place.

Other Tips:

- Don't move the microscope around after you start to view.
- Control the amount of light entering the microscope – the contrast at which you view the specimen will control how much detail you view.
- Keep the lens clean. A clean microscope will work better.
- Keep an eye on the stage while using the Image Focussing Knob so that you don't put the objective through the slide.
- Use a clean pipet and slide for each specimen. This will cut down on contamination.
- Become familiar with how the microscope works before trying to view a specimen.
- To help with finding dust: turn the eyepiece, if particles move, then there is dust on the eyepiece that needs to be cleaned. Repeat this process for each Objective lens, Condenser Diaphragm Lever, and Image and Condenser Focussing Knobs.

Name_____

Pre-Lab: Skulls & Evolution

1) Figures 8.74, 8.75, and 8.76 in the Lab Atlas show a cat skull; figures 9.12, 9.13, 9.14, and 9.15 show a human skull. Based on these figures and the chart on page Skulls-5, answer the following questions.

a) Briefly compare and contrast the size and shape of the canine teeth of humans and cats.

b) Briefly compare and contrast the size and shape of the molars of humans and cats.

Name_____

Skulls & Evolution

Purpose

- To illustrate trends in the evolution of humans.
- To demonstrate what you can learn from bones & fossils.
- To show the adaptations of various mammals to different habitiats and food sources.

Introduction

Much of what we know about evolution comes from the study of comparative anatomy. In many cases, bones (either as fossils or skeletons) have beed useful in these studies. Bone and skeletal structures can reveal how an animal moves, eats, reproduces, etc.

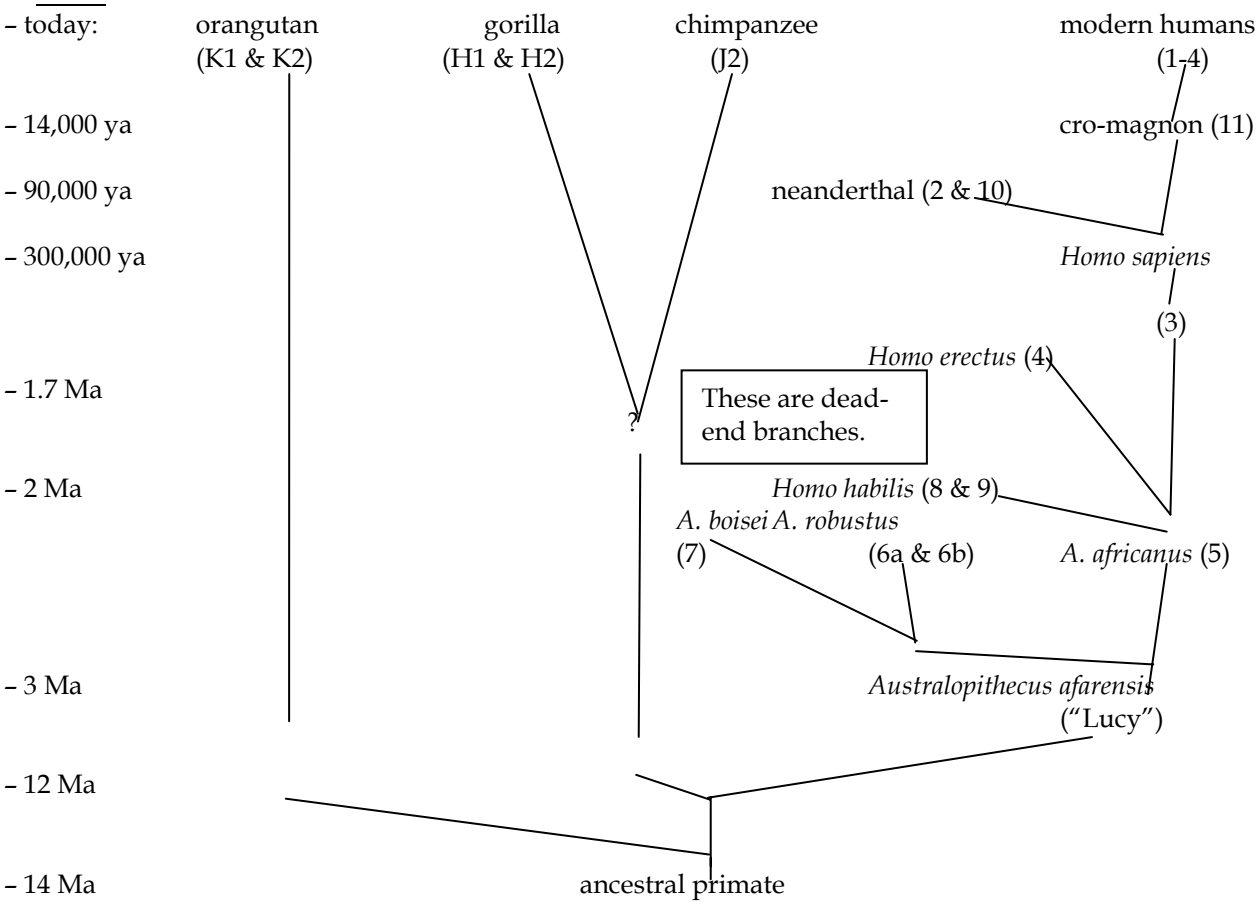
In this lab, we will look at the skulls of various mammals.

Procedure

In this lab, groups at the same table will work together.

Part I: Human Evolution

Shown below is a *very rough* outline of human evolution. While the general form is agreed on my most scientists, many of the details (exact dates & branching patterns) are still subjects of debate. The numbers in parentheses correspond to the labels on the skulls. Although gorilla, chimp, and orangutan are modern primates (and therefore have been evolving as long as humans have) they are thought to resemble ancestral forms.



From the comparison of skulls from different primates, eight (somewhat overlapping) trends in the evolution of humans have been found. Note that not all traits in a given skull will be equally 'human' – that is, you will likely find skulls where one feature is ancestral and others are modern.

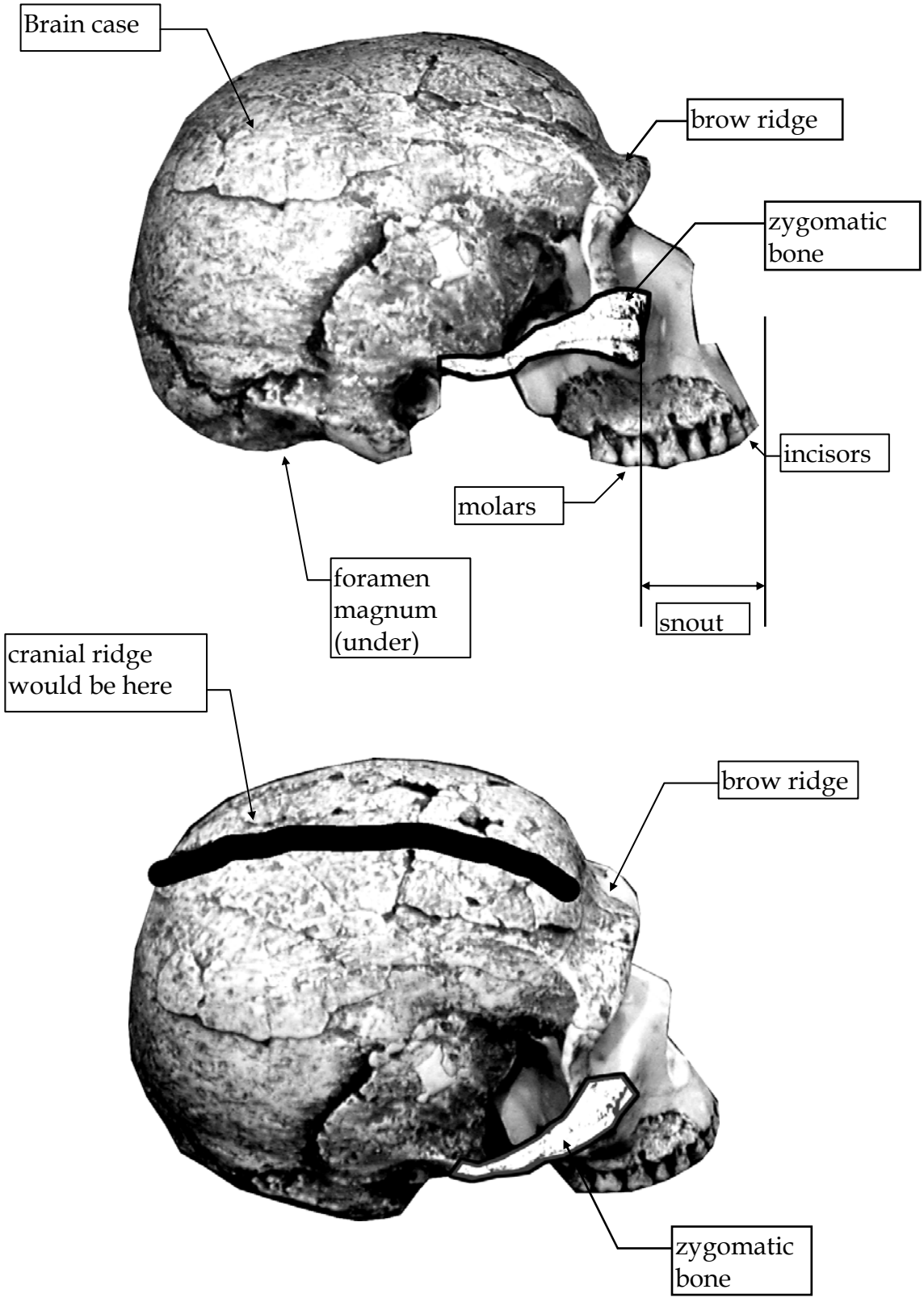
This chart describes these eight trends. The following pages illustrate the skull features described in the table.

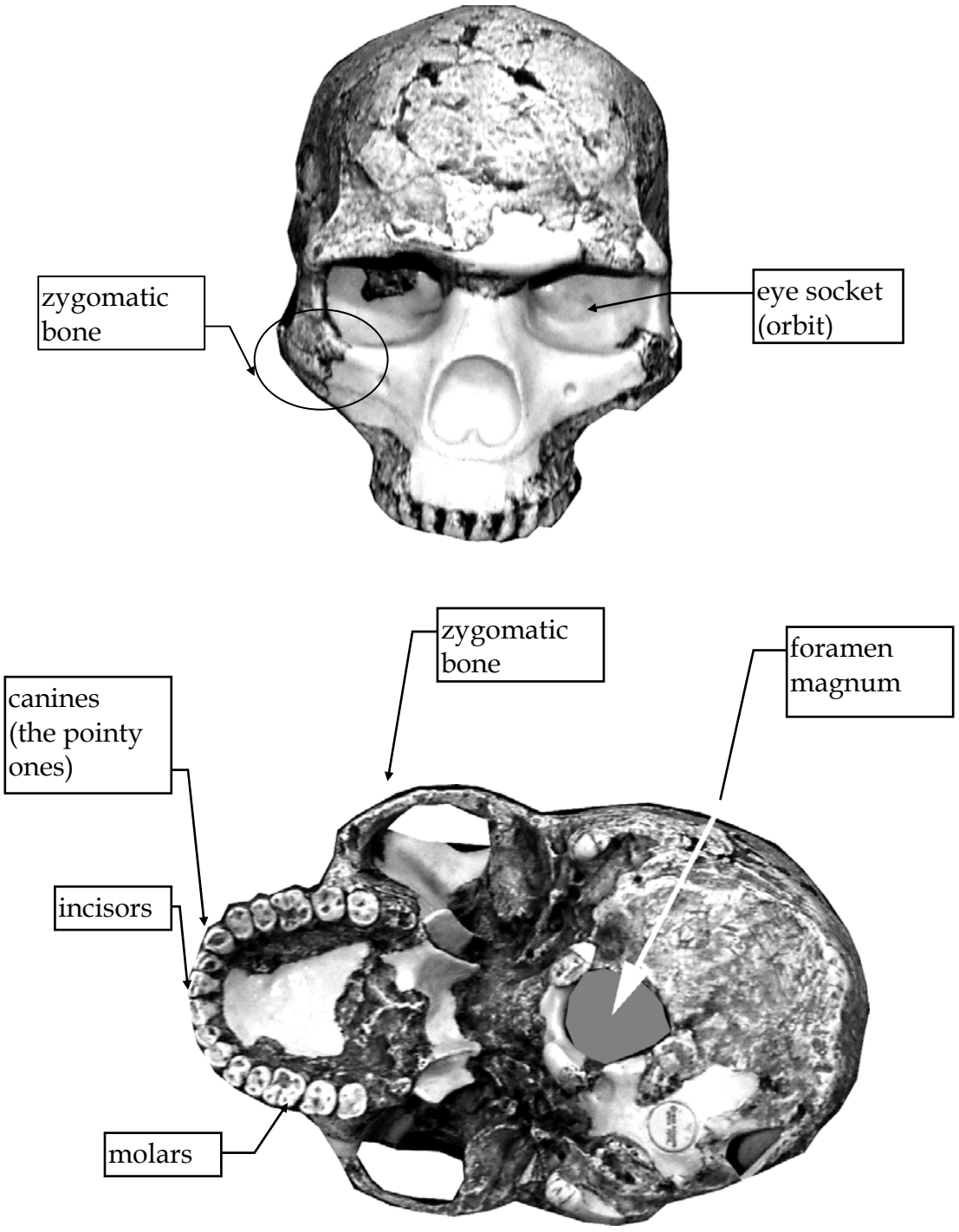
	Feature	**Details**	**Explanation**
1	Brain case	— size? — cranial ridge? — brow ridge?	The bigger brain case allows a bigger brain which, in general, allows greater intelligence.
2	Teeth	— size? — canines - large and sharp or more like incisors?	See under "Snout"
3	Palate	— U-shaped or parabolic	See under "Snout"
4	Forehead (compared to face)	— size? — height?	Related to size of brain case.
5	Location of eye sockets (orbits)	— sides/front of skull	Eyes in front allows binocular vision (seeing most objects with both eyes at once) which allows depth perception and 3-d vision.
6	Snout	— present? — length?	A reduced snout moves the molars under the rest of the skull which allows more flexibility in chewing and grinding food. This allows a more varied diet. The snout also blocks vision below the face.
7	Cheekbones (zygomatic bones)	— width of face	Wider face correlates with shorter snout.
8	Foramen magnum (where the backbone attaches)	— location - rear or bottom of skull?	Foramen magnum at bottom of skull allows walking erect, as opposed to walking on 4 legs.

You can also determine if an animal is carnivorous, herbivorous, or omnivorous (eats both meat and plants) by looking at its molars. In general (there are, of course, exceptions), blade-like molars are characteristic of carnivores and are used to shear the

meat into smaller pieces for digestion. Flat molars are characteristic of herbivores and are used to grind the plant material for digestion. The molars of omnivores (like humans) are intermediate.

Here are the parts of the skull that are important for this lab: (clearer color pictures **of a different species** can be found on pages 229-230 of the Lab Atlas as a reference point).





The palate is the lower jaw, which is not present in this skull. However, you can infer the shape of the palate by looking at the shape of the upper jaw. In this case, it is rather U-shaped.

1) Each group will be given several skulls of primates. Using the chart on the first page of this lab section, put your skulls in order from ancestral primate to modern human. Note that the orangutan, gorilla, and chimp are considered to be more ancestral than any of the other samples; the orangutan is the most ancestral, followed by the gorilla, then the chimp.

2) For each property listed in the table, determine how that property changes as you go from ancestral primates to modern humans. You should discuss this as a class.

3) To the best of your ability, try to determine when, on the chart on the first page of this lab section, humans first walked upright.

Part II: Comparing skulls of other mammals

4) Each group will be given four skulls, two from carnivores (exclusively meat-eating: leopard, cougar, wolf, great dane), one from an omnivore (eats both plants and meat: raccoon), and one from a herbivore (exclusively plant-eating: deer or sheep). The skulls will be marked with the animal they came from.

5) Consider the following features and determine the trends in these features as you go from carnivore to omnivore to herbivore.

	Feature	**Details**	**Explanation**
1	Canine teeth	• present? • large or small	Used for cutting and tearing of food.
2	Molars	• flat cross-section • pointed & blade-like	Used for grinding food.
3	Eye Sockets (orbits)	• allow for overlapping fields of vision? • allow for greater visual field coverage	Overlapping fields of vision allow for better depth perception; more visual field allows better observation.
4	Masseter muscle attachment points (see next page for description)	• large • small	Used for moving jaws when grinding food.
5	Temporalis muscle attachment points (see next page for description)	• large • small	Used for moving jaws when biting and tearing food.

Masseter & Temoporalis Muscles

These muscles are found in all mammals (although they are less clear in primates). They are different sizes and have slightly different attachment points depending on the animals diet, etc. The figure below shows the difference between the two muscles on the skull of a badger (carnivore). The figure was taken from *Skulls and Bones* by Glenn Searfoss, an excellent and very readable book on this subject.

Masseter muscle. One end of this muscle attaches at the rear of the lower jaw (mandible) and the other attaches to the zygomatic bone. This muscle is used to bring the molars together in grinding motions. The attachment points are not always as obvious for the masseter as they are for the temporalis.

Temporalis muscle. One end of this muscle attaches at the rear of the lower jaw (mandible), the muscle passes between the zygomatic bone and the rest of the skull, and the other end attaches to the temples, the top of the skull, or the cranial ridge (if present). In some cases, there is a 'tab' of bone on the mandible that fits between the zygomatic bone and the rest of the skull; the temporalis muscle attaches here. You can feel your temporalis muscle working if you put your finger on your temple as you chew something,

6) Each lab room will have at least one bottle-nosed dolphin skull. The dolphin is a marine mammal – that is, it lives in the ocean but has evolved from a land-dwelling mammalian ancestor. Compare the skull of the dolphin with that of the carnivore.

Part III: Marine Mammals III

You answered these two questions in the HMNH lab based on skeletal and morphological data and again in the Molecular Phylogeny Lab. In this part, you will use the skulls of relevant animals to look at the same questions.

You should use the techniques for looking at skulls and the features you have seen in the other skulls as you answer these questions.

We have provided you with the following skulls that may be useful in answering these questions:

Marine Mammals	Terrestrial Mammals
Dolphin	Sheep
Gray Seal	Dog/Wolf
Harp Seal	Raccoon
River Otter	Leopard
Sea Otter	Human

a) How many major different groups of marine mammals are there? A full-credit answer to this question consists of three parts:
   - The number of groups of marine mammals that you have determined. Note that, since we do not have and manatee/dugong skulls, this number may be less than the number you gave in the two previous labs.
   - An explanation of why you chose the groups that you chose. We are not interested in the "right" answer here; just a well-reasoned argument based on your observations. What are the key differences between groups? What are the key features that make members of each group similar?
   - Which of the marine mammals belong to each group? Your answer should include at least two members of each group.

b) Which is the closest living land relative of a seal? Seals evolved from land-dwelling ancestors. Although that ancestor is now extinct, it has modern-day descendants. Based on a phylogenetic tree that you construct, you must decide which order of land mammals this ancestor came from.
   A full-credit answer to this question has two parts:
   - The terrestrial mammal that you think is most closely-related to the land ancestor of seals. Choose from the list of terrestrial mammals above.
   - An explanation of why you chose that order. Again, we are not interested in the "right" answer; just a well-reasoned argument based on your observations.

In each part, we are not interested in the correct answer; we are interested in the *data* you cite and your *argument* based on that data. The more specific about the data you are and the more clear your argument is, the more credit you will get.

Lab report:

- Must be typed; handwritten reports will not be accepted. Hand-drawn and labeled drawings are fine.
- Due at the start of the lab session you are currently in during the week listed on the syllabus. This is a firm deadline.
- Although you will perform these activities as a group, each member of the group must turn in an individual lab report. Each person's report must be in his or her own words as much as possible.
- Your lab report must contain answers to the following questions.

Part I: Human Evolution

1) Describe how each of the eight properties changes as you go from ancestral primates to modern humans using specific details listed in the table on page Skulls-2. Describe the *trend*, not just the individual observations. Please format your answer as a table.

2) At which stage in human evolution did hominids first walk upright; explain your reasoning.

Part II: Comparisions of other mammals

3) Describe how each of the five properties changes as you go from carnivore to omnivore to herbivore. For each property, briefly explain how this change fits in with the animals' changed diet.

4) On the pictures of the dolphin skulls on the next pages, label the following parts:

- blowhole
- eye sockets (or where the eyes would be)
- zygomatic bone
- foramen magnum

– If a part appears in more than one picture, you need only label the one where it is shown most clearly.

– Attach the labeled pages to your lab report.

5) To which part of a terrestrial mammal skull does the blowhole of a dolphin correspond?

6) Looking at the teeth of the dolphin, which is more likely: (explain your reasoning)

– dolphins grind up their food like a herbivore

– dolphins bite off pieces of food and chew them up like humans

– dolphins grab and kill their prey with their teeth and swallow them whole or in large pieces

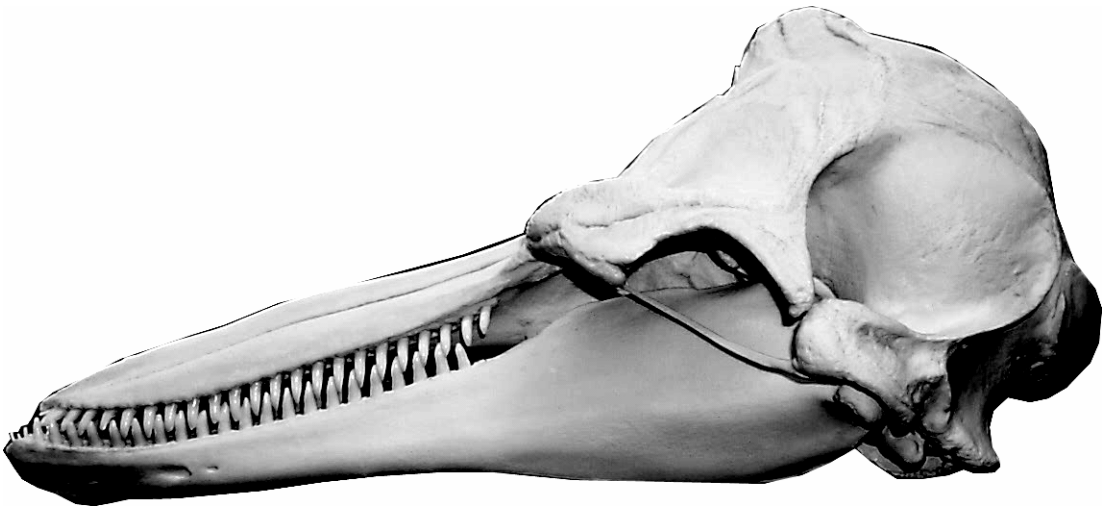
Part III: Marine Mammals III

7) The answers to questions (a) and (b) from page Skulls-7.

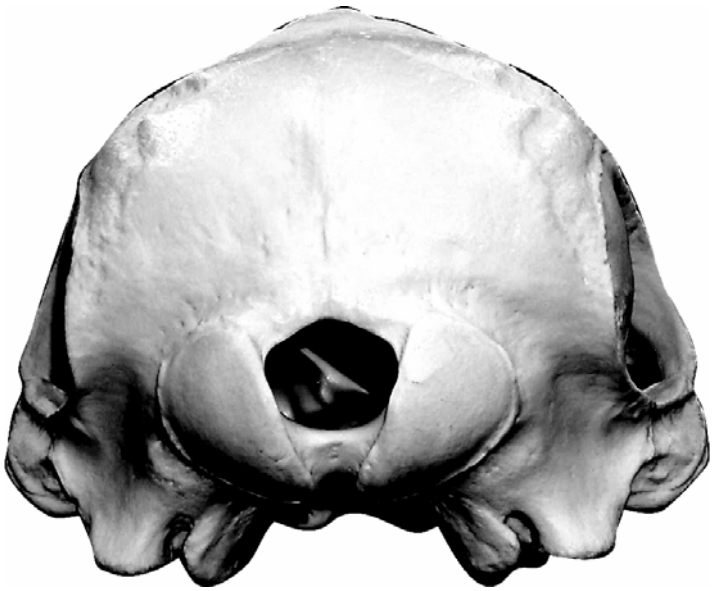
Dolphin worksheet (attach to your lab report)

An intact dolphin; left side view.

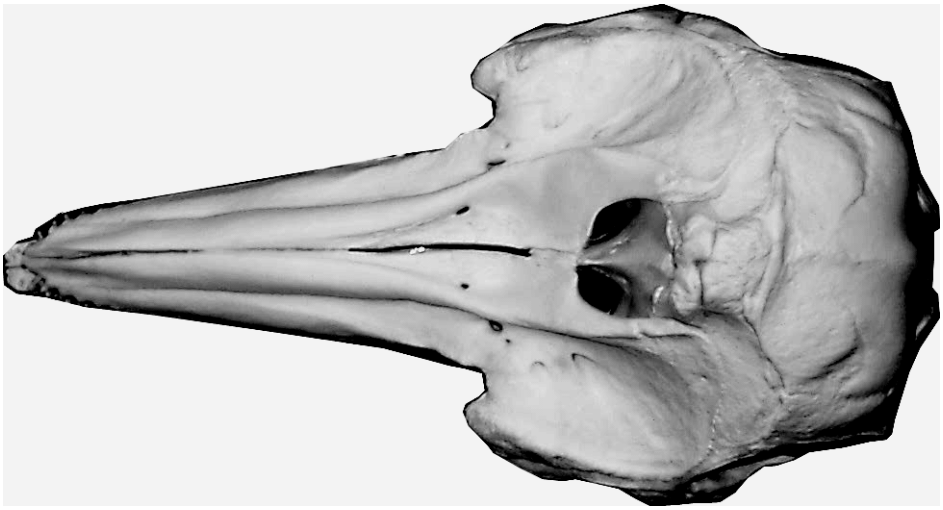
- Dolphin skull; left side view:



Rear view:



Top (dorsal) view:



Name _____

Pre-Lab: Plant Diversity

1) For each of the following, indicate whether it is a sporophyte or a gametophyte.

a) The antheridium in *Campbell* figure 29.5.

b) The archaegonium in *Campbell* figure 29.5.

c) The big green plant above the caption "FERNS" in *Campbell* figure 29.14.

d) The trunk of the Sequoia tree in *Campbell* figure 30.4.

e) The wrinkly outside shell of a peanut.

f) A blade of grass.

g) A pine needle.

2) Give an example of a gametophyte you can see without a microscope.

Plant Diversity

Objective

To observe and analyze the diversity of plants by looking at 4 major land plant groups.

Procedure

You will have 3 lab periods to complete these exercises.

Part I: c-fern Embryos

First lab session: Look at the c-ferns growing in the petri dishes you fertilized two weeks ago. You should be able to see small sporophytes growing out of the gametophytes. You may be able to tell "polka-dot" from normal (see the first c-fern lab in the Protozoa lab section); note the phenotype of the sporophyte and gametophyte as best you can.

Part II: Plant Diversity

First and second lab sessions: Look at the plants and microscope slides in the lab. Draw what you see, using the textbook as a guide. See under Lab Report for the pictures you must have.

Part III: Flower Dissection

Second session: Bring in a flower and dissect it. Draw the parts you can find and compare it to the flower in figure 30.7 of *Campbell*, pages 126 - 130 of the Lab Atlas, or the ones you can find in the on-line lab manual. See under Lab Report for the pictures you must have.

Part IV: Life-cycle Discussion

Third lab session: Complete the following table with brief descriptions, etc. as appropriate:

	S-phyte	spore	male G-phyte	female or hermaph. G-phyte	gametes	fertiliz-ation	zygote	seed
N/2N?								
# of cells								
liverwort								
moss								
fern								
pine								
angiosperm								

Part V: Greenhouse

Third Lab session: Go to the greenhouse with your lab section and answer the questions listed under Lab report.

Other resources:

Life cycle diagrams for selected plants can be found at the end of this section.

In the OLLM under Lab06, there are a set of links to Botany and Plant Diversity sites that you may find useful for reference.

Chapter 6 of the Lab Atlas contains very useful photos for this material; you should certainly bring it to lab.

Lab report

- Must be typed; handwritten reports will not be accepted. Hand-drawn and labeled drawings are fine.
- Due next week at the start of the lab session you are currently in. This is a firm deadline.
- Although you will perform these activities as a group, each member of the group must turn in an individual lab report. Each person's report must be in his or her own words as much as possible.
- Your lab report must contain:

(1) Labeled drawings with sizes indicated on each.

Your pictures should also indicate any features in {braces} in the table below: (macroscopic - how it looks to the naked eye; microscopic = how it looks in the microscope)

Type of Plant	Gametophyte	Sporophyte
Liverwort	*macro *micro {no vascular bundles}	*micro
Moss	*macro *micro {no vascular bundles}	*macro *micro {spores in capsule}
Fern	*micro {male and hermaphrodite forms}	*micro {vasculature} {spores in "sori"} *macro
Pine	*micro of both megagametophyte (in ovule) and microgametophyte (pollen) *micro showing pollen in male "cone"	*macro of female cone
Angiosperm	*micro of both megagametophyte (in ovule) and microgametophyte (pollen)	*micro of leaf cross section {vasculature}

– You must also have a sketch of each of the 5 types of plant. On these pictures, draw arrows to indicate where each of the things in the table above can be found. For example, if you were doing this with a human, and we asked for a drawing of brain cells, you would:

– You must also have drawings of the peanut and pine nut with the following labeled:

- embryo
- seed coat (if present)
- endosperm or cotyledons

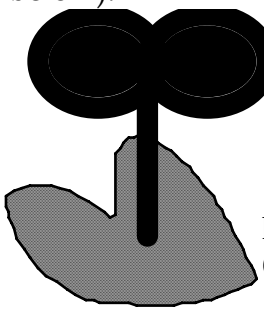
– Drawing(s) of the flower you brought in. Label all parts that you can find. Include the name of the plant and the size.

– A completed copy of the table on the previous page.

– Answers to the following questions:

c-ferns:

(2) Suppose you found a normal sporophyte growing out of a polka-dot hermaphrodite gametophyte (shown below).



a) What is the genotype of the hermaphrodite gametophyte?
b) What is the genotype of the sporophyte?
c) What will be the genotypes of the spores produced by this sporophyte and in what ratio will they be produced?
d) Could you find a polka-dot sporophyte growing out of a normal gametophyte? Why or why not?

Greenhouse Questions

(3) Leaves are not the only photosynthetic organs of plants. What other kind of photosynthetic structure have you seen in a greenhouse plant? Give two examples with genus and species names.

(4) What plants do you find in the greenhouse that are specialized for defense against herbivores and what adaptations do they exhibit? Give two different examples with genus and species names.

(5) All plants require mineral nutrients (nitrogen, phosphorus, potassium, etc.). Terrestrial and epiphytic plants obtain these in different ways. How do these plants differ in the way they get their nutrients? Give examples of each type found in the greenhouse.

(6) Give two examples, with genus and species names, of plants found in the greenhouse that you might also find in the supermarket in one form or another.

(7) The middle greenhouse contains samples of psilotum and selaginella. What phyla of plants do these represent? You may need to look these up in *Campbell*.

(8) In the greenhouses, there are several plants which are part of the *Lamiastraceae* or mint family. Surprisingly, these all look and smell very different. What can you observe that is the same in all these plants?

(9) In the greenhouse, you will find the following plants:

onion oleander sweet potato ginger

Which of these are monocots and which are dicots? Why?

(10) In the greenhouse are several succulent plants. What do they have in common? Is this an example of convergent evolution? Why/why not? How are these advantageous in dry climates?

Preparation for Exam II

Students often have trouble writing full-credit answers to the "give three differences between X and Y" questions that appear on the plant exam. The following are full-credit and partial-credit answers taken from actual exams and student responses. You should discuss these as a class to help you to understand how to write full-credit answers to these kinds of questions.

1) Give one major difference between the male gametophyte of moss and angiosperm.

Full-credit answer:
a) "The male gametophyte of angiosperm is a grain of pollen that has 4 cells whereas the make gametophyte of a moss has many cells."

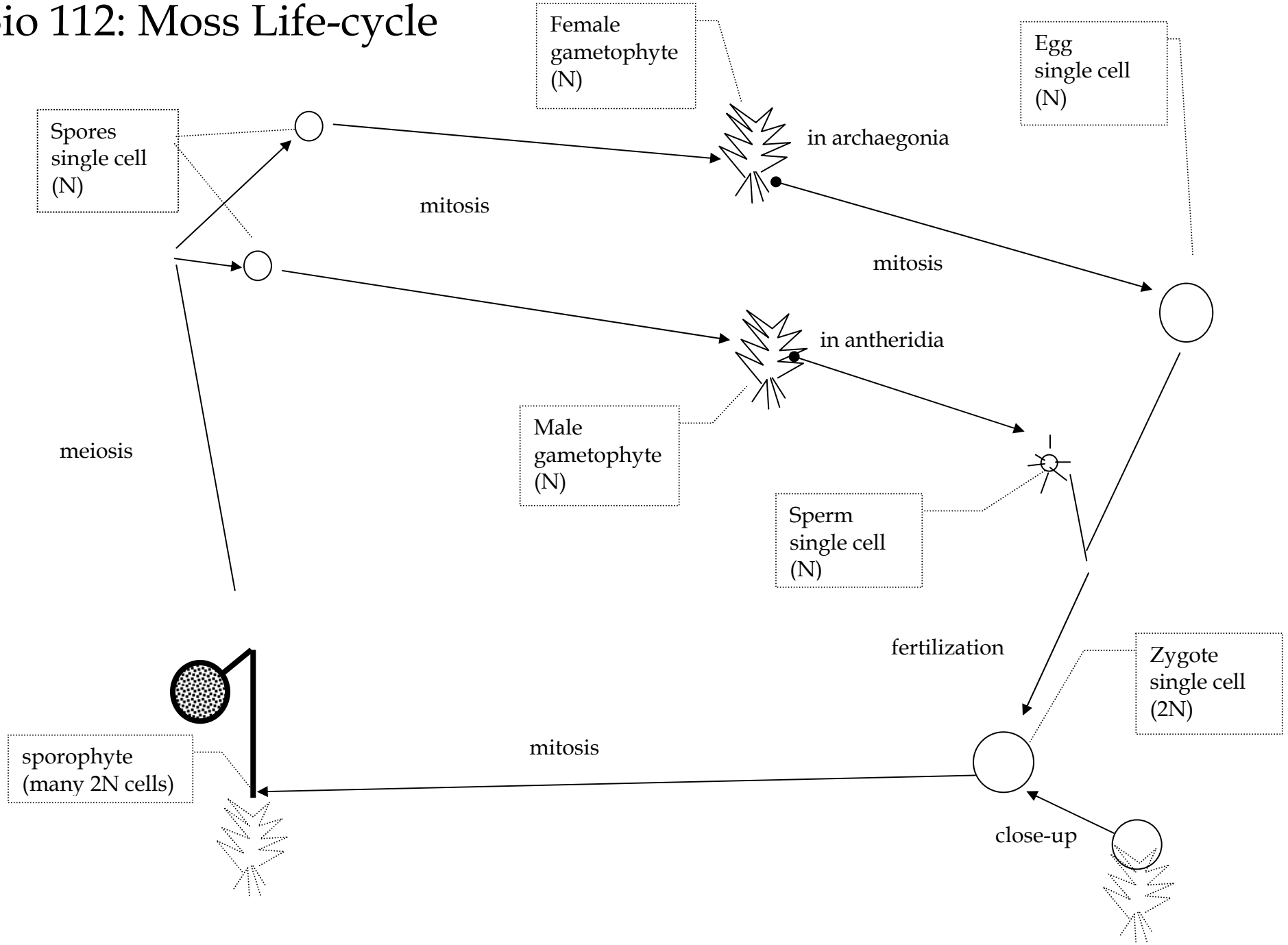
Answers that received no credit:
b) "Male gametophytes are haploid. The predominant form of angiosperm is diploid."
c) "Moss - most ancestral type of land plant, little or no vascular system therefore hard to transport H_2O/minerals throughout plant. Angiosperm: pollen made in anther an megaspore in ovary."
d) "The moss needs H_2O for fertilization; angio can use wind".

2) Give three major differences between an angiosperm seed and a moss spore.

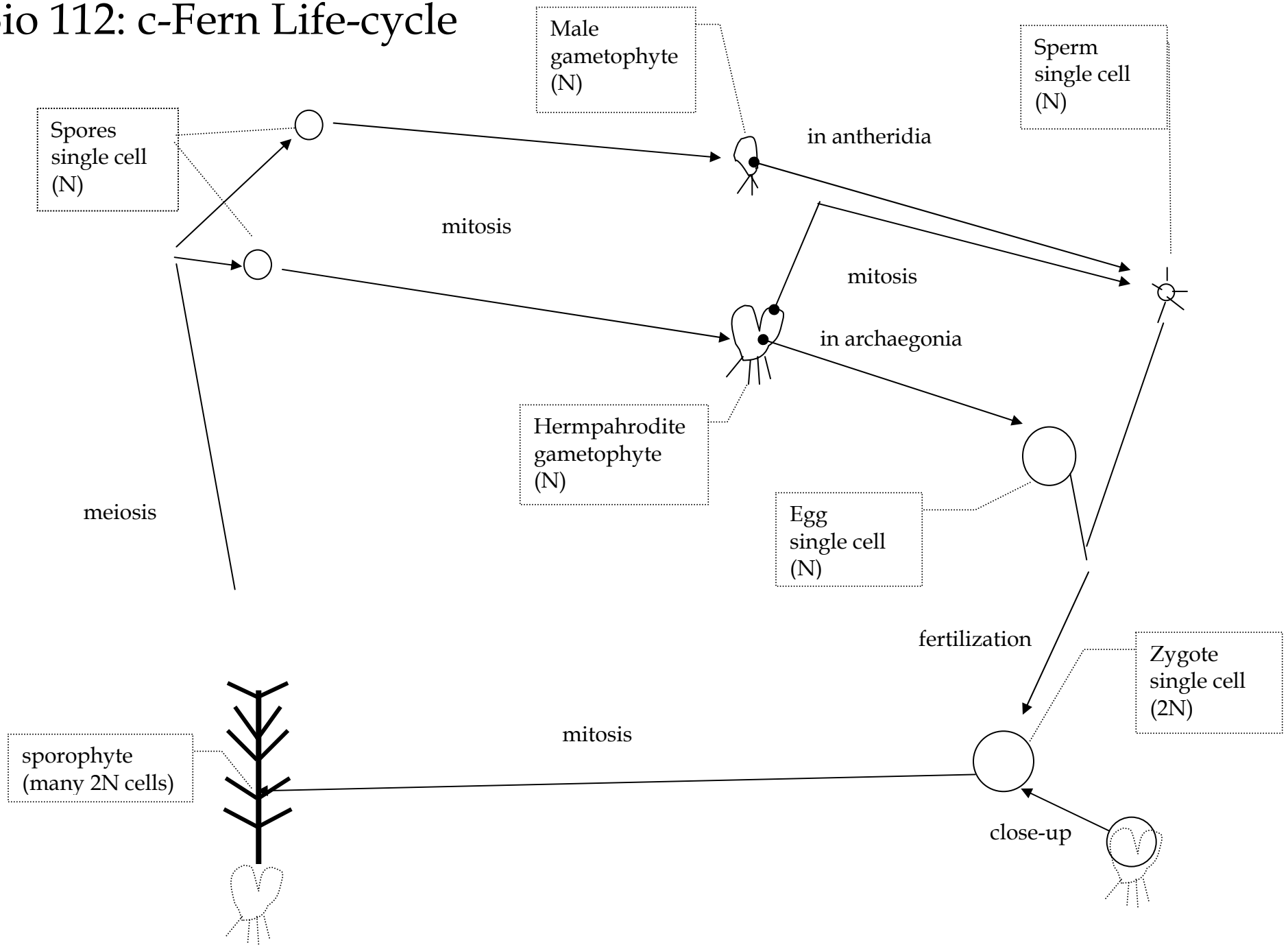
Full-credit answer:
a) "Angiosperm seed is diploid and moss spore is haploid."

Answers that received no credit:
c) "Moss spore needs H_2O for fertilization and angiosperm seed doesn't need H_2O for fertilization."
d) "Angiosperm seed: diploid immature sporophyte. Moss spore: are haploid gametophyte."
e) "Seed is made up of sporophyte and gametophyte but spore made up of sporophyte only."

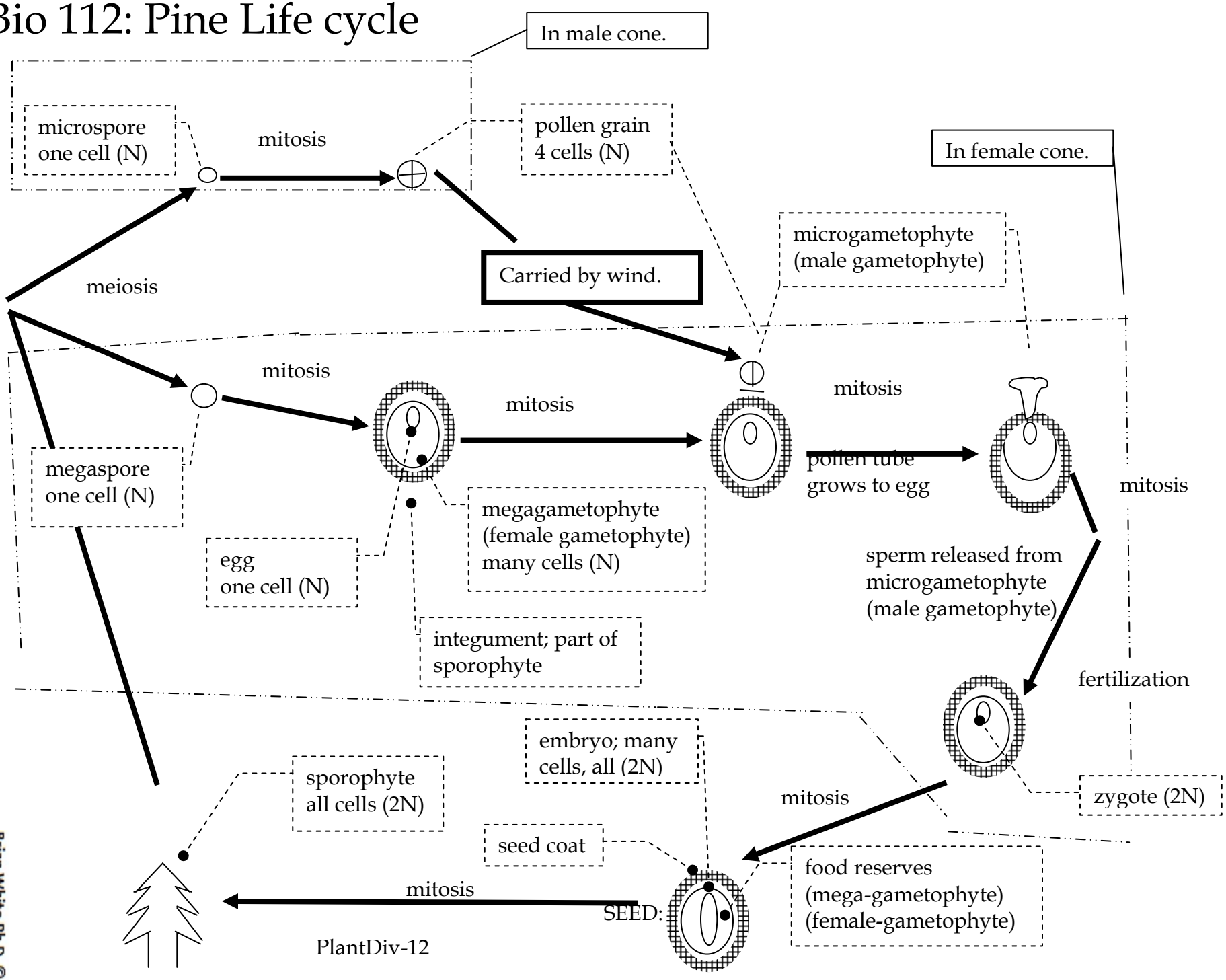
Bio 112: Moss Life-cycle



Bio 112: c-Fern Life-cycle

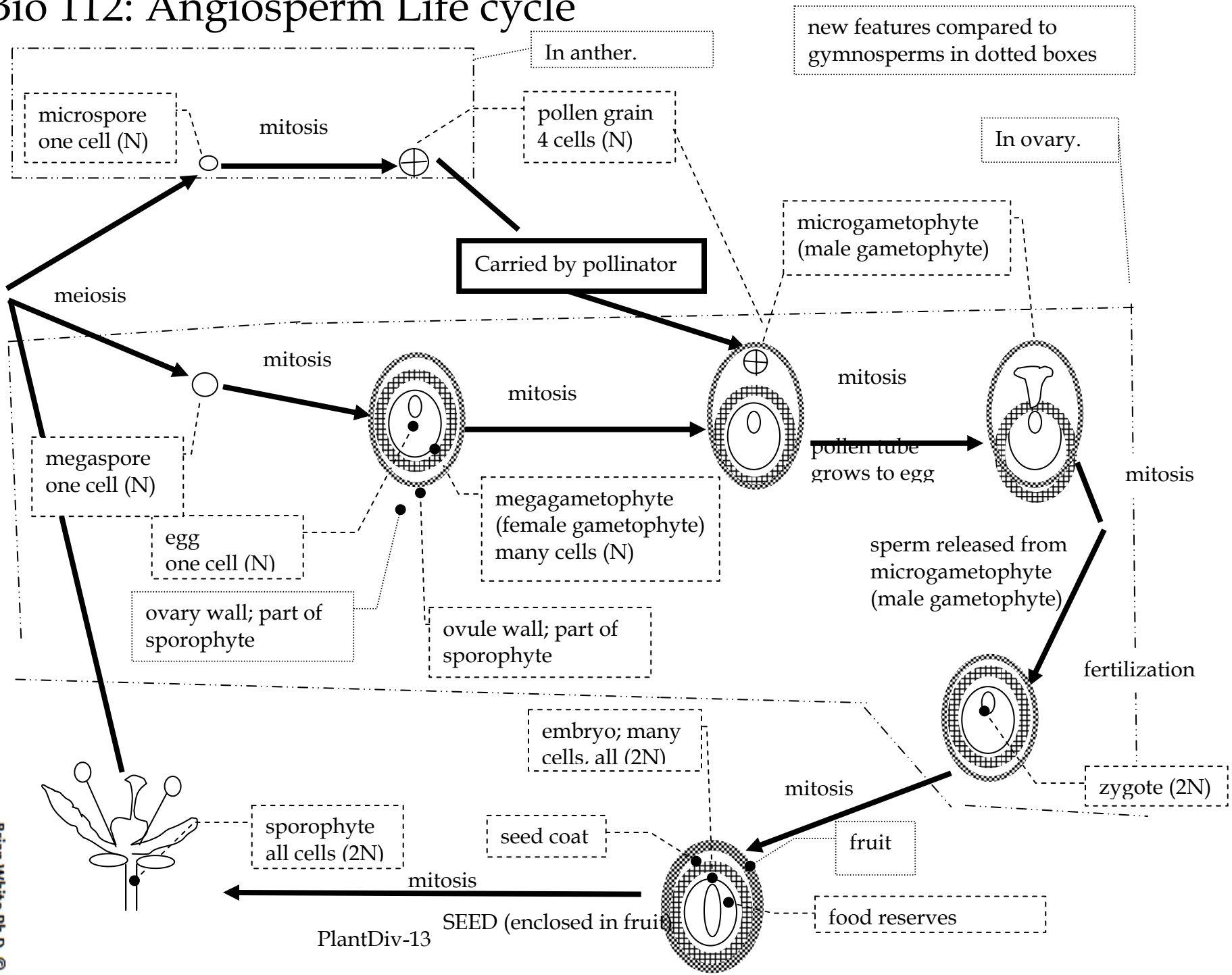


Bio 112: Pine Life cycle



PlantDiv-12

Bio 112: Angiosperm Life cycle



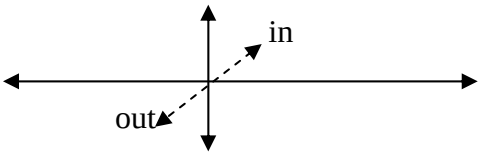
PlantDiv-13

Name _____

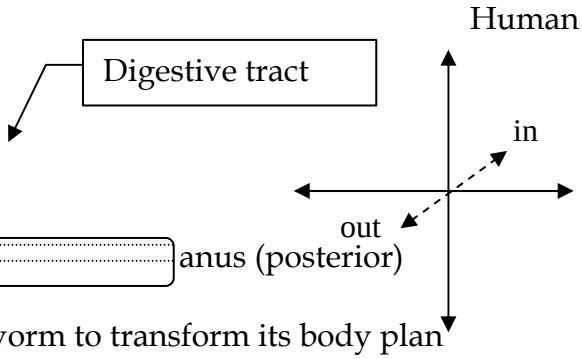
Pre-Lab: Animal Diversity I

1) On each of the figures below, label the axes with dorsal, ventral, anterior, or posterior as appropriate. Note: dashed lines indicate axes that extend out of the plane of the picture.

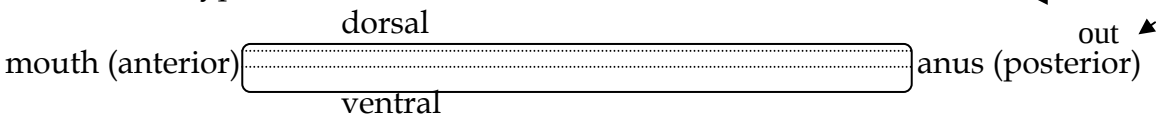
a) Squid (4 pts)



b) Shark (2 pts) & Human (2 pts) (Do the human by analogy to the shark)

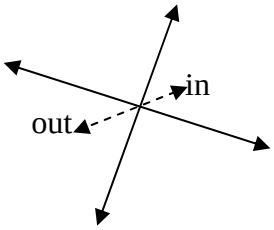


2) Consider the hypothetical worm shown below:



What would you have to do (stretch, fold, etc.) to this worm to transform its body plan (including anterior, posterior, doral, ventral, mouth, anus, digestive tract) it into an animal with a body plan like the squid? (2 pts).

Shark



Name: _____

Pre-Lab: Animal Behavioral Diversity and the Scientific Method

1) (7 pts) You just saw a dog food commercial on TV that states that dogs prefer Alpo over Kal-Kan. Think about how you could conduct an experiment to test whether or not this statement is true. State your null and alternative hypotheses. What response variable would you use in your experiment?

null hypothesis:

alternative hypothesis:

response variable:

2) (3 pts) Name three environmental cues that earthworms may respond to when selecting a specific location to live.

1.

2.

3.

Animal Behavioral Diversity and the Scientific Method

Objectives

To compare and appreciate animal diversity through the lens of behavior. To consider the diversity of behavioral adaptations that have evolved in response to selection pressures from different physical and social environments.

To understand the major components of the scientific method. To generate testable hypotheses, design experiments to test hypotheses, and analyze results from an experiment.

Introduction

Animal behavior

Broadly defined, behavior is the sum of an organism's responses to stimuli in its environment (*Campbell Ch. 51*). Behavior is what an organism does. After detecting another organism, for example, an animal may respond by attacking it, fleeing from it, attempting to eat it, courting it, freezing in place, or ignoring it, just to name a few of the possible behavioral responses depending on the identity and perhaps behavior of that other organism. To fully understand an organism's behavior, the study of animal behavior must occur in the context of the evolution, ecology, social organization, development, and sensory abilities of the subjects. Most animals possess far different sensory abilities and live in drastically different habitats than the humans studying them. Animal behaviorists study their subjects by carefully observing and experimentally analyzing behavior patterns. In your study of animal behavior in lab today, consider both the proximate and ultimate causes of the behaviors you observe. **Proximate causes** include the immediate sensory, physiological, and biomechanical events that led to the behavior. For example, did the organism use chemical, visual, or electrical cues to detect the stimulus, and what kind of nervous or hormonal events did it trigger in the organism? **Ultimate causes** refer to the adaptive value and evolutionary origin of the behavior. In other words, how does this behavior help the survival and reproductive success of the organism, and what is the pattern of behavior in the species' ancestors? To illustrate, a sparrow feeding on the ground will respond to a stalking cat by taking flight. Part of the proximate cause of this behavior might be the sight or sound of the approaching cat stimulating sensory receptors that in turn trigger nervous impulses that lead to muscle contractions in the wings. The adaptive value might include fleeing from a predator to avoid being eaten.

In today's lab you will be investigating three categories of behavior: orientation behavior, agonistic behavior, and reproductive behavior. Orientation behavior helps an organism locate the most favorable environment currently available to it. Orientation behavior includes taxis, movement directly toward or away from a stimulus. Positive taxis refers to movement toward a stimulus while negative taxis refers to movement away from a stimulus. Prefixes such as photo-, chemo-, and thermo- describe the nature of the taxis. For example, an animal that approaches light is positively phototaxic.

In addition to the need to find a favorable place to live, animals often find themselves in conflict with other organisms. For example, two bears may attempt to use the same profitable location along a stream to catch fish. Behaviors associated with conflict situations are known as agonistic and include both aggressive (attack or threatening) and submissive (retreat and avoidance) behaviors. Agonistic behavior often involves displays to make the animal appear larger or threatening, and in many cases (but not all), a conflict ends without serious injury or death. These threats and displays often help an animal to maintain a territory or social position in which it has predominant access to resources such as space, food, and mates.

Behaviors that facilitate reproduction have obvious adaptive value. Mating behaviors vary greatly among species and can involve complex and often amazing routines that assist an organism in finding, courting, and mating with a member of the same species. Can you suggest why it behooves an organism to mate with a member of the same species?

Scientific Method

The information contained in your biology textbook results from thousands of scientific investigations. Scientists express curiosity about the world and ask questions that address their desire and often society's pressing need for knowledge. Most scientists follow the same general procedure, called the **scientific method**, for formalizing questions and seeking information to address them.

The scientific method typically begins with observations of a pattern or process that suggests one or more corresponding questions to the observer. A scientist attempts to formulate alternate answers for the questions called **hypotheses**. The process of formulating a hypothesis often starts with an initial or informal hypothesis that the scientist reformulates into a formal hypothesis that ideally produces unambiguous predictions that can be tested with future experiments or observations. Formal hypotheses strive to yield mutually exclusive predictions that do not overlap with predictions from competing hypotheses. Scientists usually express formal hypotheses in two forms: a null hypothesis and one or more alternate hypotheses. A **null hypothesis (H_0)** predicts "no difference" or "no effect" between two or more experimental conditions. For example, in an experiment to test the effectiveness of a flu shot, the null hypothesis would predict that people that received the flu shot are just as likely to contract the flu as people who did not receive the shot (i.e., no difference between the two experimental groups). In contrast, the **alternate hypothesis (H_A)** predicts a difference or effect of the experimental condition. For the flu shot example, an alternate hypothesis might predict that there will be a difference between people who received the flu shot compared to those who did not in the likelihood of contracting the flu.

After formulating the hypotheses, scientists devise experiments or additional observation protocols to collect the data needed to test the hypotheses. The data typically require analysis, often statistical, before they can be judged against the predictions. If the data fail to support the predictions of the hypothesis, that hypothesis is rejected. If the data support the hypothesis, the hypothesis is not rejected. The scientist could then say that the results of the experiment support the hypothesis. However, the results do not prove that the hypothesis is true. Additional experiments need to be conducted that address the hypothesis in different ways. Several different experiments each in support of the hypothesis would strongly suggest

that the hypothesis is true. Moreover, additional experiments may reveal that the hypothesis seems to hold under certain conditions but not others.

A brief example may be useful. Imagine that you are driving around town with a couple of friends, one of whom is male and one of whom is female, and you end up lost. Your female friend wants to stop and ask for directions but your male friend does not. You joke that men hate to ask for help with directions (an initial hypothesis). To develop a formal hypothesis that men are less likely to ask for help than women, you must develop a measure of the likelihood of asking for help that works equally well for both sexes and can be measured accurately. For instance, you might conduct a survey and ask men and women how often in the past month they have asked for directions. This measure though would rely on several assumptions including the ability of subjects to accurately remember how often they have stopped for directions, the honesty with which men and women will answer the question, the number of times each subject has gotten lost and perhaps the severity and location of where they were lost, the tendency for men and women to travel beyond neighborhoods familiar to them, the frequency with which each sex drove a car in the last month, etc. If any of these assumptions differ for men and women, for example if men are even reluctant to admit that they asked for directions, then the measure will be biased.

Instead you decide to conduct an experiment in which you send men and women on an errand across town but give them the wrong directions. If given accurate directions, men and women should perform equally well on driving to the destination. You send along a spy as a passenger in the car to record how long it takes for the subject to stop and ask for directions after getting lost. Your **response variable** then is the length of time between getting lost and asking for directions. Your two mutually exclusive, opposing hypotheses for this experiment would be:

H_0: The length of time between getting lost and asking for directions will be the same for men and women.

H_A: The length of time between getting lost and asking for directions will not be the same for men and women

Before you test your hypothesis, you have to determine the number of experimental subjects (data points) you will use. This is also known as the **sample size**. Can we test with just one male and female? This approach might be acceptable if all males were the same and all females were the same (i.e., no variation), but this is clearly unreasonable. For this experiment, say you recruited 25 students of each sex from the UMass Boston campus.

Assume you have completed your experiment and obtained the following results:

Subjects	Average time to ask for directions
Female (n=25)	10.3 min
Male (n=25)	28.2 min

Can you reject the null hypothesis that men and women are the same in regards to asking for directions based on these results? Can you tentatively accept your alternate hypothesis that men and women are indeed different in this regard? This sample of men had a higher average result for this experiment than the women, but is this difference significant?

Statistical tests take into account the amount of variation within the samples and the size of the samples to allow you to estimate the probability that the results you obtained could

have been due to chance events alone. A significant difference is one that is greater than would be expected by chance. Later in this lab, we will introduce the binomial test that will be used for statistically testing today's experiments. Suppose you applied the appropriate statistical test to the results, and the test indicated that these results are unlikely if males and females are equally inclined to ask for directions when lost. The results from the test allow you to reject the null hypothesis and state that your results support the alternate hypothesis.

However, you would ideally like your results to apply to all men and women, not just to the 25 of each that you selected for your study. In order to generalize your results beyond the particular set of individuals in the experiment, you need to expand your sample to include a reasonably large number of different subjects. The experiment has not proven that men are less inclined to ask for help than women, but that under the particular circumstances for the particular group of people studied (students at UMass), males appear to be more hesitant than women to ask for directions after becoming lost. Before the evening news states that men hate to ask for help, you would want to expand your study to include different groups of people (perhaps this is only an UMass student, Bostonian, or American phenomenon), subjects of different ages, and larger numbers of subjects. You may also want to try different experimental conditions—areas that vary in familiarity to the subjects, walking versus driving, the types of places and people available to ask for help, with or without access to a map, or asking for directions before beginning a trip somewhere (not just after getting lost). Also, you might want to investigate different types of help for which people ask—directions for how to assemble something or how to use a new computer program. If all of these experiments produced similar results, you would have much stronger support for your initial hypothesis.

One more issue that can undermine the ability of your data to support your hypothesis is that of **confounding factors**. An experimental confound produces an alternate causal explanation for the observed results. For example, if you wanted to test the effects of heat on the movement of an animal and used a light to produce that heat, you could not say whether the animal was responding to heat or light even if your results were statistically significant. In our previous example, possible confounds could include the experimenter who provided the directions or the spy passenger systematically treating men and women differently. The sex of the spy passenger may also influence the results even if you always used one sex (say you always used a male passenger) for both male and female subjects; the male subjects may systematically respond differently to that man than the female subjects do (perhaps male drivers are less likely to ask for help in front of another guy).

Experimental Organisms

In today's lab, you will work with three different animal species from three different phyla: red worms (*Eisenia foetida*), crickets (*Achetus domesticus*) and Betta or fighting fish (*Betta splenda*). You will investigate taxis in red worms, mating behavior in crickets, and, as befitting its name, agonistic behavior in fighting fish. For the Betta fish and the crickets, you will perform (or your TA will demonstrate) experimental manipulations with the subjects so that you may observe some of the behaviors of interest. Based on your observations, you will then design your own experiment for either the Betta fish or crickets. After you complete your Betta fish or cricket experiment, you will design, conduct, and analyze a formal experiment on red worms. Because of the advanced nature of the statistical procedure required to analyze the

data you gathered from your cricket or Betta fish experiment and the limited number of subjects available for experimentation with those species, you will only conduct a statistical analysis on your red worm data.

Betta fish

The agonistic behavior of male Betta fish is widely known and studied, hence their common name of fighting fish. The sight of another male typically stimulates a series of agonistic behavioral displays toward the intruder often followed by physical aggression. Most pet stores, for instance, keep Betta males in separate containers because of the severe aggression that follows when the confines of the tank prevent one of the males from escaping or avoiding the more dominant male. Male Betta fish often use changes in body posture, fin and gill cover placement, general orientation, and coloration in their agonistic displays. Possible responses to a rival male include frontal approach (facing the intruder), broadside display, undulating movements, increased swimming speed, fin elevation, gill cover extension, tail expansion, and enhanced coloration in tail, fin, or body. Your behavioral experiment will investigate the use of agonistic displays by Betta fish. Think about the proximate and ultimate causes of the behavior when designing your experiment. You need to become familiar with the fish's external anatomy by locating the dorsal fin, ventral fin, pectoral fin, gill cover, and tail.

First, observe your fish subject for a few minutes to familiarize yourself with its behavior in a non-agonistic context. Next, place a tank containing another male fish against your subject's tank and observe the response. Discuss what you observed with your lab partners and ensure that you all agree on the behaviors you observed, how to record your observations, and how to quantify them (e.g., timing the duration of behaviors or recording the intensity of them).

Design your own experiment. Think about the proximate and ultimate causes driving the behaviors you observed, look at the supplies available to you, and talk to your TA if you need to stimulate your creative juices. What cues or stimuli might stimulate the fish's aggressive behavior? Under what circumstances does it benefit the male to show aggression to another organism?

Form a general hypothesis about the response of the fish subjects to your experimental manipulation. For example, if you were conducting an experiment similar to the pre-experimental procedures that you just finished, you might have hypothesized that the subject will display heightened agonistic behaviors upon seeing another male compared to the condition in which the fish could see only another empty tank. (Note: you must design an experiment different from this one.) **Answer questions 1a and b for your lab report.**

What will be your response variables? For a complex set of behaviors such as these, multiple behavioral responses will provide a more accurate picture of the response to the stimulus. You should select three behavioral responses that you can measure. Each member of your lab group can record one of your selected responses (e.g., duration of gill cover extension, length of time to react to the stimulus, occurrence of body color change, etc). Write hypotheses for each of your specific response variables. **Answer questions 1c and d for your lab report.**

Describe your experimental design. **Answer question 1e.**

Now you are ready to conduct your experiment. Have ready a pencil and paper and whatever other supplies (e.g., timer) you need. Because of the limited number of fish available for this lab, you will only be able to test your hypothesis on 2-3 individuals. Remember, this would not normally be an acceptable sample size.

In the results section, describe your results as quantitatively and detailed as possible. For example, if you measured the duration of a particular response, report the average duration for all of your trials for your various experimental conditions. Although, you will not conduct a statistical analysis of your results, do your results appear to support your alternative hypothesis? Explain why or why not? **Answer question 1f.**

What do your results demonstrate? Briefly discuss the proximate and ultimate causes of the behaviors you observed in response to your experimental manipulations. **Answer question 1g.**

Crickets

In many regions of the world, several species of closely-related crickets co-exist. Many species exhibit behavioral or morphological traits related to mating that distinguish them from spatially overlapping species. Mating behavior in *Achetus domesticus* consists of a complex series of several different behavioral patterns displayed by males and females. If the behavior display is successful, the crickets will copulate. Some of the most easily recognizable display behaviors that you might observe in your experiments include:

- Chirping: the male vibrates his wings together to produce sound
- Antenna stroking: antenna are rubbed on the head and body of the other individual
- Following: one individual closely follows the other
- Retreating: one individual leaves the presence of the other
- Ignoring: one individual appears to not respond to the other individual
- Mounting: one individual climbs on top of the other for copulation (which sex varies by species)

First, observe your crickets for a few minutes to familiarize yourself with their behavior in a non-reproductive context. You should have separate containers of males and females. Place a female with a male and observe their behavior. Discuss what you observed with your lab partners and ensure that you all agree on the behaviors you observed, how to record your observations, and how to quantify them (e.g., timing the duration of behaviors or recording the intensity of them).

Design your own experiment. (Remember you will only conduct your own experiment on either the fish or crickets.) Think about the proximate and ultimate causes driving the behaviors you observed, look at the supplies available to you, and talk to your TA if you need to stimulate your creative juices. What cues or stimuli might stimulate a cricket's reproductive behavior? Under what circumstances does it benefit a cricket to show courtship behavior to another organism?

Form a general hypothesis about the response of the cricket subjects to your experimental manipulation. For example, if you were conducting an experiment similar to the pre-experimental procedures that you just finished, you might have hypothesized that the

male crickets will display heightened sexual behaviors upon detecting a female compared to the condition in which the male crickets were only in contact with other males. (Note: you must design an experiment different from this one.) **Answer questions 1a and b for your lab report.**

What will be your response variables? For a complex set of behaviors such as these, multiple behavioral responses will provide a more accurate picture of the response to the stimulus. You should select three behavioral responses that you can measure. Each member of your lab group can record one of your selected responses (e.g., length of time to react to the stimulus, duration of chirping, occurrence of mounting, etc). Write hypotheses for each of your specific response variables. **Answer questions 1c and d for your lab report.**

Describe your experimental design. **Answer question 1e for your lab report.**

Now you are ready to conduct your experiment. Have ready a pencil and paper and whatever other supplies (e.g., timer) you need. Because of the limited time and number of crickets available for this lab, you will only be able to test your hypothesis on 5 individuals. Remember, this would not normally be an acceptable sample size.

In the results section, describe your results as quantitatively and detailed as possible. For example, if you measured the duration of a particular response, report the average duration for all of the trials for your various experimental conditions. Although, you will not conduct a statistical analysis of your results, do your results appear to support your alternative hypothesis? Explain why or why not? **Answer question 1f.**

What do your results demonstrate? Briefly discuss the proximate and ultimate causes of the behaviors you observed in response to your experimental manipulations. **Answer question 1g.**

<u>Red worms</u>

Red worms belong to the phylum Annelida which contains about 15,000 species. Like other earthworms, red worms lead primarily subterranean lives and consume organic, often decaying, matter in the soil. Red worms inhabit particularly moist soils rich in organic matter such as compost heaps and gardens. In fact, they are the primary worm used for composting. Worms have no lungs and must absorb oxygen through their skin, a process that requires their skin to stay moist. Your behavioral experiments will investigate taxis in these worms. Recall that orientation behaviors such as taxis function to place an organism in an environment favorable for survival and reproduction. When designing your experiment, you should think about the natural habitat of earthworms and the cues that they may use to detect the suitability of different areas.

<u>Hypothesis testing with red worms</u>

Based on the description of red worm natural history presented above, you will formulate hypotheses, design an experiment to test your hypotheses, conduct an experiment, and statistically examine the results. Equipment and supplies are available for your experiment. Talking to your TA or glancing at the supplies and equipment available may help inspire your creative thinking. For example, think about the habitat of the worms; you may want to examine what environmental cues they use to select where to live and feed. Say you think that temperature may represent an important influence in the lives of red worms (you cannot use this experiment).

Initial hypothesis: Red worms will move from warmer environments into cooler ones.
Response variable: The number of worms in each environment after 3 minutes of choice.
Test situation: Test 16 worms in a dish with a warm side and a cool side.
Null hypothesis: # of worms on warm side = # of worms on cool side
Alternate hypothesis: # of worms on warm side ≠ # of worms on cool side

You should now decide on an initial hypothesis, your response variable, and H_0 and H_A. **Answer questions 2a-c for your lab report and have your TA approve them.**

Experimental design

Next, you need to design your experiment. How will you test your hypothesis? You should keep in mind several factors when designing your experiment. Recall the problem of confounding factors discussed earlier. In the sample experiment on temperature preference in worms, what if you used a lamp to warm one side of the dish? If the worms move away from the source, they may be responding to light rather than heat. Perhaps you decide to use a chemical heat pack or warm water bottle instead.

If you put a worm in the middle of the dish and it moves away from the heat, you still cannot conclude that red worms, in general, are repelled by heat. Why not? Even in the absence of a heat source, you would expect that the worm may move to one side of the dish or the other 50% of the time (like flipping a coin). You must perform repeated trials of the experiment to gain confidence that the movement away from the heat source is not merely due to chance. Think about the following when designing your experiment: where in the dish do you place the worm, how long do you wait to record the result (you need to be consistent between trials), when will you start the timing? **Answer questions 2d and e.**

Data collection

You should now set up your experiment and collect the data. You will perform 16 trials using 16 different individual worms. **Fill out the chart for your lab report (question 2f) as you collect your data.**

Data analysis

To illustrate how to analyze the significance of your data, we need to revisit the sample red worm experiment. To test the red worms' preferences for heat or cool, suppose you performed 16 three-minute trials on 16 different worms and obtained the following data:

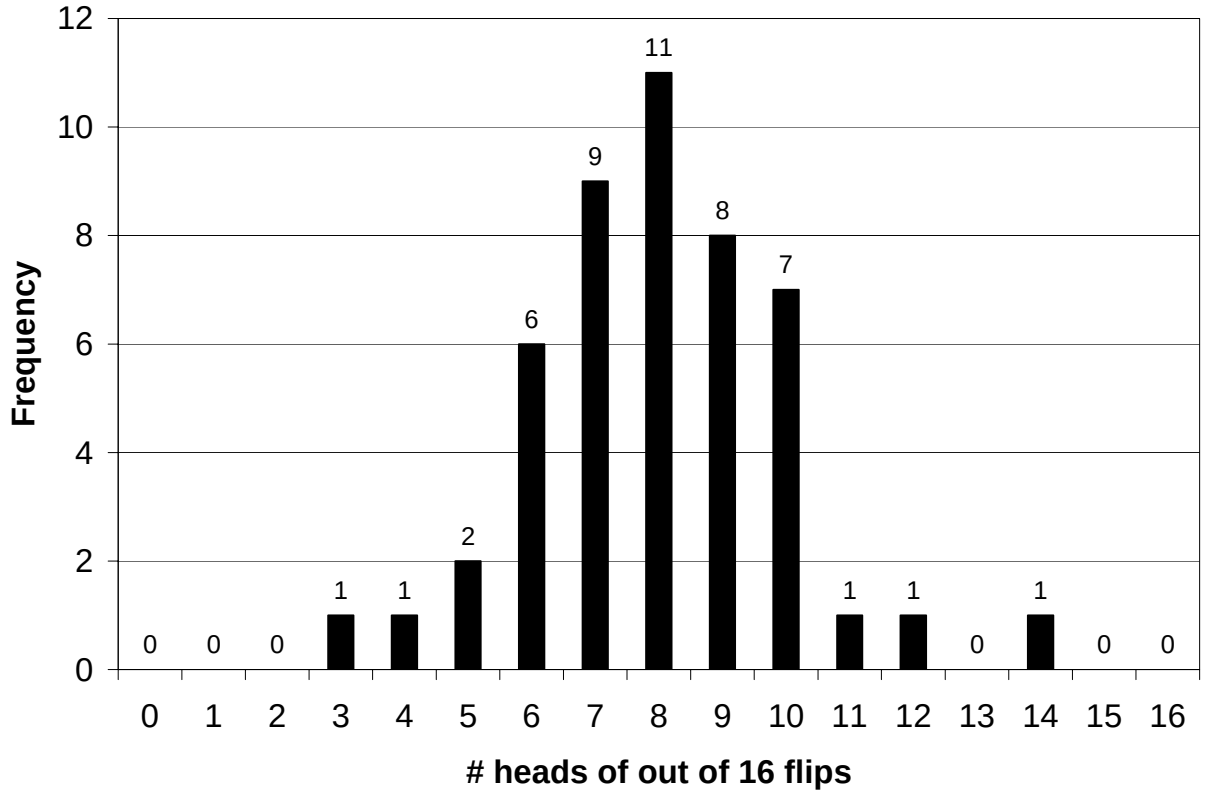
Subject #	Worm on warm side after 3 min	Worm on cool side after 3 min
1	X	
2		X
3		X
4		X
5		X
6	X	
7		X
8		X
9		X
10		X
11		X
12		X
13	X	
14	X	
15		X
16		X
Total	**4**	**12**

The data appear to exhibit a trend toward a temperature preference for the worms, but is this result significant? If the data supported the null hypothesis of no difference or no preference, you would expect to see approximately half (n=8) of the worms crawling to the worm side and half to the cool. To test for significance, the results must be compared to the distribution expected by chance.

If a worm exhibited no preference for the warm or cool side, 50% of the time it would travel to warm side and 50% to the cool. Earlier we compared this to the probability of a flipped coin landing heads up (or likewise, the likelihood that it lands tails up). On average, you would expect that a coin flipped 16 times should land heads up about 8 times and tails up about 8 times. But what if you flipped a coin 16 times and it landed heads up 12 times, does this mean the coin was loaded? Not necessarily. Because each flip is an independent event (in other words, the outcome of any given flip does not depend on the outcome of any other flip), by chance alone you could get 12 heads and 4 tails. By chance alone, you could get 16 tails and no heads although highly improbable. Statistics is concerned with calculating the probability that a certain combination of outcomes will occur by chance.

To illustrate, the class as whole will generate a distribution graph for this type of problem. Everyone will flip a coin 16 times and count the number of heads and then repeat with another 16 flips. You will have two "head" counts to report to your TA. We intuitively expect that everyone will get 8 heads and 8 heads, but other outcomes will occasionally occur. The TA will pool the class data to see how often we get unexpected results just by chance. Do this now if you haven't already. **Record the distribution generated by the class in question 2g.** You will use this distribution to test your hypothesis.

Suppose 24 students in your lab each flipped a coin 16 times, counted the number of heads, and then repeated it for another 16 flips. In all, the class would have generated 48 (= 2 X 24) trials of 16 flips. Imagine your class obtained the following distribution (**note**: do not use this distribution to test your hypothesis, use the one generated by your lab section).



What can you determine based on this distribution? We got exactly 3 heads out of 16 flips once in 48 trials. This means the probability that the number of heads=3 is 1/48 or 0.021. Eleven out of 48 flips we got 8 heads so that probability is 11/48 or 0.23. As expected, the probability of getting 8 heads out of 16 flips is much greater than 2 heads out of 16 flips. Based on the distribution, what is the probability of getting 11 *or more* heads in 16 flips? Add the number of times we got 11, 12, 13, 14, 15, and 16 heads and divide by the number of trials. Thus,

$P(\geq 11 \text{ heads in 16 flips}) = (1 + 1 + 0 + 1 + 0 + 0) / 48 = 0.0625$

Returning to the red worm temperature experiment, our mutually exclusive hypotheses were

H_0: # worms on warm side = # worms on cool side

H_A: # worms on warn side ≠ # worms on cool side

We can apply this distribution to determine the probability that our results (4 worms on warm side and 12 worms on cool side) could have occurred by chance alone. To determine this, you must look at both extremes or tails of the distribution. In other words, what is the probability due to chance alone that 12 of 16 worms would move either to the cooler side or warmer side? Based on the above distribution,

$P(\leq 4, \geq 12) = (0 + 0 + 0 + 1 + 1) + (1 + 0 + 1 + 0 + 0) / 48 = 4/48 = 0.083$

So, a little over 8% of the time we would expect worms to exhibit this degree of preference for either the cooler or warmer side of the dish just by chance alone. From this we can also calculate a **confidence level**:
100% - P($\leq$4, $\geq$12) = % confidence
100% - 8.3% = 91.7% confidence

Thus, we are almost 92% confident that the red worms are actually exhibiting a preference for either the warmer or cooler side of the dish.

Is this a statistically significant result? Where is the cutoff between statistical significance (reject H_0 but not H_A) and no statistical significance (reject H_A but not H_0)? The cutoff varies somewhat among disciplines but often is set at either $P \leq 0.1$ or $P \leq 0.05$. These respectively correspond to 90% and 95% confidence levels. $P \leq 0.05$ is the more typical standard and the one that we will use here. From our sample experiment, $P = 0.083$ which is greater than 0.05 so we must reject H_A but not H_0 and conclude that our results did not support the hypothesis that red worms show a preference between warm and cool sides of the dish. Note that the rejection of a hypothesis is somewhat subjective though. If we set our significance level cutoff at $P \leq 0.1$, we would have rejected our null hypothesis and concluded that our data support the alternate hypothesis. In practice, scientists use tables of probability calculated for a very large number of coin flips.

The Effects of Sample Size

Sample size can influence whether results show statistical significance. In our sample experiment, 75% (12/16) of the red worms displayed a preference for the cool side of the dish. Yet, at a significance level of $P \leq 0.05$, this result did not reach statistical significance, and we could not reject our null hypothesis. If instead we had tested 100 red worms and 75 had displayed a preference for the cool side (= 75% as before), would this be significant at $P \leq 0.05$? Using statistical tables generated for just this purpose, we would find that $P < 0.0001$, a *highly* significant result, and could quite confidently state that out data supported out alternative hypothesis that red worms displayed a temperature preference. Although we used a series of coin flips to illustrate a frequency distribution based on two equal outcomes (heads or tails), mathematicians and statisticians have generated standardized probability distributions which scientists use to check the significance of their results. As a side note, our sample coin-flip distribution above fairly well simulates the true distribution based on two outcomes of equal probability in an experiment with 16 trials.

Complete questions 2h-j based on the results of your red worm experiment.

Lab report

• Must be typed; handwritten reports will not be accepted. Hand-drawn and labeled drawings are fine. All drawings must indicate size.
• Due next week at the start of the lab session you are currently in. This is a firm deadline.
• Although you will perform these activities as a group, each member of the group must turn in an individual lab report. Each person's report must be in his or her own words as much as possible.
• Your lab report must contain:

(1) Observations and Hypotheses—Betta fish or crickets
(a) Which species did you experiment with?

(b) State your general hypothesis.

(c) What are your three response variables (note: these should be three different behaviors you examine to test your one general hypothesis)?

1.

2.

3.

(d) State the null and alternate hypotheses for each of your response variables.

1. H_0:

   H_A:

2. H_0:

   H_A:

3. H_0:

H_A:

(e) Describe your experimental design. Include important details about your organisms, the physical setup, number of subjects, duration of each trial, etc.

(f) What are your results? Describe them as quantitatively as possible. Do the results support your alternate hypotheses? Explain why or why not?

(g) Offer a discussion of your experiment. What do your results demonstrate about the behaviors and organisms you observed? What do your results suggest about the proximate cause(s) of the behaviors you observed in response to your experimental manipulations? What do think may be the ultimate cause(s) of the behaviors you observed.

(2) Red worm experiment
(a) State your initial hypothesis

(b) What response variable will you use to study the behavior of this species?

(c) State your null and alternate hypotheses

H_0:

H_A:

_____TA's initials

(d) Describe your experimental design. Include important details about your organisms, the physical setup, number of subjects, duration of each trial, etc.

(e) Are there potential confounding factors in your experimental design? If so, explain.

(f) Fill in the blank chart with your data. Label the chart appropriately for your experiment.

Subject			Comments or Notes
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
Total			

Why is it important to use 16 different individuals instead of testing the same subject 16 times?

(g) Fill in the distribution your class generated by coin flipping and label the axes.

0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

What is P(heads=9)? Show your calculations.

What is P(heads≥9)? Show your calculations.

(h) Using the class coin flipping distribution above as a basis for your statistical test, how likely is it that the results from your red worm experiment are due to chance? Show your calculations.

(i) Should you reject your null hypothesis? Explain. What is your confidence level? What do your data suggest about your hypotheses?

(j) Offer a discussion of your experiment. What do your results demonstrate about the behaviors and organisms you observed? What do your results suggest about the proximate cause(s) of the behaviors you observed in response to your experimental manipulations? What do think may be the ultimate cause(s) of the behaviors you observed.

Field Trip II:
New England Aquarium

Objectives

To examine the diversity of water-dwelling organisms and to consider the adaptations required for aquatic habitats. To observe living organisms (as opposed to the dead ones at the HMNH) in their habitats as they interact with other organisms. To observe live ecological systems.

Introduction

Living in water as opposed to on land changes strategies for locomotion, gas exchange, light capture, feeding, reproduction, temperature regulation, defense, and many other functions. Water can support animals that are too large and too structurally weak (for their size) to survive on land; water changes the ability of these animals to maintain body temperature economically. The diminishing of light with depth limits where photosynthesis can occur and also changes the wavelengths available. Aquatic animals may obtain oxygen either from surface air, using lungs or directly from the water, using gills. Although life began in the water and moved onto land, some land animals "returned to the water"; you will see some of them.

Procedure

VERY IMPORTANT NOTICE: This lab will take you a while to complete, especially if you are unprepared. In order to be able to complete it in 3 hours, you should **be sure to do the following before you go to the Aquarium**:

- Read up on classification systems (*Campbell* pp 496-497) and familiarize yourself with terms like kingdom, phylum, etc.
- Look up all the names listed in question (7) and make a few notes about what an organism in this group would look like - that will make it a lot easier to find these organisms. You should use *Campbell* as a reference .
- Think about how you will answer each of the questions & make some notes to help you look.

We will give you a ticket for the Aquarium this will get you a discounted admission price. You can go to the Aquarium anytime; TAs will be there during any one of the scheduled lab periods during the week listed on the syllabus. The TA will be at the information counter 30 minutes after the lab period starts and around the Aquarium for the entire period. The Aquarium is a short walk from Aquarium Station on the MBTA's Blue line.

During your visit, you should make notes from which you can answer the questions below. Your lab report will consist of answers to these questions written with the same care and thoroughness as any other lab report.

YOU SHOULD BRING A COPY OF *Campbell* FOR REFERNCE.

You should pick up a map for reference.

Lab report:

- Must be typed; handwritten reports will not be accepted. Hand-drawn and labeled drawings are fine.
- Due at the start of the lab session you are currently in during the week indicated on the syllabus. This is a firm deadline.
- Although you will perform these activities as a group, each member of the group must turn in an individual lab report. Each person's report must be in his or her own words as much as possible.
- Your lab report must contain answers to the following questions:

General notes on answers to lab report questions:

When asked to "name an organism", you must always:

- choose an organism found in the aquarium
- give the common name as well as the genus & species names; the aquarium's web site: http://www.neaq.org has a virtual tour that lists many species' names.
- give the name of the tank or exhibit where you found it

(1) Give a method of locomotion that is found both on land and in the water. Name an organism that uses it.

(2) In the large tank, you will notice that different fish use different fins to swim. There are three major types of swimming motions; describe them. What is the most common type?

(3) What method of feeding is found in the ocean but not on land? Name an organism that uses it.

(4) Various morphological adaptations have evolved for different animal species to blend into their habitat ("cryptic morphology"). Describe an example of this cryptic morphology (name and describe how the animal blends in to its habitat) from each of the following habitats:

i) sandy bottom

ii) rocky intertidal zone (also called "the edge of the sea")

iii) Some animals show "anti-camoflage" (aposematic coloring) – that is, they are colored to be especially visible so that predators will avoid them. Give an example of an organism in the aquarium that is colored in this way. How does this coloring strategy confer a selective advantage on this organism?

(5) Give two examples of mutualistic or commensal interactions between organisms in the Aquarium. You should look up these terms in *Campbell*. Good examples can be found in the Tropical Gallery; you will need to read the printed information on the wall and interpret it.

(6) Give three different methods of obtaining oxygen and one aquatic creature from the Aquarium that uses each.

i)

ii)

iii)

(7) For each of the groups of organisms below, find a water-dwelling example found in the Aquarium.

Group	**Example**
turtles	
cnidarians	
cephalopods	
bivalves	
echinoderms	
birds	
mammals	

(8) For one of the animals in the groups in question (7) that descended from land-dwelling ancestors, describe two of it's adaptations to an aquatic habitat.

Artificial Life & Evolution

Objectives:

- To explore evolution with evolving digital organisms.
- To test evolutionary hypotheses.
- To try out different evolutionary scenarios.

Introduction:

Life only evolved once on earth. In addition, for most organisms, evolution happens very slowly on a human time scale. As a result, it is difficult to explore evolution experimentally – to address questions like "What would have happened if …?" or "Did it necessarily have to happen this way?"

In the Population Genetics lab, you simulated evolution for a single simple gene with two alleles. Although this is important, it does not capture much of the complexity of evolution "in the wild".

To explore evolution in more detail, you need organisms with a more complex genotype that can reproduce rapidly. Many researchers study the evolution of micro-organisms for these reasons. In this lab, you will explore the evolution of simple digital micro-organisms as they evolve in the computer simulation, AVIDA.

In AVIDA, the "organisms" are short computer programs that carry out only one function: they replicate themselves. They are similar to computer viruses, which reproduce by copying themselves from one computer to another. Since computer viruses copy themselves exactly, they do not evolve; any changes are due to human intervention. The organisms in AVIDA are subject to random mutation and non-random selection, so they do evolve like organisms in the real world.

AVIDA was developed by researchers as a tool to study a variety of evolutionary principles and has resulted in several interesting findings. You can find links to these on the AVIDA web page; there is a link to this page on the On-Line Lab Manual for this lab. We will be using AVIDA-Ed (AVIDA for Education) developed by Robert Pennock and others. AVIDA-Ed is full-featured AVIDA with a user-friendly user interface. A link to the AVIDA-Ed home page can also be found on the On-Line Lab Manual page.

The basic requirements for evolution are:

1. Genomes. Organisms must have a genome, a complete set of genetic instructions for making themselves.
2. Self-reproducing organisms. Organisms must be able to make copies of themselves, including copies of their genome.
3. Mutation. The copying in (2) is not always perfect, so genomes can change.
4. Limiting Resources. There is only enough space, resources, etc. for a finite number of organisms, so some organisms reproduce less frequently than others.

Given these four conditions, the organisms will evolve – they will adapt to their environment by a process of natural selection. The more fit variants will out-compete the less-fit variants and the population will change over time to adapt to the given environment.

AVIDA simulates a "world" that satisfies these four requirements for digital organisms called "avidans". The AVIDA program simulates the world that the avidans live in; it simulates feeding the avidans, replicating them, and removing them when they die. In order to understand how this or any other simulation works, you need to consider each of the four requirements in three different ways:

A. **How these issues manifest themselves in the real world.** This will be shown in regular type.
B. **How is this shown in the simulation**. This will be shown in *italic* type.
C. **The underlying mechanism that the simulation uses to simulate this behavior.** This will be shown in **bold** type.

Here are the four requirements in detail:

1. Genomes. In the real world, most organisms have a DNA genome. This sequence of DNA determines the genetic properties of that organism and contains instructions for making that organism. It is not a set of instructions like a computer program, but it results in the production of a set of proteins, etc. that are capable of replication, behavior, etc. *Avidans have genomes that contain genetic information. This genetic information tells the AVIDA software how to replicate the organism.* **Each avidan has a short circular genome, like a DNA molecule. In avidans, there are 26 different kinds of "bases" in their "DNA", represented by the letters a through z. Each different base (a through z) corresponds to a particular instruction for the AVIDA program to execute as it simulates the creature containing that instruction. A given Avidan's genome is always 50 bases long. The particular arrangement of these "bases" determines if, and how, the avidan will reproduce and behave. A sample avidan genome is shown below:**
Each circle is a "base". The different letters are the different instructions in the avidan's genome. They form a simple computer program that is executed by the AVIDA software.

2. Self-reproducing organisms. In the real world, organisms make copies of themselves; they reproduce. In Bio 112, we have looked at both asexual and sexual reproduction. *Avidans reproduce asexually like bacteria and other micro-organisms. The simplest viable avidan genome contains just the sequence of bases necessary to reproduce itself. It does nothing more than copy itself. In this way, it is the simplest possible living thing in the AVIDA world.* **The AVIDA program reads the genome of each avidan and executes the sequence of commands listed in the genome. The simplest viable avidan's genome is just the sequence of instructions needed to tell the AVIDA program to make one copy of itself. Thus, in one generation, a single viable avidan gives rise to a copy of the avidan; so now there are two avidans. In the next generation, each of the two produces a daughter, giving a total of four avidans, etc.**

3. Mutation. In the real world, DNA replication is not perfect; daughter cells have genomes that differ slightly from those of their parents. This gives rise to the variation that is necessary for evolution. *When avidans replicate, their genomes are subject to*

mutation. You can control the chance of mutation when you set up an AVIDA experiment. **Each time the AVIDA program copies an instruction from a parent avidan to a daughter avidan, there is a small (and adjustable) chance that the copied instruction will be different from the original (for example, changing an "a" to an "e"). This results in a mutation that will be passed to the offspring of the daughter.**

4. Limiting Resources. In the real world, there is not enough space, food, light, etc. for all organisms that are born to survive. As a result, some organisms reproduce more than others, so succeeding generations have a higher frequency of advantageous alleles. *In the world simulated by AVIDA, there is plenty of food, but space is limiting. Therefore, only a fixed number of avidans can be alive at any given time. This number is adjustable, but it defaults to 900.* **When an avidan is "born" (copied from parent to daughter), it replaces a randomly-chosen neighbor of its parent. Open spaces result when avidans die. Avidans are chosen randomly for death independent of genotype. Avidans are deleted randomly from the petri dish.**

In this lab, you will explore the evolution of avidans and make connections between this simulated world and evolution in the real world.

Note:

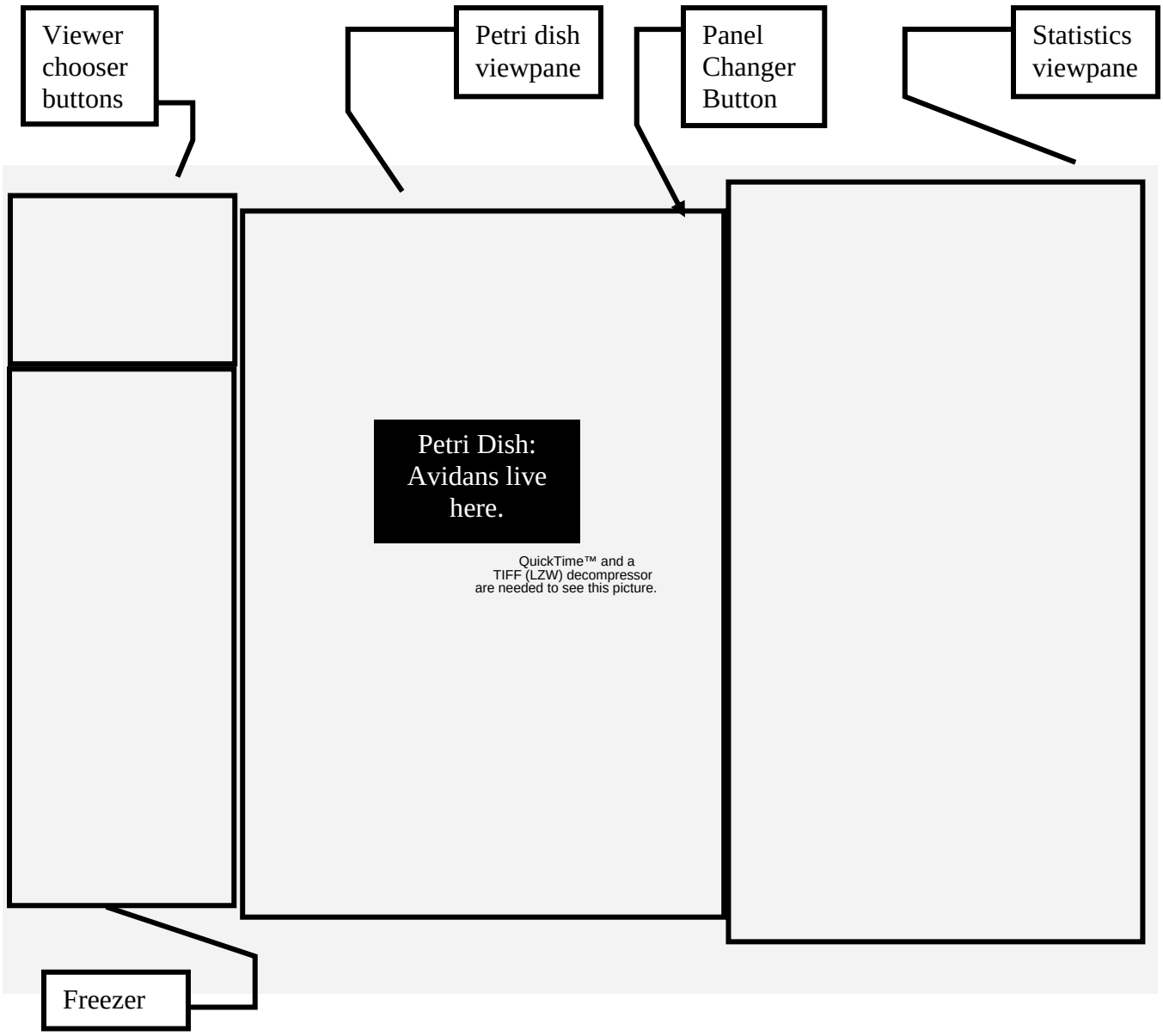
This is new software; it was just released this spring. This has three consequences. First, it may have bugs. Please report them to your TA and try to be patient. Second, the authors would like us to help them evaluate the software. You will be asked to fill out a survey and sign a consent form to have your work anonymously analyzed. This is completely voluntary, but I encourage your participation. Finally, the lab is new, so it is a little vague in places; please be patient.

Procedure

Part I: Warm-up Exercises

1) Start up AVIDA-Ed from the dock. It takes a little while to get started.

2) You will see something like this:



This is the view you will observe as your population of avidans evolves.

You now need to set up the petri dish environment, add a starting avidan, and run a simulation.

3) Click the **Panel Changer Button** (it is at the upper right of the **Petri Dish Viewpane** and is marked "Flip to Settings" to get to the settings panel. You should see this in the center panel:

This allows you to set the environmental parameters for the simulation run. The are:

- **Per site mutation rate**: This rate reflects the percent chance that an instruction is incorrectly copied. So, if the per site mutation rate is 1%, there is a 1% chance that when an instruction is copied, it will end up as any one of the 26 possible instructions (one of which is itself, so it could 'mutate' back to itself). With a 1% per site mutation rate, if 100 instructions are copied one of them will be mutated on average (although this number could be higher or lower in any instance).
- **World size**: Sets the maximum number of Avidians that can exist in the population. The two numbers specify the number of Avidians per row, and per column. So, 10 x 10 = a population of 100 organisms.
- **Ancestral seed organism(s):** The organism(s) the population begins from. Drag in organisms from the **Freezer** at the beginning of a run.
- **Environmental Resource Settings**: Avidians can receive extra energy and have increasedf fitness if they evolve the ability to "metabolize" nutrients. Here you can set what nutrients are available in the environment.
- **Exact Repeatability**: Many steps in an Avida evolutionary run happen randomly (e.g. what mutations will occur in the genome, into what cell a new organism will be placed at division), so each run will be slightly different even with the same general environmental values, as in nature. This is the default setting. However, if you need to repeat a run (e.g. for a demonstration) you can switch this to exactly replicate the sequence with the same mutations and values.
- **Offspring placement**: When an offspring is born, it can either be placed (at random) in any of the eight cells adjacent to its parent, or anywhere (at random) in the population. If the cell is already occupied the organism there is overwritten.
- **Pause Run Manually/Automatically**: If you set a specific number ahead of time, the run will pause when this many updates have passed. If you set the run to stop manually, it will continue indefinitely until it is paused using the button under the Petri dish.
- **Freeze Petri Dish Button**: Push snowflake button to save either just the environmental configuration (by saving an 'empty') Petri dish, or else the environment plus the organisms (by saving a 'full' Petri dish).

For this lab, the most important one is the **Environmental Resource Settings**. This models different resources available in nature and allows you to see how evolution would proceed if conditions were different. Some of these resources are more nutritious than others. *Although all avidans in the petri dish receive sufficient nutrition, if they are able to metabolize certain additional nutrient "sugars" (notose, nanose, etc.) they receive a substantial increase in fitness. For example, an avidan that can use notose has twice the fitness of an otherwise identical avidan that cannot. Other sugars have even higher fitness "rewards"; these are listed above the buttons in this part of the pane. Avidans that are more fit reproduce more often than those that are less fit.* **In the AVIDA**

system, an avidan is "able to use a sugar" if it can perform a particular simple numerical calculation. The AVIDA system tries to send a number to each avidan, if that avidan can read in that number and send back an appropriately-modified number, then AVIDA gives that avidan a fitness boost.

Importantly, it is easier to evolve the ability to utilize some sugars than others. That is, it takes more alterations of the starting avidan to allow it to utilize equose than to allow it to use notose. In the real world, some nutrient sources require more enzymes, or more highly-modified enzymes, to be utilized by an organism. *The ancestor avidan cannot use any of the sugars in the* ***Envoironmental Resource Settings****. Some mutant versions of the ancestor can use some or all of these sugars. It requires only a few mutations to make an avidan that can use notose; it requires many independent mutations to use equose.* **In order to use any sugar, the avidan must include instructions for getting a number from AVIDA and returning it to AVIDA. It must also include instructions for the particular mathematical manipulation. Some manipulations are simple, like the one for notose, and take only a few more instructions; others are more complex, like the one for equose, and take many additional instructions.**

You should leave all the other settings at their default values in this part of the lab. You may want to play with some of them in Part II.

4) For this first run, you should **turn off all of the sugars in the Environmental Resource Settings**. Click the checkboxes on all of them until they are all unselected. In this state, all avidans receive minimal nutrition and there is no added fitness associated with being able to use any of the sugars.

5) Load an ancestor avidan into the petri dish. In the **Freezer**, look under "Organisms". Click on "@ancestor" and drag it into the **Ancestral Organisms** pane described above. You should see this in the pane:

Now you are ready to run a simulation and let these organisms evolve.

6) Click on the "Flip to Petri Dish" button in the upper right of the **Environmental Settings Panel**. This will take you back to the petri dish view.

7) Start the simulation. At the bottom of the **Petri Dish viewpane**, you will see these buttons:

Click on the Start/pause button and the simulation will start.
You will then see several things happening:

- Colored squares will start appearing in the petri dish - these are avidans being born.
- The **Time (Updates)** will start to increase to indicate that the simulation is running.
- The graph at the lower right of the **Statistics Workpane** will start being drawn to show the average fitness of the avidans in the petri dish.
- The **Population Statistics** numbers at the upper right of the **Statistics Workpane** will start to change.
- The arrow button will change to a "||" - pause - button.

8) Quickly pause the simulation by clicking the pause button after about 50 updates.

9) You should look at the various displays and discuss as a class what they mean:

- **The Average Fitness Graph**. The ancestor has a fitness of 0.25. You will note that the average fitness of the population has fallen. Provide a plausible explanation for this observation.

- The **Color Scale Legend** - the rainbow stripe just below the petri dish. This is the color code for the avidans in the petri dish - their color depends on their fitness. This is useful because, in addition to showing the fitness of each avidan, it is constantly updated as the fitness of the creatures increases. Therefore, if you look at the maximum value of this legend, it gives you the fitness of the most fit avidan currently in the petri dish. Click on an avidan with a low fitness and look in the **Statistics Workpane** under "Org. Clicked on Report" to find the exact fitness of the avidan you clicked on.

10) Click the start (arrow) button to continue the simulation. Let it run until the petri dish is full and the **Population Size** is about 900 and then click the pause button. From the menu under the graph, choose **Number of Organisms** and you will see a graph of the number of organisms over time. What kind of growth does that show (linear, exponential, logistic)? Why?

11) Click the start button and let the simulation run until about 300 updates have passed, then click the pause button. Look in the **Population Statistics**, you should see something like this (your numbers will be different):

This display shows the number of avidans in the population that are able to use each of the different sugars. You can click on the buttons to identify the organisms that are able to use that sugar. If you click on one of the identified avidans, you will see that its fitness is not higher even though it is able to use the sugar. You should discuss the following questions as a class:

a) Why is it that the avidans that can use notose do not have significantly higher fitness here? What does this have to do with evolution in the real world?

b) How is it possible that these avidans have evolved to use these sugars even though the sugars are not present? What does this have to do with evolution in the real world?

c) Pool the class' results of how many avidans could use each of the sugars into a table on the blackboard (one column for each group; one row for each sugar). Why aren't the numbers the same for all groups? What does this have to do with evolution in the "real world"?

d) What is the range of fitness (lowest to highest) of the organisms in your population?

e) Note the **Average Fitness**; we will use this data later.

12) Now it is time for a new run. Select **Start New Experiment** from the **Control** menu (or hit apple-R) and click "Discard and Start New Experiment". The petri dish will clear.

13) Click the "Flip to Settings" button.

14) Set up for your next run:

- Start with one @ancestor as in step (5).
- Set the environment to contain one and only one sugar: **notose** (upper left of the list). Be sure that notose and only notose is selected.
- Click the "Flip to Petri Dish" button.

15) Click the run (arrow) button and let the simulation run for about 300 updates and then click the pause button. You should then answer the following questions as a class:

a) What is the **Average Fitness**? How does it compare to the average fitness you observed in Step (11e)? Provide a plausible explanation for this result.

b) What is the range of fitness values in your population? Does it differ from your answer to (11d)? Provide a plausible explanation for this result.

c) Pool the class' results of how many avidans could use each of the sugars into a table on the blackboard (one column for each group; one row for each sugar).
    - How do these numbers differ from those you saw in (11c)? Provide a plausible explanation for this result. What does this have to do with evolution in the "real world"?

    - Why aren't the numbers the same for all groups?

Part II: Your Own Experiments

In this part, you will use AVIDA to address an evolutionary issue. You will choose an issue to address from the list below or devise one of your own. You will then devise a strategy to use AVIDA to address this issue. After your TA has approved your plans, you will carry them out and answer the following questions; you should type these up on the computer using Microsoft Word and print them out for your TA. This will be your **Lab Report**.

1) The names of the members of your group.
2) The issue you decided to address.
3) Your strategy for addressing it.
4) Your results.
5) Your conclusions.
6) How your conclusions relate to evolution in the real world.

Here are some sample issues; choose only one from either list or think of one of your own.

The first set are common misconceptions about evolution:

a) *Individual* organisms increase in fitness over their lifetimes.
b) Mutations always make organisms *more* fit.
c) Mutations always make organisms *less* fit.
d) The presence of the selective agent (for example, the presence of a new nutrient) *causes* mutations that make organisms more fit.

The second set are questions based on what you have observed:

e) What is the effect of changing the mutation rate on adaptation to a new nutrient?
f) Will mutations to allow use of a "hard sugar" like ornose happen faster if an "easy sugar" like notose is present?
g) What is the effect of different combinations of sugars on the numbers and sugar using abilities of the avidans that evolve?

Part III: Exit Survey

You should fill this out and return it in lecture next week.