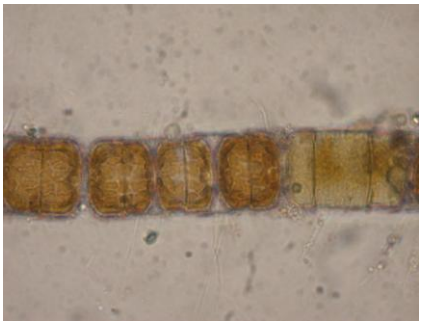
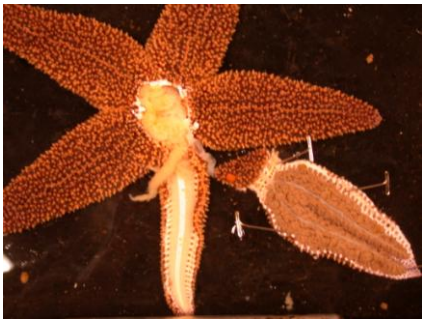
Laboratory Manual
Biology 112
Fall 2011

Name _____

TA _____

Lab Section/day and time _____

Biology 112 Laboratory Syllabus Fall 2011

* Prelab assignments are located at the beginning of the corresponding section in the lab manual and are due at the scheduled start of lab.

Week of	**Laboratory Exercise**	**Page(s)**	**Prelab assignments*due**	**Assignments due**
September 5	None			
September 12	HMNH Field Trip	3-16		
September 19	Skulls and Evolution Molecular Phylogeny	17-40	Skulls and Evolution & Molecular phylogeny prelab (10 pts)	HMNH Field Trip questions (30pts)
September 26	Aipotu: Evolution	41-60	Aipotu: Evolution prelab (10 pts)	Skulls and Evolution & Molecular Phylogeny assignment (30 pts)
October 3	Microbial Diversity	61-70	Microbial Diversity prelab (10 pts)	Aipotu: Evolution assignment (30 pts)
October 10	Plant I Diversity: Life Cycles & Greenhouse Tour	71-84	Plant Diversity prelab (10pts)	
October17	Plant II Structure & Flower Dissection			
October 24	Plant III Growth	85-92	Plant Growth prelab (10pts)	
October 31	Animal Diversity I	93-112	Animal Diversity I prelab (10pts)	Plant Growth assignment (20pts)
November 7	Animal Diversity II: Trout dissection Squid dissection	113-132	Animal Diversity II prelab (10pts)	
November 14	Practical Exam			(100pts)
November 21	None		None	
November 28	Animal Behavior	133-150	Animal Behavior prelab (10pts)	
December 6	Phylogenetic Collection	151-164		Animal Behavior lab report (20pts) Phylogenetic Collection (20pts)
December 12	None		None	Phylogenetic Report (30pts)
			80 points	280 points
				360 points total

Field Trip: Harvard Museum of Natural History (HMNH)

Note: There is no pre-lab for this lab.

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; tear it out and take it with you). 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. Tickets will be given out in class to the HMNH; this will get you free admission (it is normally $7 for students). You can go to the HMNH anytime that the museum is open. TAs will tell the class when they will be at the museum. The HMNH is open daily 9:00 AM to 5:00 PM. Admission is free (even without a ticket) Sundays from 9 to 12.

YOU SHOULD BRING YOUR TEXT FOR REFERENCE.

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 (see your text) and familiarize yourself with terms like kingdom, phylum, etc.
• The following phyla can be found at the HMNH; you should go through your text 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 • coniferophyta
• arthropoda • platyhelminthes
• cyanobacteria • lycophyta • mollusca

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

Phylogenetic Data Gathering and Expression

When studying evolution, it is very important to choose the *characters* - the particular features of the organisms under study - very carefully. It is important to start thinking rigorously in this regard and you will notice that it is useful when making arguments based on observations of organisms. You should strive to be very specific about the characters and traits you are comparing and to specify these in a table format.

First, some definitions:

• **Character** - a feature of an organism. For example, "leg form" or "number of eyes".
• **Trait** - a particular form of a character. For example: the character "leg shape" could have the traits "long", "bent", and "none"; these would be used to describe organisms with long legs, bent legs, and no legs. Similarly, the character "number of eyes" could have the traits "two" and "none".

When answering the questions in the lab manual that require this format, you should first examine the organisms in question, then make a list of the characters you will study, and finally compile a table it could be like the one below (a hypothetical table based on comparing some small animals). The table has one row for each organism and one column for each character; the cells in the table contain the traits.

Organism	**Segmented body?**	**Legs**	**Exoskeleton**
Honeybee	Yes	6	Yes
Ant	Yes	6	Yes
Millipede	Yes	250	Yes
Slug	No	0 (1?)	No

When making tables like this you should use at least 4 characters; you can use more if you like. You could then make an argument that there are two groups of organisms based on this data. It could go something like this, "There are two groups of organisms here. One has an exoskeleton, segmented body, and 6 or more legs - the honeybee, ant, and millipede are all part of this group. The other group lacks these features and includes the slug. The reason these are two different groups is that members of the first group share three of the characters listed with each other while the other does not.

Thus, the members of one group are more similar to each other than they are to the slug."

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 on your lab manual from which you can study the answer to the questions below. You will not hand in these questions, but you do need to think about and answer the questions; it is not necessary to assemble your answers into a larger essay. This material could be on your practical exam. Your TA will go over them with you next week, and may call on your particular examples to be discussed.

Assigned Questions:

• Important note: these questions are difficult and involve some speculation and interpretation on your part.

Our purpose is to get you thinking about these issues rather than to emphasize a specific right answer. Your answers should be reasonable and clearly-explained, so you can recall them for in class discussions. Do not plagiarize, your answers must be your own and cite any references used.

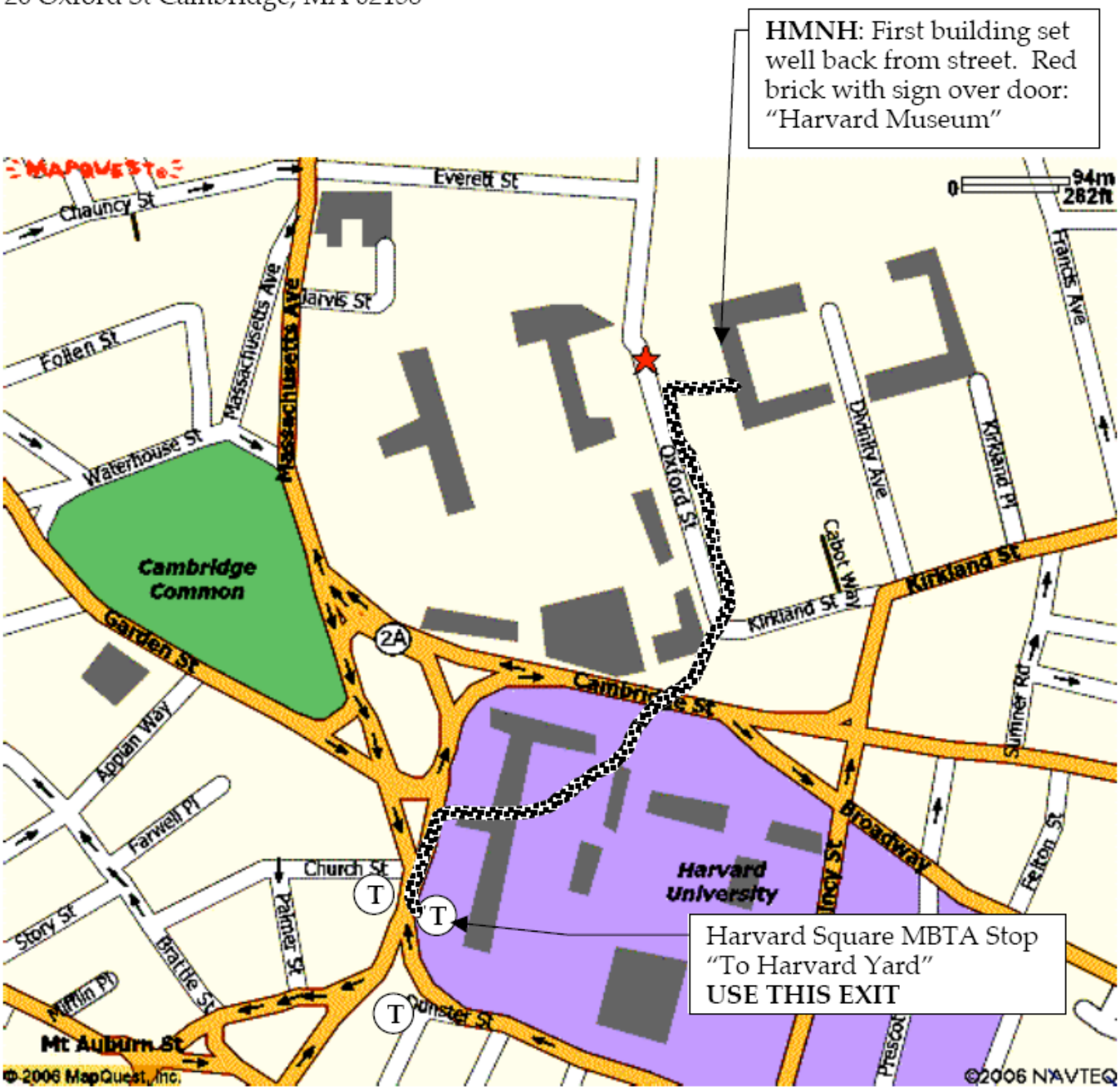
• Though not to be handed in, you may want to type out your answers to make it easy to study them and use them in a class discussion include hand-drawn and labeled drawings. This information could be on the practical and will probably help you in lecture.

• Although you will perform these activities as a group, each member of the group should be able to recall and discuss the answers to the questions.

• Answer all the questions in the lab manual.

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) Flowers and Pollinators For this question, you should visit the *Glass Flowers* Exhibit gallery. This is the first gallery you come to at the top of the stairs by the Gift Shop. The Glass Flowers are FRAGILE. Please do not lean on or bump the cases.

Flowers are so variable because they have evolved to attract certain pollinators. There are many different types of pollinators: bees, butterflies, moths, ants, beetles, flies, birds, and even mammals. Some pollinators feed on the pollen itself. Many seek another reward — nectar, which the plant makes just for them. As they feed on nectar, these animals are dusted with pollen and inadvertently carry it from flower to flower, thus allowing the plants to mate without having the ability to move. The flowers you will look at could be pollinated by one or more of the following pollinators:

Hummingbird

Wants: Nectar from the base of the flower. Can feed while hovering — doesn't need to land.

Sees: Reds and oranges.

Uses: Its long beak to suck nectar.

Bee

Wants: Pollen and/or nectar. Likes something to land on.

Sees: Some colors — white, yellow, blue. Stripes, dots, or bull's-eye patterns help guide the bee to the center of the flower.

Uses: Pollen sacs on its legs to carry pollen, and its mouth to eat nectar.

Butterfly

Wants: Nectar and a surface to land on for feeding (can't hover while feeding).

Sees: Bright colors, including pink, red, yellow, orange, and purple.

Uses: Its proboscis (long tongue) to sip nectar.

Look at the flowers listed below. Using the descriptions above and your observations of the flower, choose which pollinator(s) you think would pollinate that flower. Explain your reasons why. Pollinators can be used more than once or not at all.

Plant name	Pollinator	Explanation
Blue flag, *Iris versicolor* C21		
Milkweed, *Asclepias syriaca* L63		
Trumpet creeper, *Campsis radicans* M76		
Black-eyed susan, *Rudbeckia speciosa* O90		

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 bird (Northern Harrier) skeleton can be found in case C6 on the balcony in the Hall of Mammals with the hawks.
- 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.

All three wing structures are based on the same tetrapod vertebrate arm and five-fingered hand structure that is shown in *Campbell*, 8th ed. figure 22.17. Using figure 22.17 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 22.17}
- 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 22.17, you would say something like, "The cat's foot is like a human hand, but it walks in its tiptoes."

3. The Functions of Color in Animals

Animals show tremendous diversity of color and pattern in their bodies. Why? View the exhibit "The Language of Color" at the museum and answer the questions below.

a. List 4 different ways that animals use color. State a specific example of each using genus and species names of the animals.

b. From the list of 4 ways animals use color above, state which one you think gives an individual an increased probability of survival. Explain your answer.

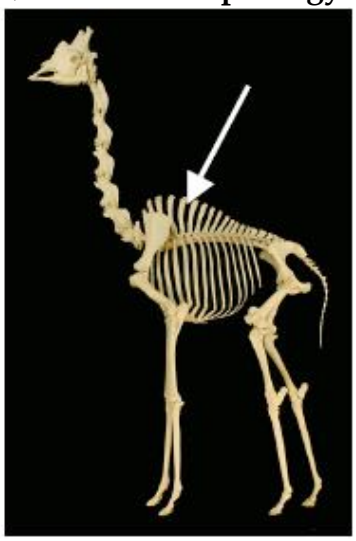
c. From the list of 4 ways animals use color above, state which one you think gives a population an increased probability of survival. Explain your answer.

d. Some animals use "mimicry" as a technique for survival. Give a specific example of an animal that uses this with genus and species names and describe how a species could evolve to "mimic" another species.

e. Describe 2 different ways that animals derive their color.

f. How is a bird's vision different from a human's? What can they see that we can't?

4) Skeletal Morphology and Function A giraffe skeleton is shown at the left. 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. 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 and very long and heavy neck, has very large neural spines.



For each of the following animals: (Moose, Whale, Human)
a) state whether the neural spines are **large** defined by being **larger** than the corresponding projections on the lumbar vertebra like the giraffe's, or **small,** not much larger than the corresponding projections on the lumbar vertebra. Note that it is *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*** *in comparison to its size.*
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.**

	a) **Large** neural spines relative to lumbar spines ?	**b)** Plausible explanation for why or why not
Moose		
Whale		
Human		

5. The Arthropod Exhibit

Choose 3 types of arthropods from different classes. Describe the characteristics that show they are all arthropods. Describe the differences between them. In what class does each of your organisms belong? What is the habitat of each organism and what adaptations allow it to survive in that habitat?

Arthropod name			
Class to which it belongs			
Arthropod Traits			
Class Characteristics (differ)			
Habitat			
Survival Adaptations			

6) Invertebrates: These include all animals without a back bone. They range from sponges to insects, and greatly outnumber the vertebrates in both number of individuals and number of species.

6a) What are at least two major problems that confront an animal without some form of internal skeleton?

Many invertebrates, such as lobsters and insects, have a skeleton, but rather that being inside the body, the skeleton forms a shell on the outside. It is called an exoskeleton and is composed of hard proteins, a cellulose type substance called chitin, and a very thin layer of lipid.

6b) What is one obvious problem that an exoskeleton causes?

6c) List by phylum and scientific or common name three invertebrates that do not have a head. What important features of their mode of life are associated with the absence of a head?

6d) What functions are located in heads?

7) Fish:

After you have examined some of the bony and cartilaginous fish, return to the rather primitive coelacanth (pronounced "seal'-a'-kanth"). This fish is an example of an animal at an extremely important stage in the evolution of vertebrates. It represents the potential for the vertebrates to leave the water and invade the land. If you look carefully at the so-called lobe-fins of the fish, you will notice that they are in approximately the same position as the limbs of any four-footed terrestrial vertebrate. It is thought that fish such as the coelacanth (once thought to be extinct, but recently found to be still living) gave rise to those forms that first moved from the water to the land. The first land vertebrates were still essentially fish, but they had lungs as well as gills and could breathe air rather than having to extract oxygen from the water. They also had fleshy lobe-fins which differed considerably from the fins of the modern fishes that are more familiar to us. These lobe-fins not only had a primitive musculature but the distribution of the bones within the fin is similar to the patterns of bones in the locomotion once they moved to the land.

7a) What are the basic structures and modifications that you observe in modern fish that are adapted to their life in the water?

8) Reptiles: Look carefully at the examples of reptiles. The development of a scaly skin in these animals prevents their bodies from drying out. They also developed internal fertilization, and eggs with a tough, leathery covering, so that even the eggs no longer need the presence of water. In the history of life, reptiles were the first vertebrates to live entirely on land.

8a) What are some other adaptations that differentiate the reptiles from the amphibians?

For a long time, (about 100 million years), reptiles were the dominant forms of life on the earth, especially during the age of the dinosaurs ("terrible lizards"). The dinosaurs were dominant for many millions of years, but except for some related forms (like turtle, alligator, and the giant Komodo lizard), all died out. The cause of the dinosaurs' extinction is still debated. This museum has the world's oldest egg, from a dinosaur, located in the room with the coelacanth. Its age is estimated at 225 million years.

8b) Which reptiles do not have four limbs?

8c) Do all reptiles look alike? Name at least four reptiles that differ from each other and list the characteristics that make them different.

8d) Mentally compare the reptiles you have seen to vertebrates known for high-speed running. Do you think reptiles would be efficient runners? Why or why not?

9. **Hunting for Headgear exhibit: True Horns, true Antlers and other head structures.**
9a. Define Artiodactyl. (use your phylogenetic knowledge for a full description)

9b. Find an example of an organism with true horns, one with true antlers and one with a structure that looks like a horn but doesn't form in the same way. Is it sexually dimorphic in regards to the headgear? Give the class and phylum. How are these structures useful to these organisms?

	Name of organism, Class and Phylum	Sexually dimorphic headgear ?	Advantage of the structure?
True Horn			
True Antler			
Horn like structure but not a true horn			

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Name_____ Pre-Lab: Skulls and Evolution

1. How do the brow ridge, cranial ridge, brain case size and forehead size compare between the skulls of *Australopithecus afarensis*, *Homo erectu*s and *H. sapiens* in the figures found in your textbook? Use general observations.

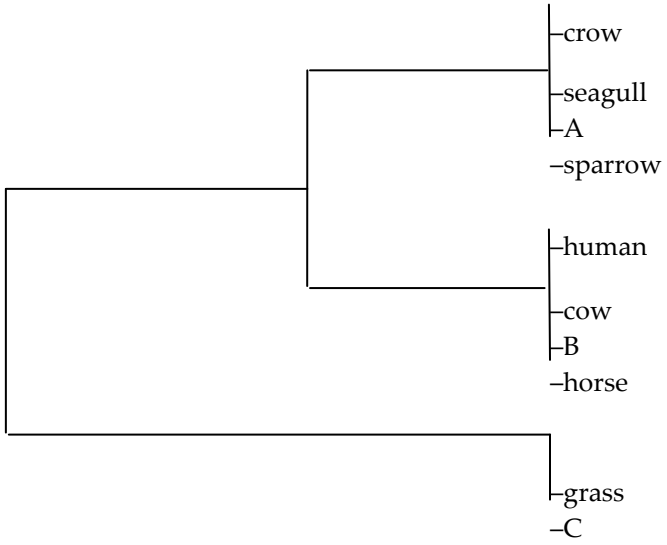
2. Given the following data:

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



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 habitats and food sources.

Introduction

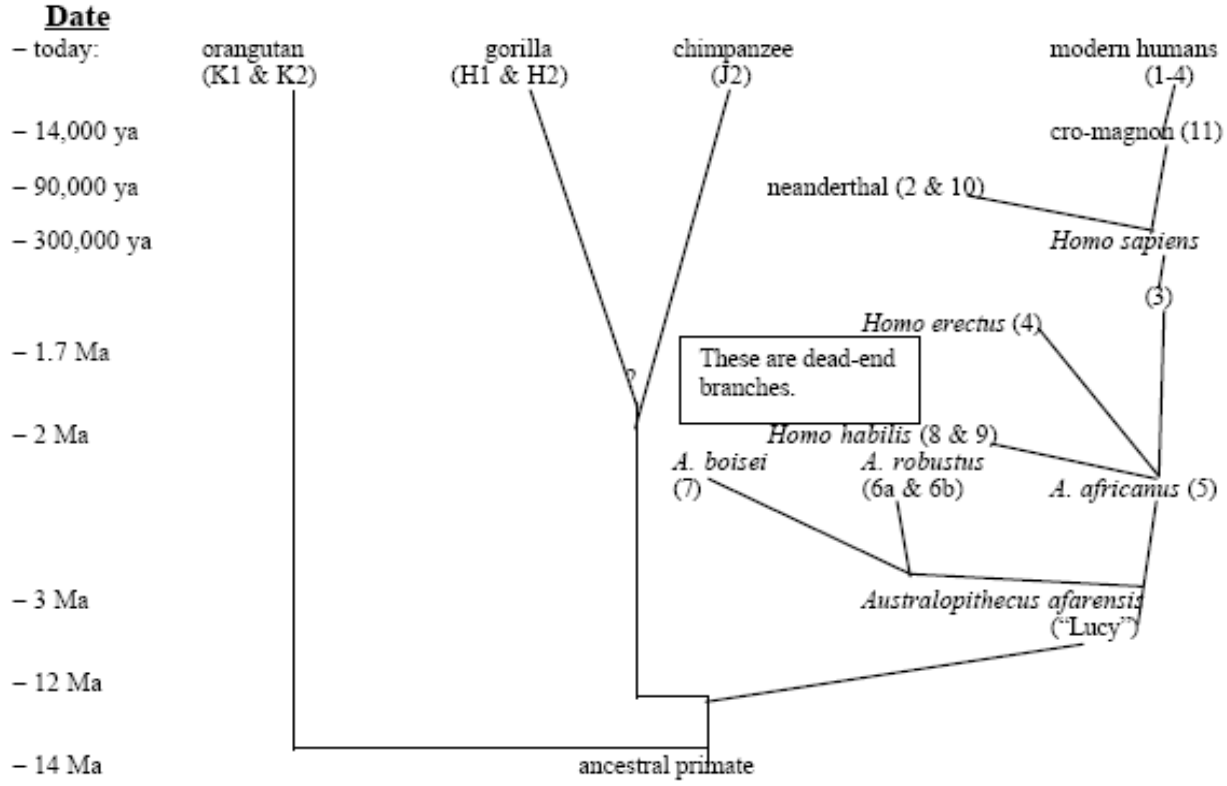
Much of what we know about evolution comes from the study of comparative anatomy. In many cases, bones (either as fossils or skeletons) have been useful in these studies. Bone and skeletal structures can reveal how an animal moves, eats, reproduces, etc. In this lab, we will look at the skulls of various 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 by most scientists, many of the details (exact dates & branching patterns) are still subjects of debate. 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.



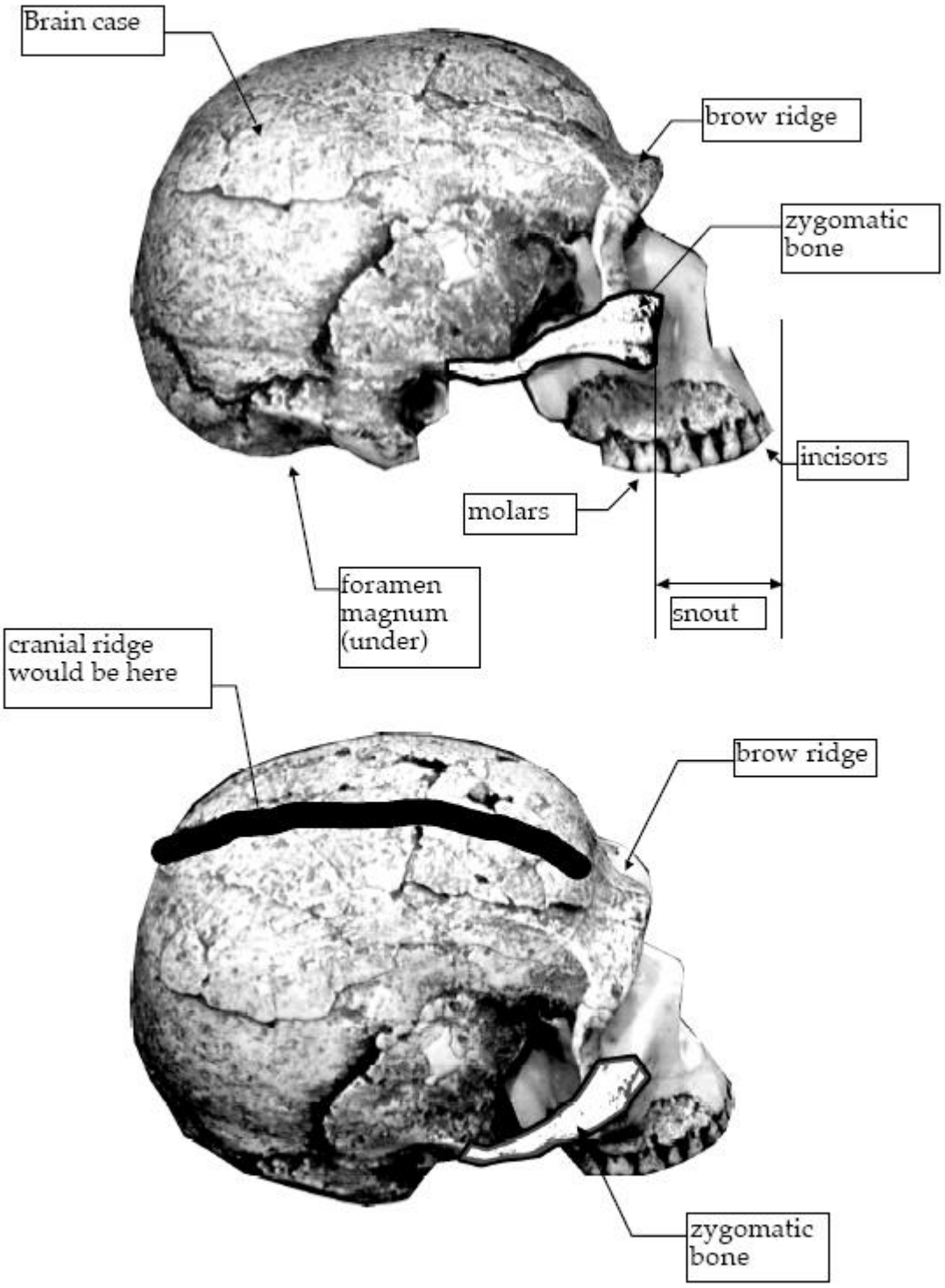
From the comparison of skulls from different primates, eight (somewhat overlapping) trends in the evolution of humans have been found. Note that not all traits in a given skull will be equally 'human' – that is, you will likely find skulls where one feature is ancestral and others are modern. This chart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

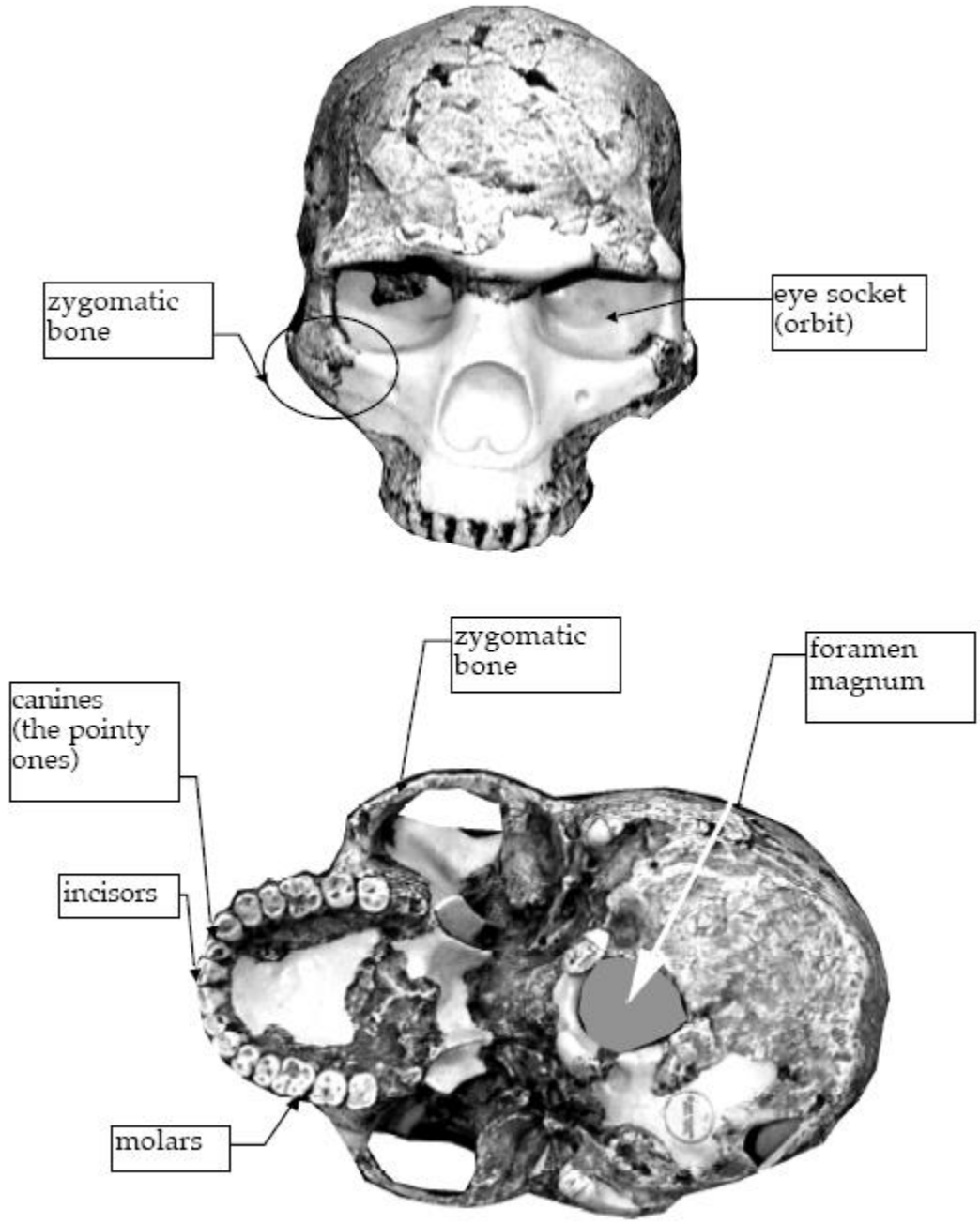
Table 1.

	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 rectangular	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:





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, chimp, and gorilla are considered 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 three skulls, one from a carnivore (exclusively meat-eating: leopard, or cougar), one from an omnivore (eats both meat and plants: wolf or Great Dane), and one from an herbivore (exclusively plant-eating: deer or sheep). The skulls will be marked with the animal they came from.

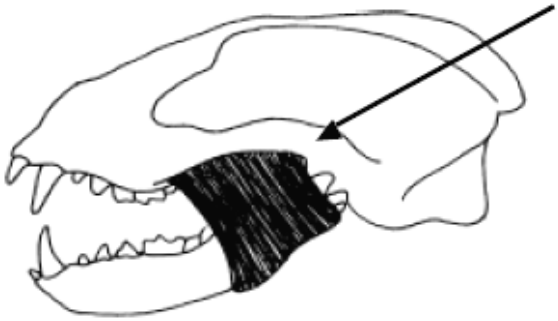
5) Consider the following features and determine the trends in these features as you go from carnivore to omnivore to herbivore.

Table 2.

	Feature	Details	Explanation
1	Canine teeth	• present? • large or small	Used for cutting and tearing of food.
2	Molars	• flat • pointed	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 & Temporalis Muscles

These muscles are found in all mammals. They are different sizes and have slightly different attachment points depending on the animal's 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,

Dental Formula

The dental formula is a way of expressing the number and types of teeth that an animal has. There are 5 types of teeth (adapted from http://animaldiversity.ummz.umich.edu/site/topics/mammal_anatomy/kinds_of_teeth.html):

- **Incisors** (I) These are the most anterior teeth. Incisors are usually simple teeth, though the crown is sometimes lobed. In many species, incisors are used as pincers for grasping or picking, both in feeding and in grooming; they are also used for biting, cutting, and stripping.
- **Canines** (C) All mammals have a single canine in each quadrant, if they have canines at all. These teeth are often absent; when present, the canines are the first tooth in the maxilla. They tend to be moderately to very long, and most commonly they consist of a single cusp with one root (but there are exceptions). Canines are most often used for stabbing and holding prey, and it is in herbivorous species that they are often reduced in size or missing altogether. Canines are used by some species as weapons in social displays or fighting.

- **Premolars** (P) The premolars lie immediately posterior to the canines. In the upper jaw, they are found in the maxillary. Premolars are usually, but not always, slightly smaller and simpler than the molars that follow them. They are distinguished from molars because premolars are deciduous; that is, there is a milk set that is later replaced by an adult set.
- **Molars** (M) The most posterior teeth in the jaws of most mammals are molars. As with premolars, they vary tremendously in size, shape, and function. The completion of their eruption is usually delayed until the individual reaches near adult size.
- **Post Canines** (PC) These are found posterior to canines in seals, dolphins, and whales instead of molars. A dental formula specifies the teeth, reading from anterior to posterior of one half of the jaw. You start in the middle of the two front teeth and work your way back. The number of teeth in one side of the upper jaw is written over the number in one side of the lower jaw.

For example, consider the human skull shown at the bottom of page Skulls-4. You start with the midpoint between the incisors at the front of the skull and move down one side to the rear of the skull. There are 2 incisors, 1 canine, 2 premolars (much narrower than the molars), and 3 molars. Although it is not shown, the lower jaw has the same pattern.
Therefore, the dental formula would be: I 2/2 C1/1 P2/2 M3/3 .

This translates as "on one side of the upper jaw, there are 2 incisors, followed by 1 canine, followed by 2 premolars, followed by 3 molars; the lower jaw is the same." That gives a total of 16 teeth on one side of the skull; multiply by 2 to get the total number of teeth in that skull = 32 which is typical for an adult human.
It will not be possible to determine the type of some of the teeth you find today (especially molars vs. premolars since we are only looking at skulls of adult animals), so you should try your best and discuss your conclusions with your lab mates. *We will therefore grade this part of your write up generously.*

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
In this part, you will use the skulls of relevant animals to collect data to answer the two questions that follow. You should use the techniques for looking at skulls and the features you have seen in the other skulls as you formulate an answer to these questions.
We have provided you with the following skulls:

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

a) How would you group the skulls of the marine mammals ? A full-credit answer to this question consists of two parts:

• An explanation of why you chose the groups that you chose. We are not interested in one "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? This part must include a *data table* with an explanation of how you used the data in the table to draw the conclusions you drew.

b) Which of the skulls listed that you have in lab 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 your observations of the skulls, you must decide which land mammal is most closely-related to seals. 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 mammal. This part must include a *data table* with an explanation of how you used the data in the table to draw the conclusions you drew. Again, we are not interested in the "right" answer; just a well-reasoned argument based on your observations.

The more specific about the data you are and the more clear your argument is, the more credit you will get.

Lab Write Up:

• Must be typed; handwritten assignments 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 write up. Each person's report must be in his or her own words as much as possible.
• Your lab write up 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. **Include in your answer your data table**.

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

Part II: Comparisons 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 these labeled pages to your write up.

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

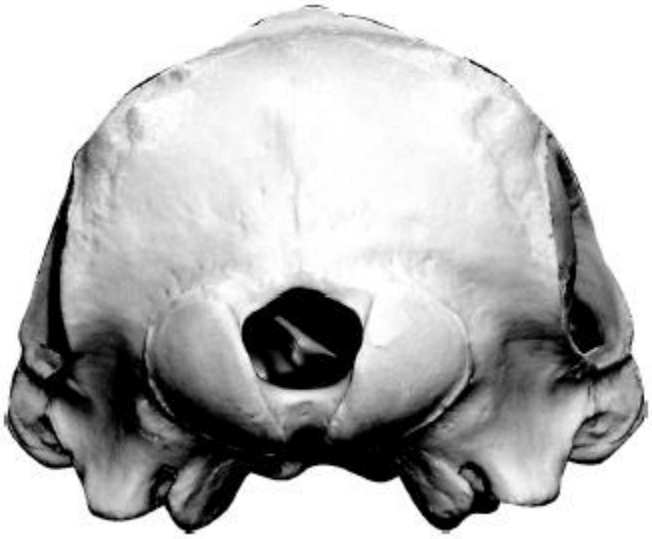
7) The answers to questions (a) and (b) from page Skulls-10.

Dolphin Worksheet (attach to your write up)

An intact dolphin;
left side view.

• Dolphin skull; left side view:

Rear view:



Top (dorsal) view:

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 respiring 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. 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.

You will use a computer program called clustalw, which takes a group of protein or DNA sequences and determines the most likely phylogenetic relationship between them. This software takes into account the number of differences between the sequences as well as the locations and nature of the differences. There are many such programs that use different methods and assumptions. You should remember that clustalw generates the most likely tree, but not necessarily the way the organisms actually evolved.

Procedure

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

The amino acid sequences of cytochrome c from many organisms (as well as many other protein and DNA sequences) are stored in a database that is accessible from the web. In general, the software runs SLOWLY, so be patient. You can also access all of the resources for this lab from any computer with www access.

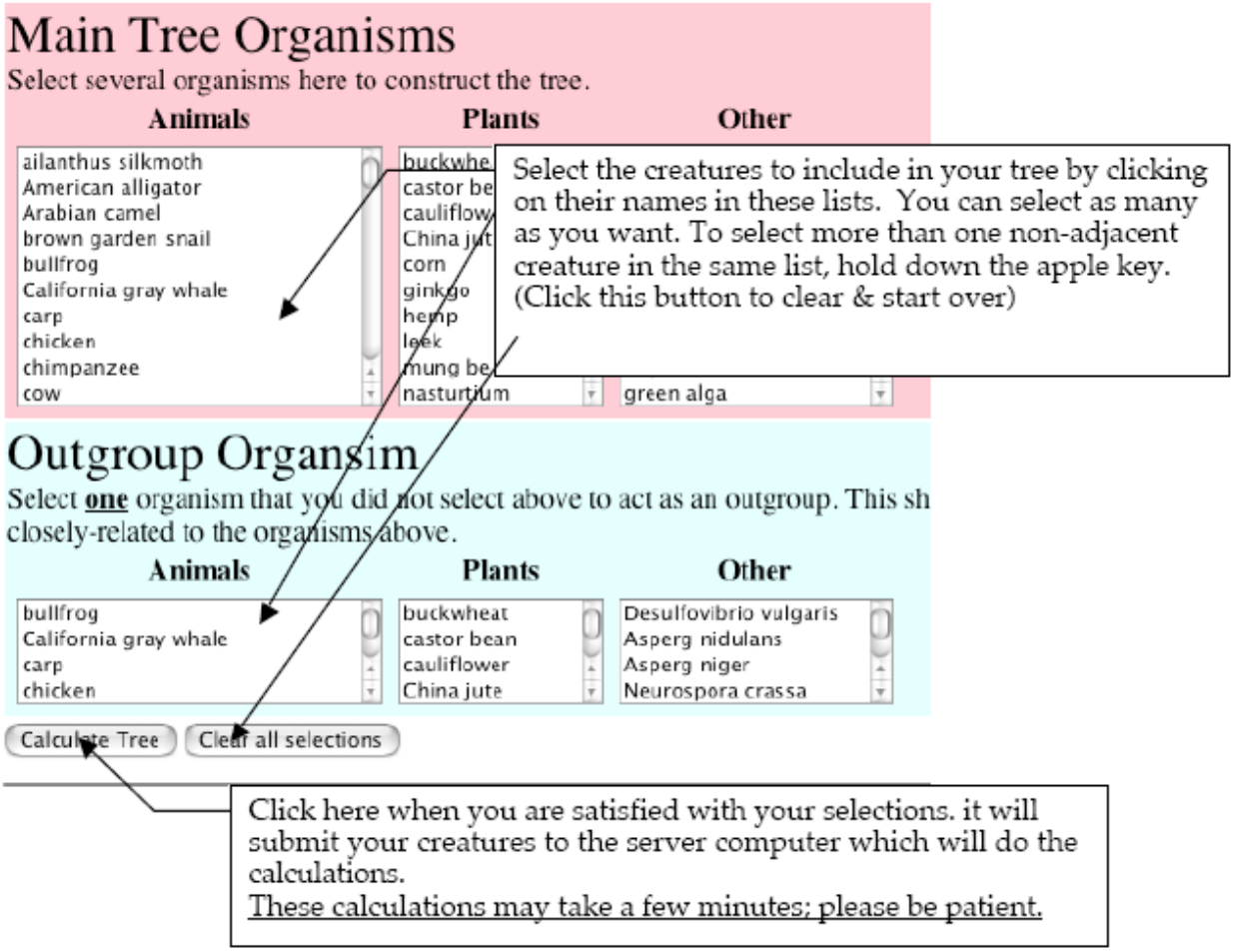
In this part of the lab, you will use the software to show you the number of differences between two protein sequences - this will help you to understand how this information is generated. You will then use this information to construct a simple tree manually.

Part I. Draw a phylogenetic tree for 3 organisms of your choice.

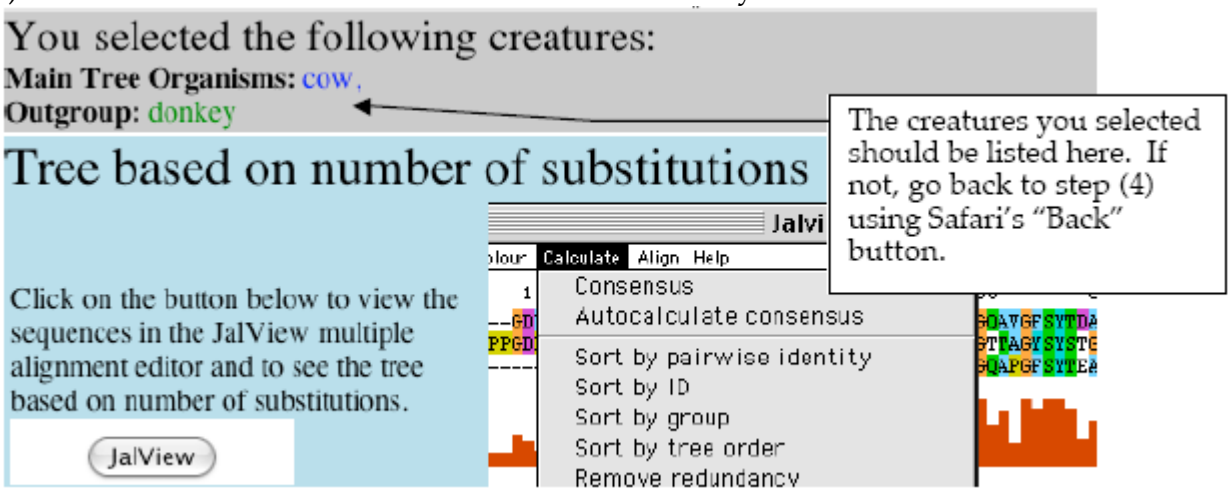
1) To access the "Tree Constructor", it can be found on the course lab website, which is linked to the main biology website (http://www.bio.umb.edu) look for "Bio 112 Fall 09" and click on it.

2) Find the title to this week's lab listed and click on it (Molecular Phylogeny). "New Phylogenetic Tree Constructor" should open.

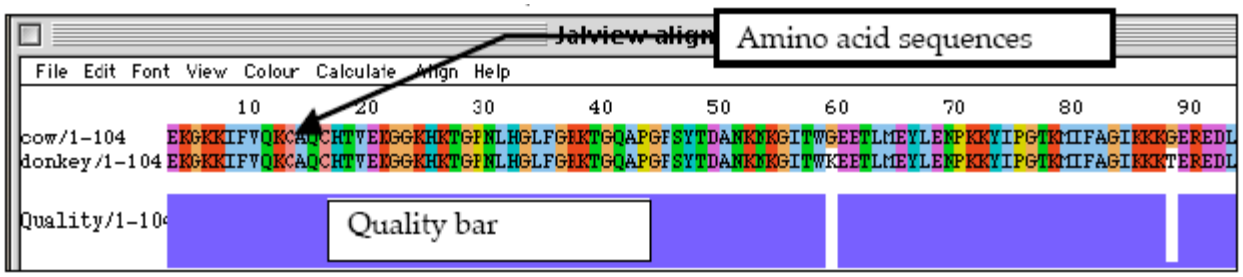
3) Choose two organisms that you think are closely-related. Select one in the "Main Tree Organisms" and one in "Outgroup Organism". You have to select one in each set or the program will complain. In this example, I have chosen "cow" and "donkey". You should choose two other organisms that are closely-related.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



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



5) 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).

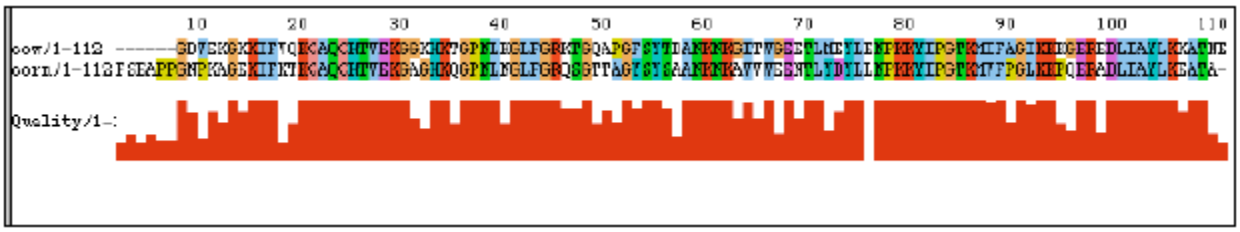
From this, we can conclude that there are two amino acid differences between the cytochrome c's of cow and donkey. We would then say "cow and donkey differ by 2 substitutions".

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

7) 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.

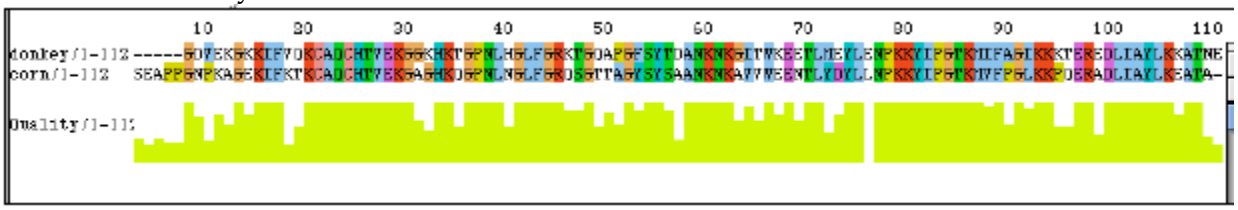
I chose corn as my distantly-related organism. Here are the results I got:

• 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.

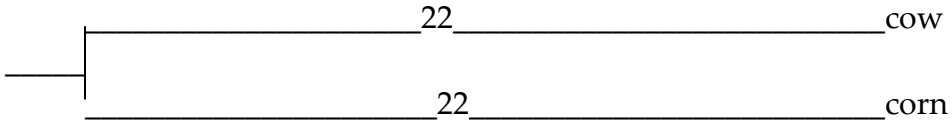
• corn vs. donkey:



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

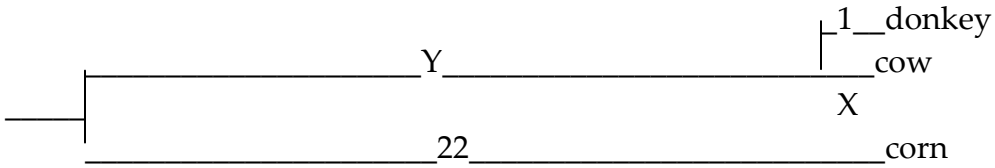
8) Make a phylogenetic tree of your three organisms based on the substitution data. Here is a simple way:

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.

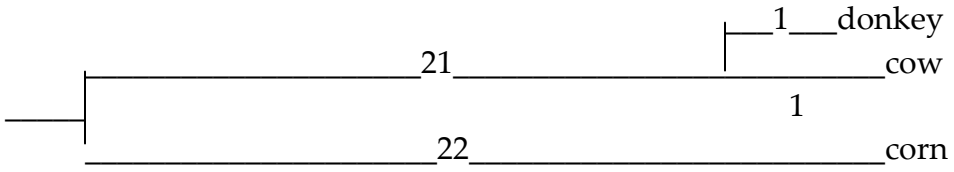


Note that the total distance between cow and corn is 22 + 22 = 44.

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:

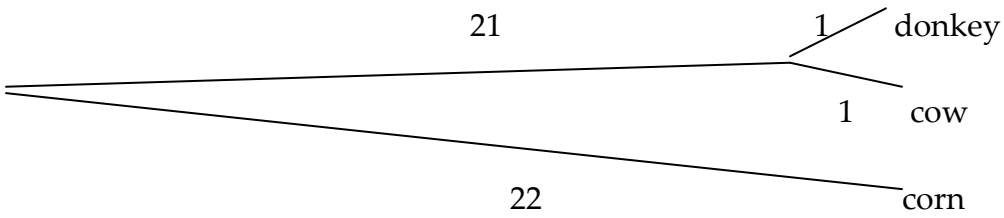


iii) But what about the "X" and "Y"? Since the distance between cow and donkey must be 2, X + 1 must = 2. Therefore X = 1. Since the total length from the branch at the left to cow must equal 22 and X = 1, Y = 22 –X or 22 – 1, or 21. This gives the final tree:



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

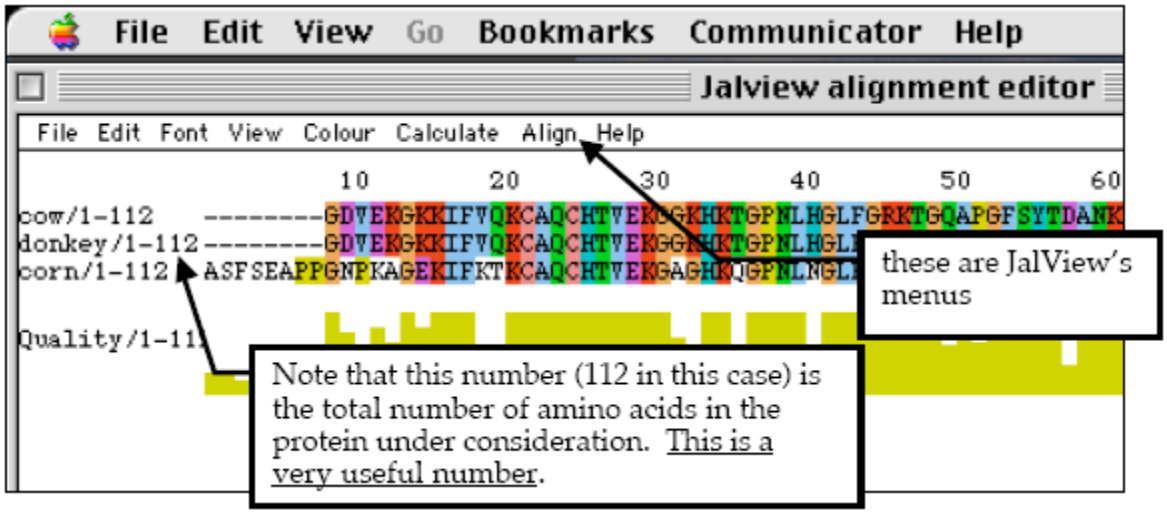
- The lengths of the vertical lines are not counted in the branch lengths. Therefore it is identical to this tree:



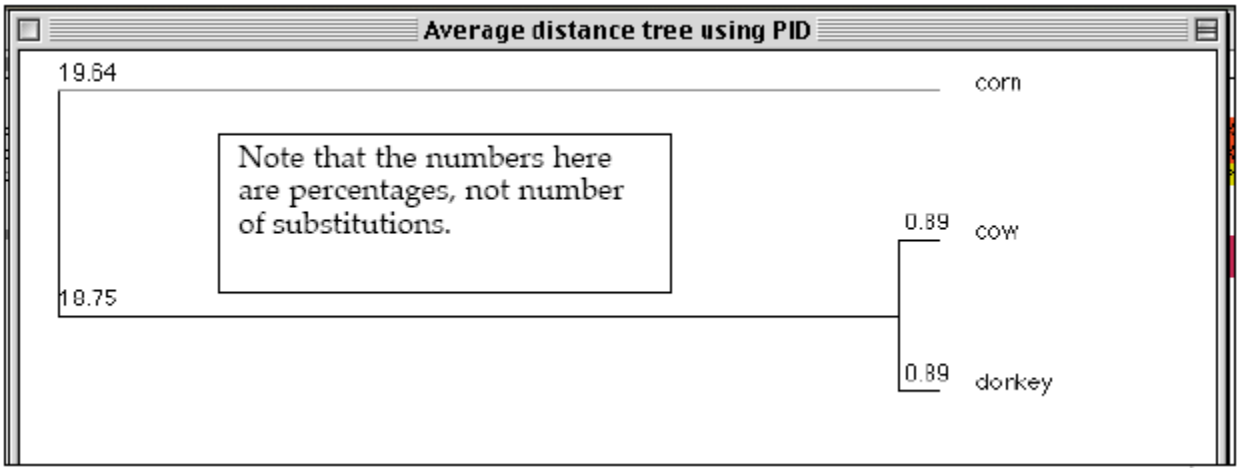
- It is approximate! The distance from donkey to corn should be 44 substitutions (as measured from the sequences) and that is what the tree shows. Sometimes, it comes out like this and sometimes the numbers don't add up properly. This is what we call "close enough for government work".

9) 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 this:



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 0.196 x 112 = 22 (which exactly matches 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)

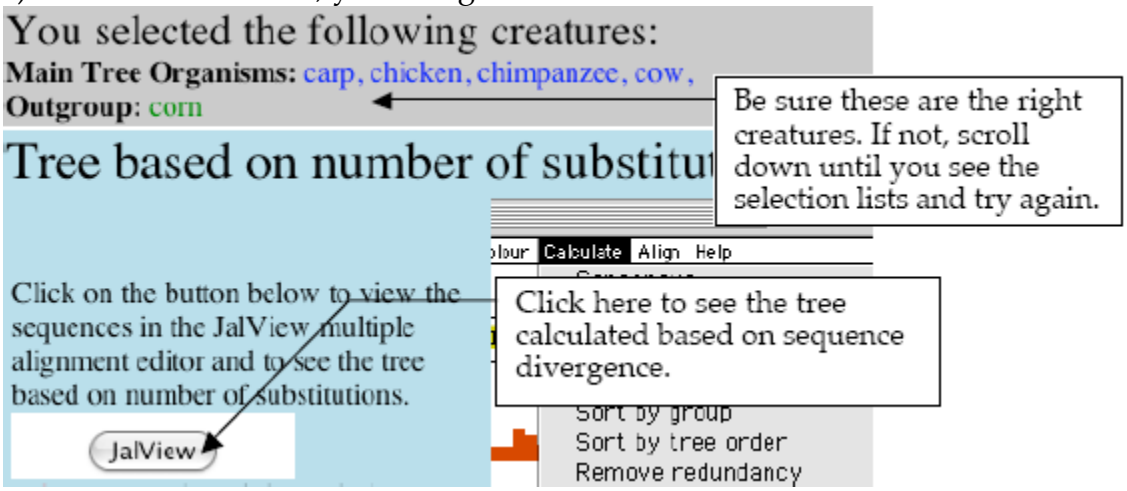
Part II: Draw a phylogenetic tree for 5 organisms of your choice.

a) 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).

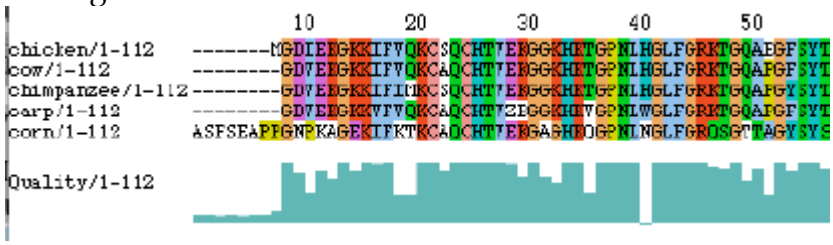
b) Select the 4 "Main Tree Organisms" as you did previously. Use shift-click to select more than one organism at a time. If you want to select non-adjacent organisms in a list, use appleclick. Once you have made your selections, click the "Calculate Tree" button. In the example below, I selected: Main tree: Carp
Chicken
Chimpanzee
Cow
(all of these are vertebrates)
Outgroup: Corn
(this is very different from a vertebrate!)

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

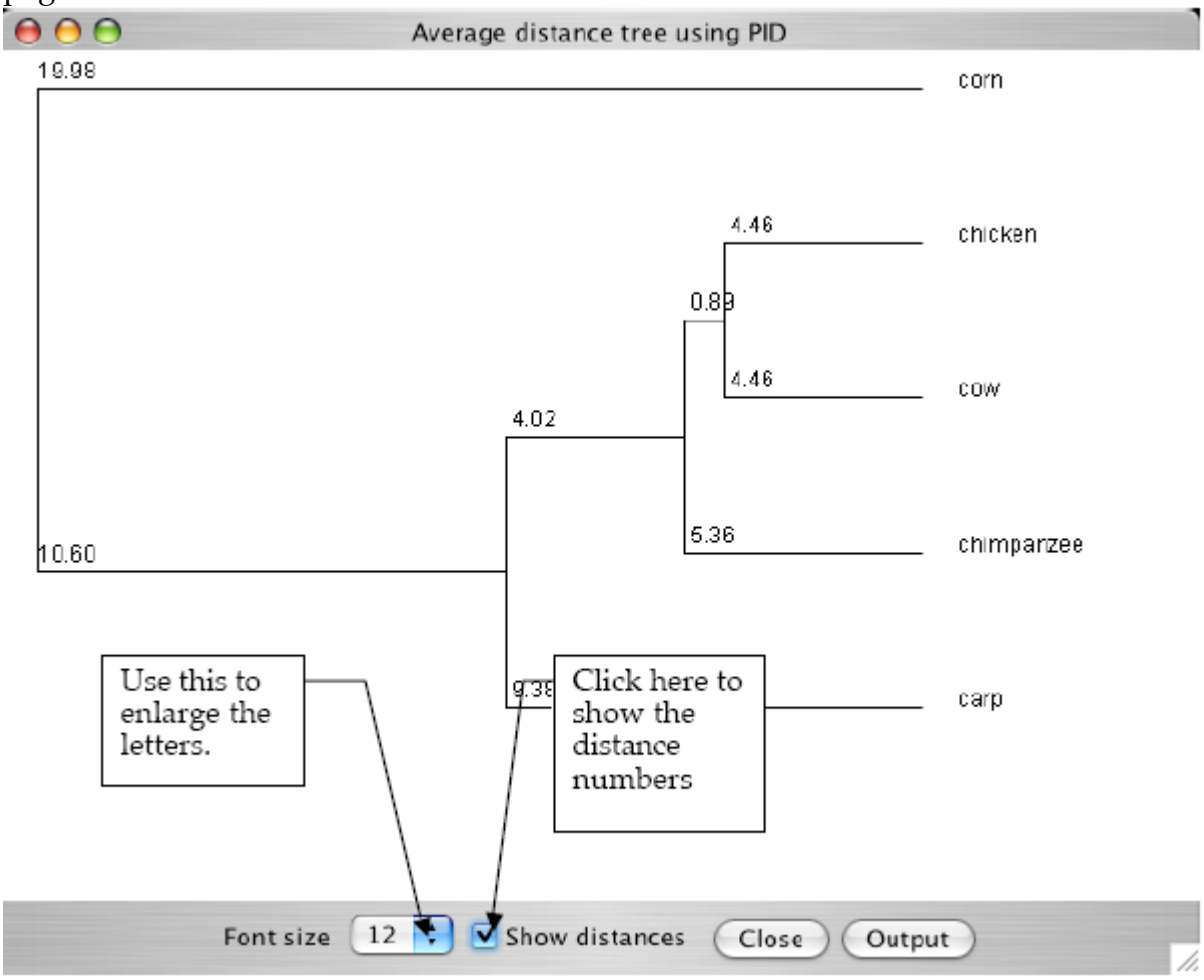


[The only time you should really worry is if you get a message like "server not responding" at this point. In this case, contact TA ASAP.]

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:



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:



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).

Assignment: Pass your phylogenetic tree into your TA. Make sure all group members' names are on the sheet.

Name: _____ Pre-Lab: Aipotu: Evolution

1) On page Aipotu:Evolution-3, the Lab Manual described a color trait with two alleles, red (R) and white (r). On page Aipotu: Evolution -7, the Lab Manual describes a population that consists of 100 Rr individuals only. Is this population at Hardy-Weinberg Equilibrium (HWE)? Justify your answer mathematically.

2) The following is a student's response to the "How did the cheetah get so fast?" question from the first day of class: "*The anatomy began changing because they needed to get the food so they began changing to get the food and other resources. They had mutations in order to get their needs.*" This statement contains at least one of the misconceptions listed on page Aipotu IV-11. Give the number of one of the misconceptions it contains and explain how the answer demonstrates the misconception. (We will grade this based more on your explanation than the misconception you choose).

Misconception # Explanation:

Aipotu:Evolution - 2

Aipotu: Evolution

Objectives:

To explore evolution with evolving digital organisms.
To test evolutionary hypotheses.
To try out different evolutionary scenarios.
To address several misconceptions about evolution.

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?"

To explore evolution in detail, you need organisms with a complex genotype that can reproduce rapidly. In this lab, you will explore the evolution of simple digital organisms as they evolve in the computer simulation, Aipotu.

Aipotu simulates the production of color in a hypothetical species of simple diploid flowers. This software allows you to do look at the genetics, biochemistry, molecular biology and evolution of flower color in simulated flowers. In this lab, you will look specifically at a model of the evolution of flower color. In Aipotu, color results from the particular color protein genes an individual possesses. Since these genes are subject to mutation (visible in the molecular biology component of Aipotu) and we can model differential fitness based on color, Aipotu can simulate the evolution of colors in these flowers. Since the flowers in Aipotu are subject to random mutation and non-random selection, they evolve like organisms in the real world.

As a reminder, color is controlled as follows in the Aipotian flowers:

•**Molecular Biology**: Each organism carries two copies of a gene that can produce a pigment protein. Each of these genes is a DNA sequence. The simulation attempts to express each gene by looking for the appropriate signal sequences (promoters, terminators, splice sites, start codons, and stop codons -just like real genes) in the DNA sequence and producing any protein encoded therein.

Mutations alter the DNA sequence randomly by changing bases, deleting bases, or inserting bases. These mutations can lead to the production of altered proteins.

•**Biochemistry**: Proteins encoded by the DNA sequence are folded in 2-dimensions using the usual rules of protein structure: hydrophobic side chains cluster out of the water; hydrophilic side chains are in contact with the surrounding water; side chains can form hydrogen bonds or ionic bonds if they have the appropriate structure and location in the chain.

Proteins with a particular shape – a hexagonal hydrophobic core of seven amino acids with six hydrophilic amino acids nearby – can be colored; proteins without this shape are white (colorless). The particular color depends on the content of the hydrophobic core: if the core contains phenylalanine, the protein is red; if it contains tryptophan, it is

yellow; if it contains tyrosine, it is blue. These colors combine as paint pigments do: red + yellow = orange; red + blue = purple; blue + yellow = green; red + blue + yellow = black.

•**Genetics**: The flower color genes are inherited as any other genes are in a diploid organism. Each individual has two copies of the gene for flower color and passes one of them to each of its offspring. The final color of a flower depends on the genes present. White is recessive to all other colors while the other colors combine as above.

That is: a heterozygote with one red allele and one yellow allele would be orange. A heterozygote with one orange allele and one green allele would be black since it contains all three colors: red from the orange allele; blue from the green allele; and yellow from both alleles. Furthermore, a heterozygote with one orange allele and one red allele is orange (not red-orange).

It is important to note that this is **not** the way colors work in nature. In nature, proteins fold in 3 dimensions and flower colors are typically due to small molecules synthesized by several enzymes. Although the Aipotu simulation is unrealistic in these key details, it still retains many of the features necessary for understanding evolution, as you will see.

In this lab, we will use the evolution portion of the Aipotu simulation:

- **Evolution**: To simulate evolution, you need to have a large population of Aipotian flowers. We will use a population size of 100 – this is small by real-world standards, but large enough for our purposes without being so large that the program runs too slowly.

In Aipotu, the flowers are annuals – they live for only one year and die after producing seeds for the next generation. Each year is one generation where the seeds from last year grow and produce flowers. These flowers are subject to natural selection based on their colors.

Each generation, flowers grow from last year's seeds. The flowers contribute to the 'gene pool' for the next generation based on their fitness: those with colors that have high fitness contribute more copies of their color genes to the gene pool than those with colors that have low fitness; flowers with colors with zero fitness do not contribute any of their alleles to the gene pool. All the parents then die. Each individual in the next generation is produced by drawing two alleles randomly from the gene pool; these alleles are then subject to random mutation. The next generation grows from these individuals and replaces their parents with exactly 100 individuals.

The simulation does not model *how* the natural selection based on color occurs. It could be due to predators' or pollinators' color preferences, or something altogether different. In the simulation, all that matters is that fitness depends on color and color depends on genes. We thus have all the requirements for evolution: *variation* in color; that variation is *genetic*; and *fitness* depends on color. Thus, alleles of the color gene that contribute positively to fitness will increase in frequency, while those that

contribute negatively will decrease in frequency. All the time, mutations will be busily creating new alleles.

You can change all of the relevant parameters: starting population, mutation rates, and relative fitness. This gives you a 'laboratory of evolution' where you can watch evolution happening and see what would happen if …

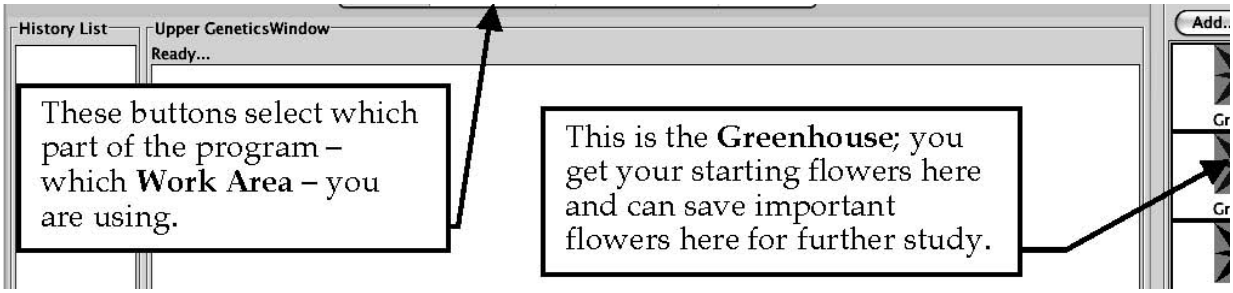
We will begin by looking at some population genetics scenarios with mutations turned off for simplicity. We will then turn mutations on to explore more complex evolutionary scenarios.

Part A: Population Genetics

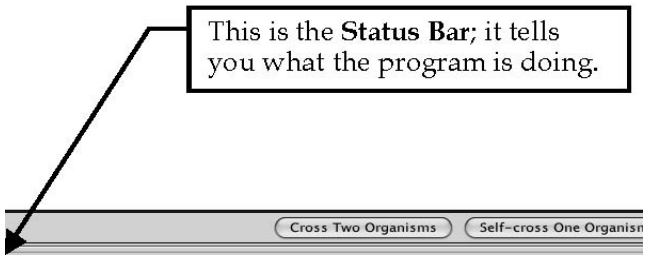
In this part of the lab, we will use Aipotu to simulate Hardy-Weinberg Population Genetics. We will begin by looking at Natural Selection in the absence of mutation. In this part of the lab, we will only look at red and white colored flowers. In this case,
flower color is controlled by one gene with only two alleles:

Allele Contribution to Phenotype	Genotype	Phenotype
R red (dominant)	RR Rr rr	red red white
r white (recessive)		

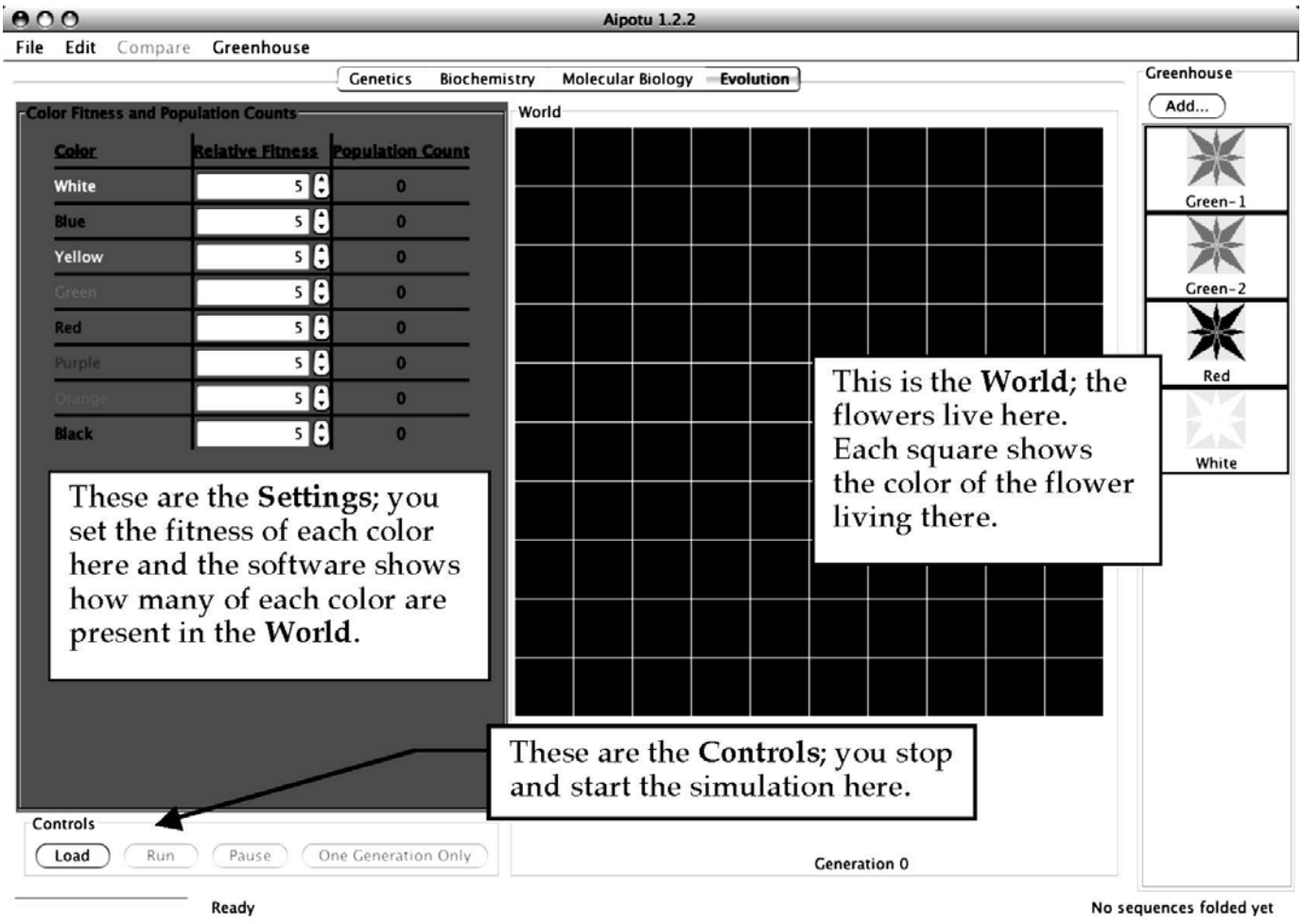
1) Start Aipotu by clicking on its icon in the Dock. You should see something like this:



The Genetics, Biochemistry, and Molecular Biology **Work Areas** are the same as that for the Evolution component of the simulation program. If you don't see this or see other flowers in the **Greenhouse** besides those shown above, restart the computer to get a fresh copy of the program.

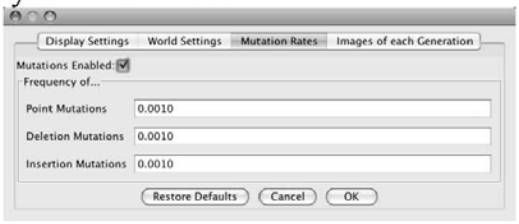


2) Click on the "Evolution" button at the top to go to the **Evolution Work Area**; you should see this:



3) You must first turn off mutation for this part of the lab. From the **File** menu, choose **Preferences...**; you will see this:

Click on **Mutation Rates**, and you should see this:



Uncheck the **Mutations Enabled** checkbox (the mutation rate numbers should gray out), and click OK. Mutations are now off.
Now, you will run some simulations of Natural Selection. First, to get a qualitative feel for what happens, and then more quantitatively. Note that, since mutations are off, no new alleles will be produced, so no new colors will appear. If they do, you haven't turned off mutations…

A) Select for Red.

A1) Click on the **Red** organism in the **Greenhouse** to select it; its border will turn green. While holding the shift key, click on the **White** organism in the **Greenhouse**; now, both should be selected.

A2) Click the **Load** button in the **Controls**. The **World** will fill with a roughly 50:50 mix of red and white organisms. Note the count of red and white in the **Settings** panel; it should be about 50 of each.

A3) Set the **Fitness** settings in the **Settings** panel to select for red. Set the fitness of red to 10 (the maximum) and all the other colors to 0 (the minimum).

A4) *Prediction*: What should happen to the number of red and the number of white flowers after several generations with this selection?

A5) *Test*: Click the **One Generation Only** button in the **Controls**. This will run one generation only. First, the starting flowers will contribute to the gene pool based on their fitnesses. Then the starting flowers will die off and be replaced by exactly 100 offspring. Each offspring flower will get two alleles randomly chosen from the gene pool.

A6) *Result*: What happens to the counts of red and white flowers as you simulate more generations? Roughly how many generations does it take to get to pure red? Be careful, as some all red generations can have some white offspring (why?).

B) Select for White.

B1) Click on the **Red** organism in the **Greenhouse** to select it; its border will turn green. While holding the shift key, click on the **White** organism in the **Greenhouse**; now, both should be selected.

B2) Click the **Load** button in the **Controls**. The **World** will fill with a roughly 50:50 mix of red and white organisms. Note the count of red and white in the **Settings** panel; it should be about 50 of each.

B3) Set the **Fitness** settings in the **Settings** panel to select for white. Set the fitness of white to 10 (the maximum) and all the other colors to 0 (the minimum).

B4) *Prediction*: What should happen to the number of red and the number of white flowers after several generations with this selection?

B5) *Test*: Click the **One Generation Only** button in the **Controls**. This will run one generation only. First, the starting flowers will contribute to the gene pool based on their fitness. Then the starting flowers will die off and be replaced by exactly 100 offspring. Each offspring flower will get two alleles randomly chosen from the gene pool.

B6) *Result*: What happens to the counts of red and white flowers as you simulate more generations? Roughly how many generations does it take to get to pure white?

Why does it take more generations to get to pure red than it does to get to pure white? Hint, you can see the genotype of each flower by checking the **Show colors of both alleles** in the **World Settings** part of the **Preferences…** .

Now, we will get quantitative.

C) Hardy-Weinberg Equilibrium & Natural Selection

C1) Load the **World** with only the **Red** organism from the **Greenhouse**. The **World** should be entirely red.

C2) Show the colors of both alleles in each organism by checking the **Show colors of both alleles** in the **World Settings** part of the **Preferences…** if you haven't already. You should see little red and white rectangles in the upper left corner of each organism in the **World** – this indicates that each has one red and one white allele = genotype Rr.

C3) Set all **Fitness**es to 5.

• Is this population at Hardy-Weinberg Equilibrium?

C4) Calculate the allele frequencies in the starting

population: Genotype Number #R's #r's

RR

Rr

rr

TOTAL:

frequency of R (p) =
frequency of r (q) =

C5) Calculate the genotype frequencies expected at HWE:
frequency of RR = p^2 =
frequency of Rr = 2pq =
frequency of rr = q^2 =

C6) Is the population at HWE? Why or why not?

C7) Run one generation only. Is that population at HWE? You may find it useful to pool the class results to get an answer that is less subject to small-sample-size fluctuations.

C8) Set the **Fitness** settings in the **Settings** panel to select for red. Set the fitness of red to 10 (the maximum) and all the other colors to 0 (the minimum).

C9) *Prediction*: What should happen to p and q after several generations with this selection?

C10) *Test*: Click the **One Generation Only** button in the **Controls**. Do this a few times.

C11) *Result*: Calculate p and q as you did in part (d):

Genotype Number #R's #r's RR Rr rr

TOTAL:

frequency of R (p) =

frequency of r (q) = C12)

Does the result match your prediction? Why or why not?
Now, we will turn mutations on and observe the evolution of new alleles and new colors.

4) Choose **Preferences…** from the **File** menu and click on the **Mutation Rates** button. Click **Enable Mutations** and then click **OK.** Mutations are now enabled.

Note: in nature, mutation rates vary but a rough figure is that any given base in DNA has a 1/500,000,000 chance of being mutated in a given individual in each generation. In this simulation, the mutation rate is about 3/1000. Taking into account the population size of 100, this is roughly 15,000 times higher than the 'natural' rate; therefore, one generation – one "year" – in Aipotu is roughly equivalent to 15,000 years in real life. You should know that this is a *very approximate calculation!*

Part II: Misconceptions about Evolution

In this section, you will perform a series of experiments that address several common misconceptions about evolution. For each experiment, you will begin by conducting the experiment and observing the result. You will then determine which misconception(s) it addresses. Finally, you will make predictions about what the outcome of the experiment would have been if the misconception were *true*.

Your TA will assign you a misconception number from the list below. You should be sure that you understand your misconception; ask your TA if you have any questions. Write your misconception number below:

Misconception #

Misconceptions

You will be responsible for one of these misconceptions during the lab and in your lab write up.

1. *Selection causes mutations that are adaptive.* For example, the presence of an antibiotic causes the mutations that make the bacteria antibiotic resistant. {In fact, the mutations are always random and occur *before* the selection}
2. *Evolution has a goal.* If the world were somehow started over, the result would be the same world we see today. {In fact, chance plays a huge role in evolution and the outcome would likely be very different}
3. *Mutations cannot produce new features.* Since mutations are random and destructive (see #7), they cannot *create* new features. {In fact, that is how all the amazing diversity of life originated}
4. *When a trait (or organ) is no longer beneficial for survival, the offspring will not inherit the trait*. Parents will only transmit *beneficial* traits to their offspring. {In fact, parents randomly pass on traits to their offspring; *any* traits, beneficial, neutral, or harmful, can be passed on.}
5. *There is only one mutation that can cause a given phenotype.* For example, there is only one particular DNA change that could change a slow cat into a faster cat. {In fact, although this is true for some phenotypes, in most cases, any given phenotype can be caused by several different mutations}
6. *Variations only affect outward appearance, do not influence survival.* The variations we see in nature (different colors, beak sizes, finger lengths, etc.) do not influence survival. {In fact, all variations are potentially subject to selection.}
7. *Mutations always reduce the fitness of organisms.* Since mutations damage genes, they can only impair their function and must therefore reduce the fitness of the organism. {In fact, mutations can be neutral, beneficial, or deleterious}
8. *Changes in a population occur through a gradual change in all members of a population*. For example, *all* the giraffes present in one generation gradually get longer necks over their lifetimes; this continues generation after generation. {In fact, individuals in the population can be quite different from each other (some giraffes have long necks; some shorter) and the change happens from one generation to the next (the frequency of long-neck giraffes in generation 1000 is higher than in generation 1; none of the giraffes from generation 1 is present in generation 1000)}.

Each of the following experiments will address one or more of the above

misconceptions. Also, a given misconception may be addressed by more than one experiment. **Note** that time you click **Run** in Aipotu, it is like starting evolution all over. Each run is a new earth, started with slightly different conditions and/or different random events.

Experiments

IMPORTANT For these experiments, you must turn mutations on! The easiest way to do this is to quit and re-start Aipotu. That will restore all the default settings.

D) Starting with Green-1; no selection.

Here, you will start with **Green-1**, which is a homozygote – it has two identical green alleles. You will let it reproduce with random mutations, but *no selection*. That is, all colors, including white, will be equally fit.

D1) Quit and re-start Aipotu to enable mutation.

D2) Go to **Evolution** and load the World with **Green-1** from the **Greenhouse**.

D3) Click **Run** and let the simulation run for about 5 generations.

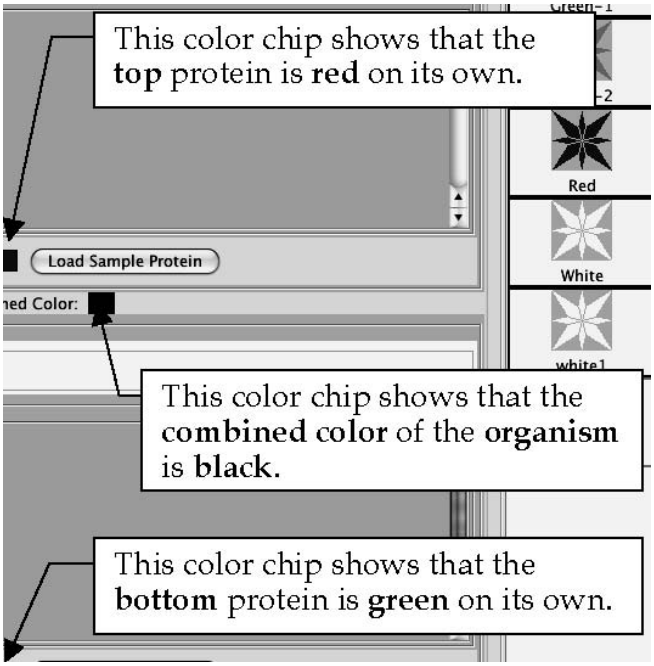
D4) What colors do you see? Specifically:

-What colors besides green are present in your World?

-What colors are present in the World's of the other groups in your lab? Based on these class results, which colors occur often and which are rare?

-Which misconception(s) does this address? For each, what would the result have been if the misconception were true?

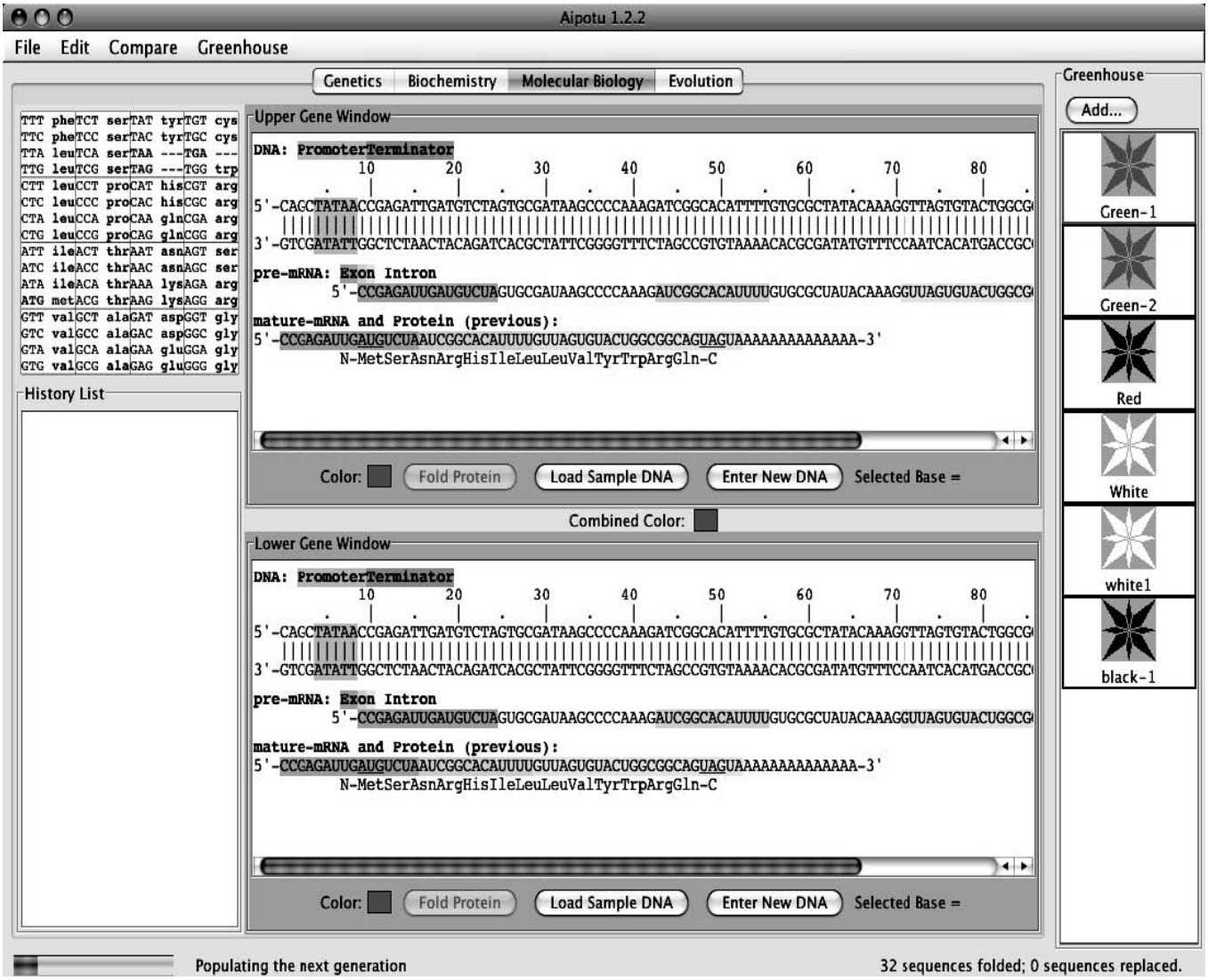
D5) Save one of the black flowers to the **Greenhouse**. To do this: choose one of the black flowers from your **World**, click on it to select it (its border will turn black) and click the **Add…** button at the top of the **Greenhouse**. Give it a name when the program asks you and it will appear in the **Greenhouse**. You can now examine it using the other tools in Aipotu.
D6) Switch to **Biochemistry** and double-click the organism you just saved in the **Greenhouse**. The program will then show you the proteins encoded by the two copies of the pigment protein gene in this organism along with their individual and combined color. A sample is shown below; yours will likely look different:



Note that the proteins have different shapes – that is why they have different colors.

Typically, the black mutants that occur early on are heterozygotes containing one un-mutated green allele (the bottom one in the example above; yours may be different) and one mutant red allele (the top one in the example above; yours may be different).

Look at the black mutants of your classmates; they should almost all be red/green heterozygotes. All the green proteins should be the same (since they're likely to be un-mutated); the red proteins are likely to be different from each other.
To see how the red protein came to be, you will need to look at the DNA sequences in the **Molecular Biology** work area.

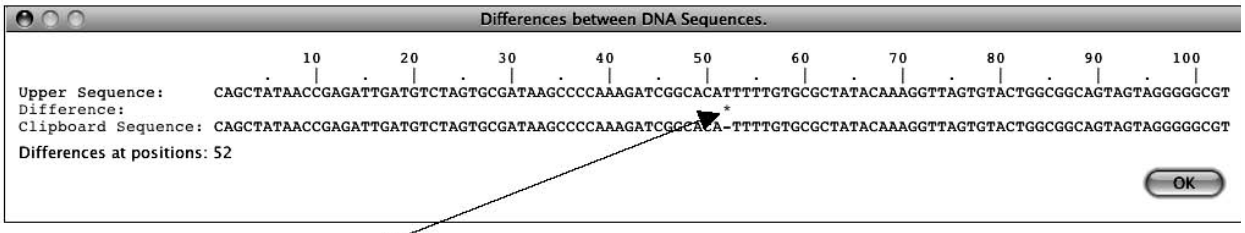


D8) From the **Edit** menu, choose **Copy Upper Sequence to Clipboard** (be sure not to choose either of the "image" options). This copies the upper DNA sequence – the un-mutated green allele that all the mutants started from – to the program's memory.

D9) Double-click on the black mutant organism you saved in the **Greenhouse**. You will see its two copies of the pigment protein gene in a window like the one above. One will be green and one will be red. You want to look at the red one – note whether it is the upper or lower sequence.

D10) From the **Compare** menu, choose **Compare Upper vs Clipboard** (or **Lower**, whichever is the red one) and you will see a display of the differences between the two DNA sequences like the one shown below (if no differences are shown, you compared two identical green alleles; if you tried **Upper**, try **Lower** and vice-versa). Yours will be

similar, but not identical, to this.



Note the * at position 52. This indicates the single difference between the clipboard sequence

(the green allele that you started with) and the red mutant; that is, the mutation that made the allele red. In this case, the "-" in the clipboard sequence indicates that the T in the red mutant is *not present* in the original green allele. Therefore, the mutation that made the red allele was an *insertion* of a T between bases 51 and 52. This resulted in a frame-shift mutation that led to the production of a red protein. Your mutation will likely be different.

Make a list of the 'red mutations' from your class. Are they all the same? How is it possible that more than one mutation can lead to the same phenotype? How does this explain why some colors are rare and others are not?

-Which misconception(s) does this address? For each, what would the result have been if the misconception were true?

E) Starting with Green-1; selecting for Black; then removing selection.

Here, you will see the effects of a change in natural selection.

E1) Load the **World** with **Green-1** from the **Greenhouse**.

E2) Select for black. Set the fitness value for black to 10 and all the others to 2.

E3) Click **Run** and run until the field is mostly black.

-Are all the organisms in the **World** black? Why or why not?

-Which misconception(s) does this address? For each, what would the result have been if the misconception were true?

E4) Click **Pause** to stop the simulation.

E5) Remove all natural selection. Set all the fitness values of all colors to 5.

E6) Click **Run** and run the simulation for about 10 more generations.

E7) Observe the colors of the organisms in your **World** and those of your classmates.

- Are there any black organisms remaining?

-Which misconception(s) does this address? For each, what would the result have been if the misconception were true?

-Did all groups get the same results?

-Which misconception(s) does this address? For each, what would the result have been if the misconception were true?

F*) Starting with Green-1; with and without selection for Orange.*

Here you will pool class results to see how many generations it takes for Orange to evolve with and without selection for orange.

• *First run; without selection for orange.*

F1) Load the **World** with **Green-1** from the **Greenhouse**.

F2) Set all the fitness values to 5 (default value).

F3) Click **Run** and watch for the first appearance of an orange flower. Note the generation number when it appeared in the space below. If you get to Generation 10 and still have not seen an orange flower, click **Pause** and write ">10" in the space below. Generation # -Did it take the same number of generations for all groups? Would you expect it to? Why or why not?

-Roughly how many generations did it take to get the first orange in the absence of selection for orange?

• *Second run; with selection for orange.*

F4) Load the **World** with **Green-1** from the **Greenhouse**.

F5) Set the fitness values to select for orange; that is, set orange to 10 and all the others to 2.

F6) Click **Run** and watch for the first appearance of an orange flower. Note the generation number when it appeared. If you get to Generation 10 and still have not seen an orange flower, click **Pause** and write ">10" in the space below. Generation # -Did it take the same number of generations for all groups? Would you expect it to? Why or why not?

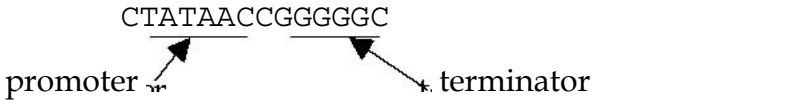
-Roughly how many generations did it take to get the first orange when selecting for orange?

-Which misconception(s) does this address? For each, what would the result have been if the misconception were true?

G) Evolution of color from colorless proteins.
Here, you will start from a colorless protein of your own devising and see if you can evolve colored proteins from that via random mutation and non-random selection. First, you must build a working gene that encodes a short protein. Don't use the white flowers from the **Greenhouse**; the protein in these flowers is only one mutation away from color, so using it would be unfairly 'stacking the deck'.

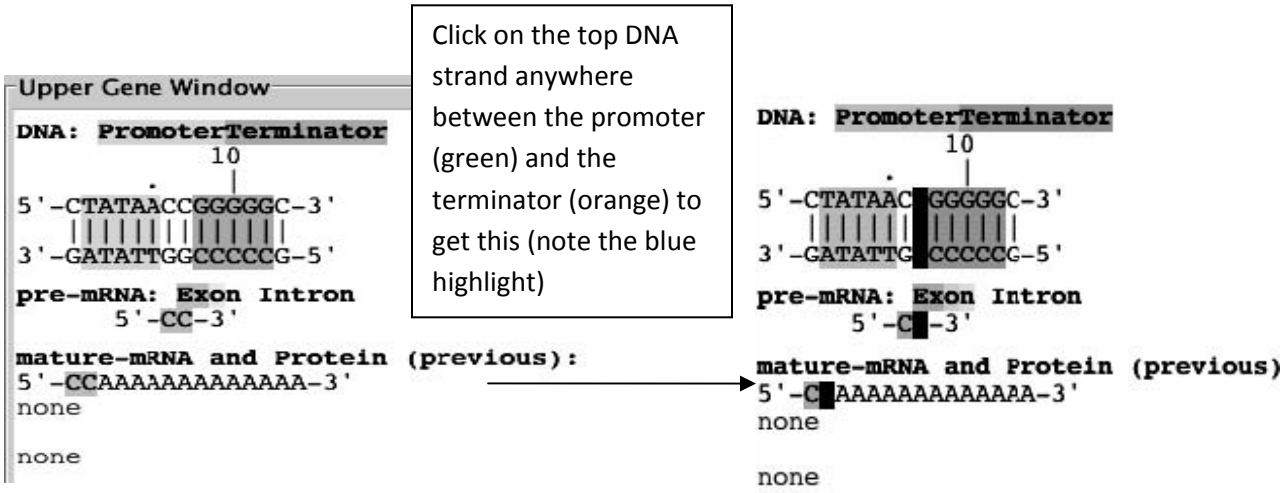
G1) Switch to **Molecular Biology** and click on **Enter New DNA Sequence** in the top workpanel. You will be presented with a dialog box into which you can enter your DNA sequence. Build your gene step by step.

G2) A gene needs a promoter (TATAA in Aipotu) and a terminator (GGGGG) to make an mRNA. If you type something like the sequence shown below and click **OK**, it will create a gene that makes a small mRNA.



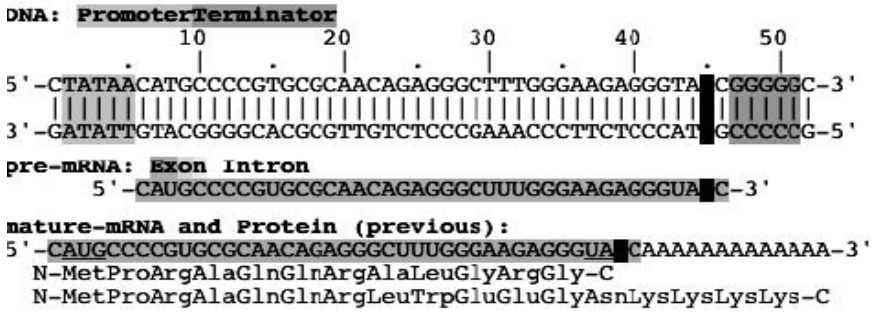
the extra C's are just spacers – they can be any sequence

You should see something like this when you click **OK**:



G3) Now you can type in your coding region directly. Just hold the shift key while you type the DNA sequence (A, G, C, or T) and the bases will be inserted. Type a start codon (ATG), codons for 10-12 amino acids (this is where you get to be creative), and a stop codon (TAA).

If you've got it working, you should see something like this (your protein should be different):



G4) Once you've got a working gene, you should fold the protein to be sure it isn't colored (remember that you want to start with a colorless protein for this experiment). Click the **Fold Protein** button. An item will appear in the **History List** showing the shape of the protein and the color chip in the **Upper Folding Window** will show its color. You can further edit the DNA and fold the new protein until you are happy with your starting protein.

G5) Now, you need to make an organism that is homozygous for your gene. Double-click on the item in the **History List** that corresponds to the desired protein and choose **Send to Lower Panel** from the menu that pops up. You should now see two identical genes, one in the **Upper** and one in the **Lower Gene Window**.

G6) Now, save the organism to the **Greenhouse** so you can use it for evolution. Click the **Add…** button over the **Greenhouse** and give your organism a suitable name. It will appear in the **Greenhouse** – it should be white.

G7) Switch to **Evolution**. Set the fitness values to select for colored proteins. Set the fitness of white to 1 (you want it low to favor colors, but not 0 or you'd kill all your starting population!)and all the other colors to 10.

Alternatively, if you want to select for a *particular* color, you can set that fitness higher than the others. However, it will evolve to your color faster if you set the fitness of the other colors to something higher than 0. Why?

G8) Load your organism into the **World** and start it evolving. Wait and see if you get colored flowers. This may take a while… If and when you do get a colored organism, save it to the **Greenhouse** and compare the protein and DNA to your starting gene as you did in A8-A10. If you don't, you can try another protein sequence or just wait a long time… It is likely that at least one of your classmates will be successful.

-Which misconception(s) does this address? For each, what would the result have been if the misconception were true?

Lab Write Up

- 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 write up must be in your own words.

Your lab write up must include:

Your TA will have assigned you one of the misconceptions described on page Aipotu Evolution-11; you should answer the following questions about **that misconception**.

1) Which misconception were you assigned? Give the number.

2) Although misconceptions are not correct, they often seem reasonable if you don't know all the details. Explain what might lead someone to think that the misconception you chose was plausible.

3) Explain how the data you collected in lab shows that the misconception you chose is incorrect. Be sure to include the *relevant* data from your lab and explain how those data are relevant.

4) A skeptic could argue, "Aipotu is just a computer simulation. It has nothing to do with real organisms. Any conclusions you draw from it are not relevant in the real world." How would you argue that, although Aipotu is a computer simulation, the results from it are still relevant to the misconception you chose? In other words, "In what relevant ways is Aipotu similar to the real world so as to allow one to draw meaningful conclusions about this misconception?"

5) Describe a hypothetical evolutionary scenario that involves (the *correct* version of) your misconception. This scenario can be similar to the "how the cheetah got so fast" scenario we discussed in lecture. It need not be correct, but it should be reasonable and is must specifically address the correct version of your misconception.

For example, suppose that your misconception had been "physical fitness - strength - is the same as evolutionary fitness" (note that this is not on the list; it is only an example). The correct version of this misconception is "evolutionary fitness is reproductive success". A full credit answer to this question would be something like: *The correct version of my misconception is, "Evolutionary fitness is reproductive success". A scenario involving this would be as follows. A species of bird prefers mates with bright red feathers. As a result, red-feathered birds reproduce more than those without red feathers. Over time, the population becomes majority red-feathered, even if the red-feathered birds are physically weaker than those without red feathers.*

Lab 4 Pre-Lab **Name**_____

Microbial Diversity & Microscopy

Protozoa

1) Using Campbell, draw a rough sketch and briefly describe the following organisms:

Amoeba

Paramecium

Vorticella

Spirogyra

Diatoms

2) Using the information in your text book, draw a phylogenetic tree that includes the following organisms. You do not need to include information about when the last common ancestor existed. Do include the names of the kingdoms and phyla. Please draw it on the back of this sheet.

humans

fish

c-ferns (they are regular ferns)

Paramecium

Volvox

Vorticella

3) Which two organisms from question 2 are the most closely-related?

Microbial Diversity & Microscopy

Objectives:

To become familiar with the proper use of a compound microscope in order to comparatively study and measure cells.

To learn how to make a wet mount of a living culture and observe with a microscope.

To observe eukaryotic unicellular organisms (protozoa) and their subcellular components.

To look at some organisms on the border between plants and animals and to contrast them with organisms of other domains.

Introduction:

All living things are made up of cells, but as life varies greatly so do the cells that make it up. **Prokaryotes** (Archaea and Bacteria) are organisms in a group which lack true nuclei and contain few organelles. **Eukaryotes** (Fungi, Plants and Animals) on the other hand have true nuclei, cytoplasm, and a plasma membrane surrounding their cells and contain a variety of other organelles. They also differ in that some are unicellular organisms and others are multicellular. Whether eukaryotic or prokaryotic, a general term for any life form needing magnification in order to be seen is "microbe" and many (but not all) of these are single-celled organisms rather than multicellular.

The average eukaryotic cell is much larger and easier to observe with a microscope than the average prokaryotic cell. You will observe examples of prokaryotes with the microscope and will see how small they are compared to eukaryotic cells, which you will spend much more time observing. Protists are all contained in the Domain Eukarya within different kingdoms and phyla reflecting their great diversity. They are unicellular creatures and some are animal-like and called Protozoa (*Paramecium* and *Amoeba*); others are more like plants (green algae, diatoms); and still others seem to be both plant and animal at the same time (*Euglena*).

Plant cells are often easy to identify in that the typical plant cell, in addition to nuclei, cytoplasm, and a plasma membrane, has a cell wall - a rigid structure made up chiefly of cellulose that surrounds the plasma membrane. Plants also possess chloroplasts - structures within the cell that contain the green pigment chlorophyll. The typical plant cell has much of its volume taken up by a large vacuole containing water,

salts, sugars, and other compounds whereas most animal cells are largely filled with cytoplasm.

In this lab, look at and learn to recognize some representatives of the major microbial groups. These include bacteria, some fungi, and within the protists, protozoa and some algae. To do this you will need to learn to use a microscope, in order to distinguish the basic cellular structures: cell wall, nucleus, vacuoles, flagella, chloroplasts. As you examine each species, try to determine its method of movement and nutrition, and check out its phylogenetic classification. This material will be on the **Lab Practical** so take good notes.

Cells				
Cell Parts & Organelles	**Prokaryotes**	**Eukaryotes**		
	Bacteria	**Fungal**	**Plant**	**Animal**
true nucleus	no	yes	yes	yes
cell wall	yes	yes	yes	no
cell membrane	yes	yes	yes	yes
chloroplasts	some	no	yes	no
vacuole	no	yes	yes, large	yes, small
flagella	some	no	no	some

Part I: How a Microscope Works

To see small things like cells one must learn to use a microscope. 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.

A. First, familiarize yourself with the parts of the microscope and their function. Locate the main parts named in the diagram. These include the stand (arm and base), the light, the condenser lens with its diaphragm, the movable stage or the non-movable stage with slide clips, the objective lenses, the nosepiece, the body tube, and the ocular lens.

B. Plug in the light cord, turn on the light, and then move the diaphragm lever as far to the left (closed) 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 move the condenser up and down while observing the light on the piece of paper (do not look through the microscope yet, just 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.

C. 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 close two points can be and still be distinct. Contrast is the distinction of a particular detail against its background.)

D. 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 (ocular) lens. Basically, the objective lens magnifies the object and forms an image in the tube which is further magnified by the eyepiece lens. 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.

E. 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 objective 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 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. 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.

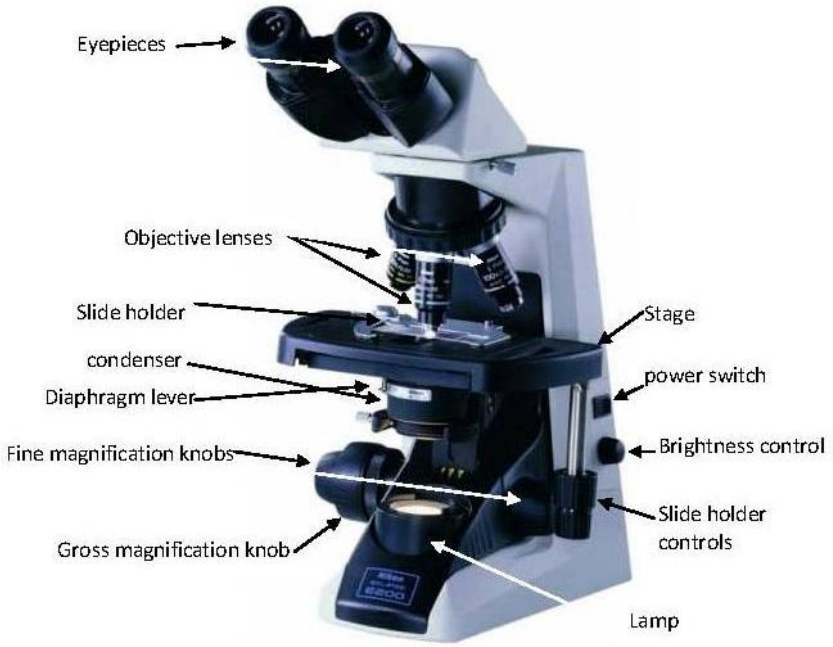


Diagram of a typical bright-field compound microscope. Though various styles exist, all compound microscope have the same basic components and they are labeled in the picture above.

F. Only when the object is in focus under low power should you go up to higher power. Move the object to the center of the field of view, and then rotate the 40X objective into place. You may need to adjust the light strength, condenser height and the diaphragm opening. When you adjust the focus, use only small fine movements, or you run the risk of hitting the coverslip with the lens and damaging both.

Part II. Observing Prokaryotic Life

Sampling & Inoculation Procedure:

1. During this lab, inoculate a bacterial growth plate with a sample from some common environment. You may expose the plate to room air, or dust, or a drop of water from the fish tank. (Do not use human samples because we are not equipped to diagnose possible pathogens). Seal the plate with parafilm, turn it upside down, label it with your name and the date, and what sample was taken. Give it to your instructor to leave on a shelf in the lab room. Next week, you can look at it and describe the different kinds of colonies present, and their relative numbers. You can view the cells under the microscope to see their structure.

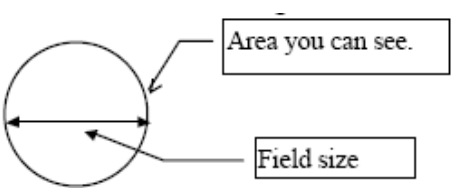
2. Look at prepared slides and images of several prokaryotes and Archaea. Draw what you see, note the size of the organisms. What structures are visible? Do you see nuclei?

Part II. Observing Unicellular Eukaryotic Life: Protists

1. You will be given prepared slides as well as live samples of the following protozoa to look at. Names in *italics* are genus and species names. Those in normal type are names of phyla. The abbreviation *sp.* means *"species"*, that is, the genus is known but the exact species is not. Familiarize yourself with the species using the prepared slides and then make a wet mount of the live cultures using the directions to follow. Draw, label and measure (see #2) the following organisms and the organelles you can find.

Amoeba proteus	*Euglena sp.*	*Paramecium caudatum*	
Vorticella sp.	*Volvox globator*	*Spirogyra sp.*	Diatoms

2. How big is it? You can use the microscope to measure the size of the cells you are looking at. For each magnification, the table below gives the diameter of the field of view (field size). For the microscopes we use:

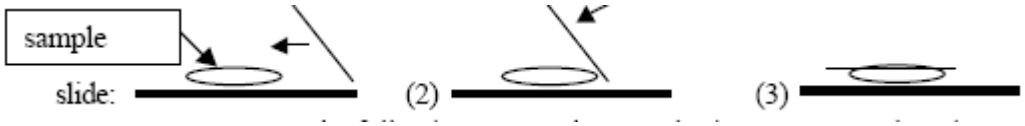


Magnification shown on objective lens	Actual magnification		Field size (millimeters)	Field size (microns (μm))
3.5x	35x	⇒	5.1	5100
10x	100x	⇒	1.8	1800
40x	400x	⇒	0.45	450

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.

3. **Preparing a Wet Mount Slide and Making Observations**: Your lab instructor will show you how to make a slide. The great art here is to avoid air bubbles when you lower the coverslip! A useful trick for this is to:

(a) put a drop of sample on the slide
(b) while holding the coverslip at an angle, slide the edge of the coverslip to the edge of the drop
(c) slowly let the coverslip fall flat:



(d) Place the wet mount on the (dry) microscope stage, and use the slide clamp to hold it. Move the slide holder until an edge of the coverslip is right over the center of the condenser.
(e) Start with the low power (10X) objective, and focus up and down until you see the edge of the coverslip. This puts you in the right focal plane. Adjust the

light strength, the condenser height, and the condenser diaphragm for good contrast.
(f) Move the slide so the drop is under the objective and look for cells. When you find one, put its image in the center of the field of view, and only then rotate the high power (40X) objective into place. Do not use the 100X lens for most purposes because it requires special immersion oil between the lens and the object.

4. **Looking at a mixture of organisms in pond water or a plankton tow from Boston Harbor.** Take a drop of water from the pond or sea water samples and place it on a clean slide with a cover slip. Quickly scan the slide with low power to find an observable living organism, ideally unicellular. Look at it under all magnifications you have on your microscope. Try to determine what type of cell it is by its components and its behavior. Draw your cell or multicellular organism; label all parts you can and determine its size.

Points & Tips to Remember:

To slow down fast-moving protozoa, as you set up the wet-mount:

Take a clean slide & make a small ring about 1/2 inch in diameter of "Proto-slow" – viscous methyl cellulose that slows protozoa because it is thick and difficult for them to swim through. Drop a drop of the protozoa in the middle of the ring. Put on the coverslip and observe. The protozoa will gradually slow down as the proto-slow reaches them.

Compound Microscopes are good for looking at small things. Adjust the magnification by changing the objective lens, grasp the ring above them to rotate, not the objectives themselves. Be sure it clicks solidly when changing magnification or you won't see anything. Helpful quick microscope tips:

1. Use the condenser and diaphragm correctly, too much light removes your image.
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. Do not use any other paper to clean it, only lens paper.
7. You should look at pictures in your text book and online to familiarize yourself with what you will be looking at.

References:

Campbell et al., 1999. pp 502-517,520-543, 583.

Van De Graaff, K. M., and J. L. Crawley, 1996. A Photographic Atlas for the Biology Laboratory. Morton, Englewood CO. pp 24-44.

Brocks JJ, Logan GA, Buick R, Summons RE, 1999. Science 285:1033. Archean molecular fossils and the early rise of eukaryotes.

Name_____ Pre-Lab: Plant Diversity

1) For each of the following, indicate whether it is a sporophyte or a gametophyte. See the life cycle diagrams in your lab manual for help.

a) The antheridium of mosses.
b) The archaegonium of mosses.
c) Fern fronds.
d) The trunk of a tree.
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 I

Note: There are four parts to this lab: the diversity of plant life cycles set up in lab, the greenhouse visit, comparing Monocots and Eudicots and preparing your experiment for growth measurements in week 3. There will be a laboratory practical exam towards the end of the semester and everything you learn from now until then in lab could be on it. Focus today on plant life cycles.

Purpose

To observe and analyze the diversity of plants through studying four major land plant groups
Identify the parts of plants at different life stages
Dissect a flower (second week) Bring one in next week!
Identify structure of plants: Stems. Leaves, roots, xylem, phloem

Introduction

The Kingdom Plantae is characterized by multicellular, autotrophic (primarily but there are exceptions), sexually reproducing eukaryotes. Their cells have pigment-containing plastids. The main photosynthetic organelle contains chlorophyll a and b and is called a chloroplast. Plants have well defined cell walls made of cellulose. Reproduction in plants is characterized by alternation of generation which you will exam in different plant phyla. There are 10 extant phyla (see *Campbell's* Table 29.1)

Photosynthesis:

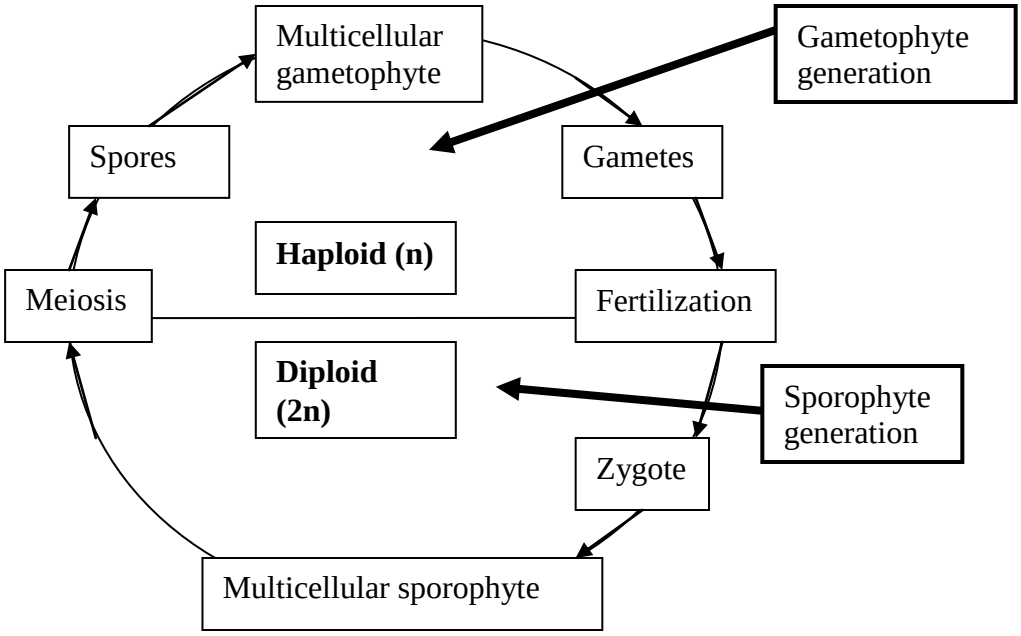
$$6CO_2 + 12H_2O + \text{light energy} \longrightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$$

Plants are the dominant form of photosynthetic life on land. Algae, both microscopic forms and larger seaweeds, are the dominant photosynthetic life forms in fresh water and marine environments.

The best context within which to examine the plant material in this lab exercise is the increasing complexity of plants as they have become better adapted to terrestrial environments. The earliest land plants were obligated to live in moist environments. They didn't need or have water-conducting roots or vascular tissues like xylem. Modern mosses and liverworts are still like that. Reproductive strategies and structures have also changed with increasing adaptation to terrestrial environments. The gametophyte stage is less pronounced in the more evolved plant taxa. Plant embryos in these taxa are packaged in containers that can withstand prolonged desiccation. We call these

containers seeds. General characteristics of the plant groups are listed in the table below followed by a generalized life cycle diagram.

Plant	True Roots?	Dominant generation	Haploid N or Diploid 2N	Spores? N or 2N?	Seeds?	Flowers?
Moss	No	Gametophyte	Haploid –N	Yes – N	No	No
Fern	Yes	Sporophyte	Diploid -2N	Yes – N	No	No
Pine	Yes	Sporophyte	Diploid – 2N	Yes – N	Yes	No
Lily	Yes	Sporophyte	Diploid – 2N	Yes – N	Yes	Yes



All plants have life cycles that continually alternate a sporophyte generation with a gametophyte generation. The sporophyte produces spores through meiosis which germinate and grow into the gametophyte. The gametophyte produces gametes through mitosis that fuse during fertilization to form the zygote which grows into the sporophyte. All parts of the plant in the sporophyte generation are diploid and have two sets of chromosomes in each cell except for the spores. All parts of the plant in the gametophyte generation are haploid and have one set of chromosomes in each cell. Organisms that have the alternation of generations must have multicellular individuals in both generations.

You will have two weeks to learn the life cycles of these land plant groups and draw diagrams of them. You will also learn about the structure roots, stems and leaves and the structural differences between Monocots and Dicots. You will answer also questions in the greenhouse and perform the plant growth lab, which follows this one.

Part I: Plant Diversity Drawings: Look at the plants and microscope slides in the lab. Draw what you see, using the textbook as a guide.

Lab assignments: You must draw the 22 pictures listed below today and hand them in next week in lab to be checked off by the TA at the end of the plant diversity labs. Label drawings with sizes indicated on each. The table below helps to identify by plant type what should be included, 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**
Moss: Bryophyta	*macro Leafy green plant *micro slide of stem x-section with no vascular bundles	*macro stalk and capsule *micro spores in capsule (cross section of capsule with spores)
Fern: Pterophyta	*micro male c. fern gametophyte on plates and hermaphrodite forms	*micro {stem vasculature} {fronds with "sori" containing spores} *macro fern plant
Pine: Coniferophyta	*micro of both megagametophyte (in ovule) and microgametophyte (pollen)	*macro female cone & male cones Pine branches Stem vasculature
Angiosperm : Angiospermae	*micro of both megagametophyte (in ovule) and microgametophyte (pollen)	*macro flowering plant *micro leaf cross section vasculature of stem

Lab Check list of Drawings

1) Male g′phyte c-fern_____
2) Hermaphrodite g′phtye c-fern_____
3) Sporophyte c-fern_____
4) Moss macro g′phyte_____
5) Moss micro g′phyte {no vascular bundles}_____
6) Moss macro s′phyte_____
7) Moss micro s′phtye {spores in capsule}_____
8) Fern micro male g′phyte_____
9) Fern micro hermaphrodite g′phyte_____
10) Fern micro s′phyte {vasculature}_____
11) Fern macro s′phyte {spores in sori}_____
12) Pine micro mega g′phyte (in ovule)_____
13) Pine micro micro g′phyte (pollen)_____
14) Pine macro female cone_____
15) Angio micro mega g′phyte (in ovule)_____
16) Angio micro micro g′phyte (pollen)_____
17) Angio micro leaf cross section {vasculature}_____
18) Dissected flower_____
19) Fern life cycle_____
20) Moss life cycle_____
21) Pine life cycle_____
22) Angiosperm life cycle_____
23) Brought in a flower_____

PART II. Greenhouse Tour

Diversity of Plant Adaptations

The greenhouse is on the fourth floor of the Science Building. As you enter the potting room and work area, the first glasshouse on the left is a tropical room, the second and third contains teaching and research materials. **Before visiting the greenhouse,** look over the questions below. When you get to the greenhouse, wander through all the rooms before you look for plants that will enable you to answer the questions. Some of the questions require the use of your text or other reference for their answer. The answers to these questions will be due in your next lab.

1.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.

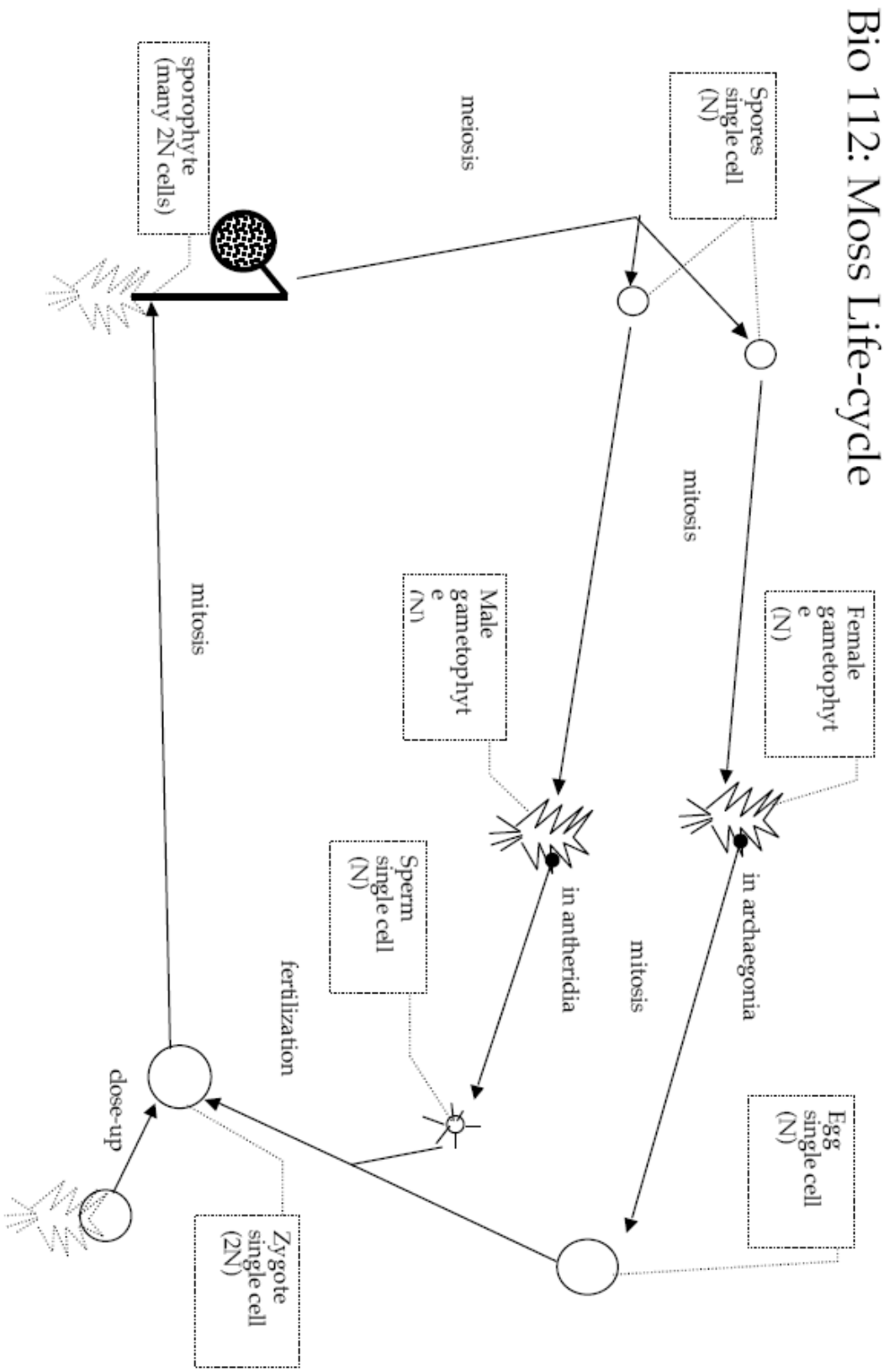
2. What plants in the greenhouse do you find that are specialized for defense against herbivores and what adaptations do they exhibit? Give two different examples with genus and species names.

3. 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.

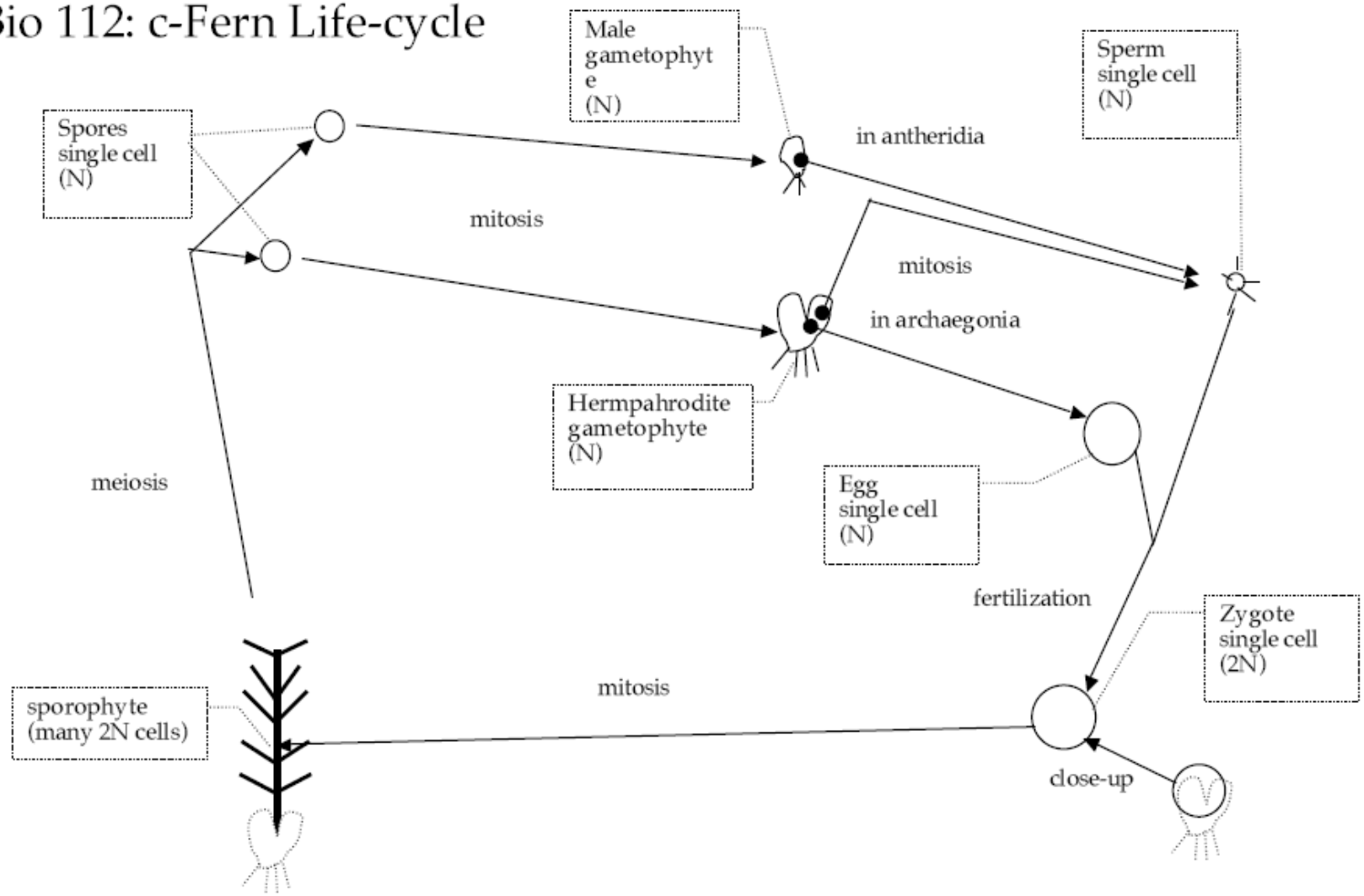
4. Give two examples, with genus and species names, of plants found in the greenhouse that you might also find in the supermarket in one form or another.

5. 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?

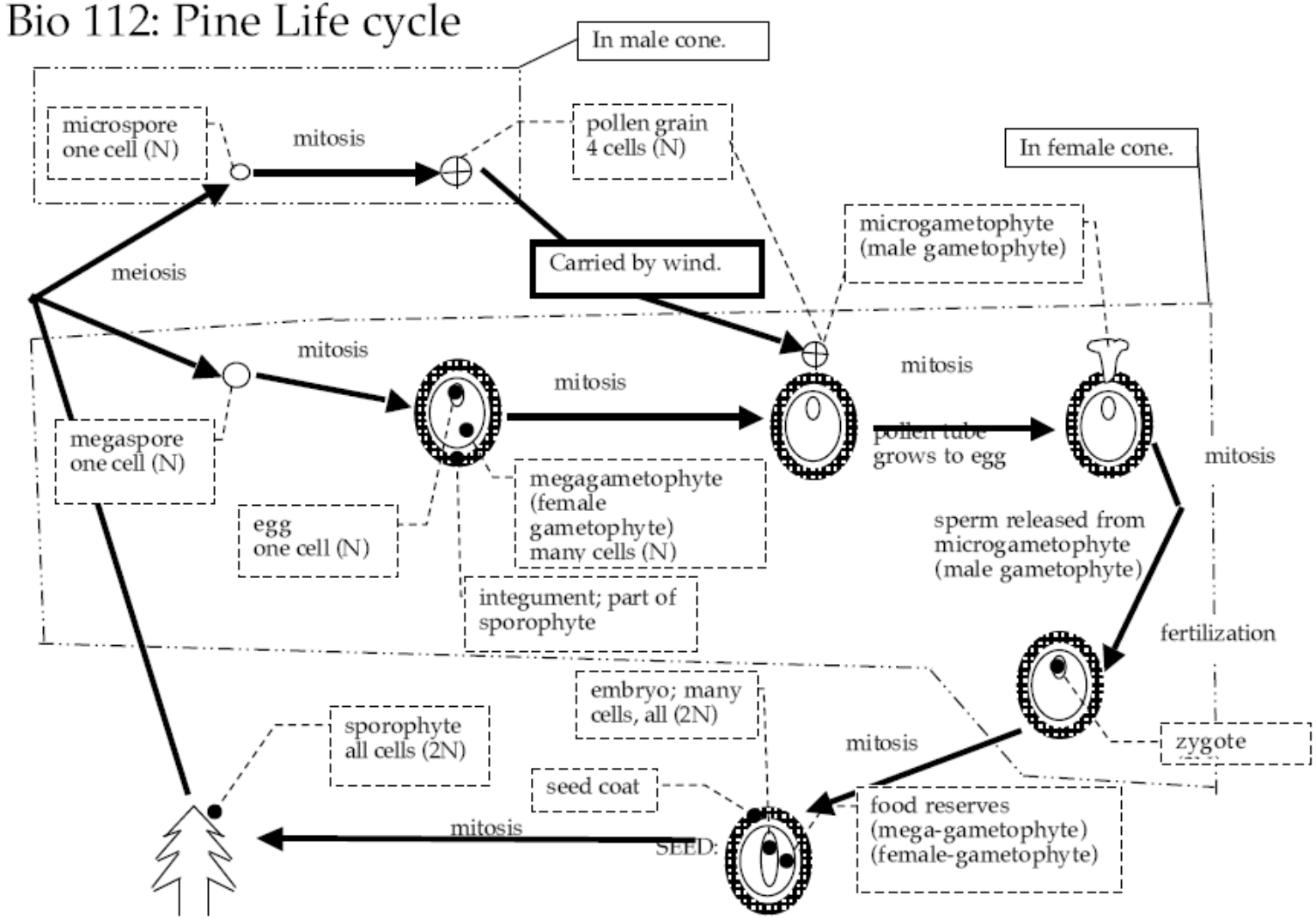
6. In the greenhouse are several succulent plants. What do they have in common? Is this an example of convergent evolution? Why/why not? How are these advantageous in dry climates?


Bio 112: Moss Life-cycle
Spores
single cell
(N)
meiosis
sporophyte
(many 2N cells)
mitosis
Female
gametophyt
e
(N)
in archaegonia
Egg
single cell
(N)
mitosis
Male
gametophyt
e
(N)
in antheridia
Sperm
single cell
(N)
fertilization
Zygote
single cell
(2N)
close-up

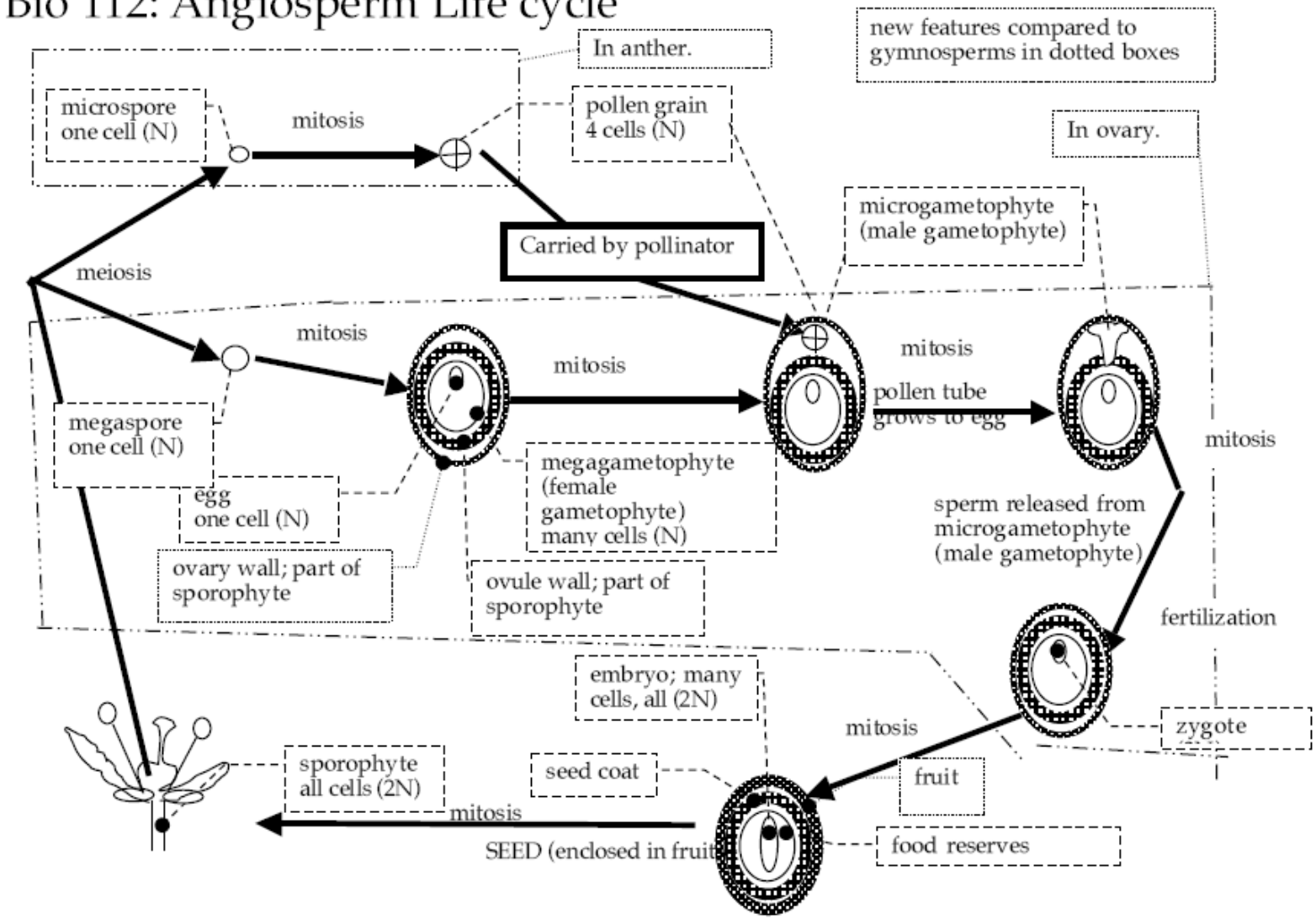
Bio 112: c-Fern Life-cycle



Bio 112: Pine Life cycle



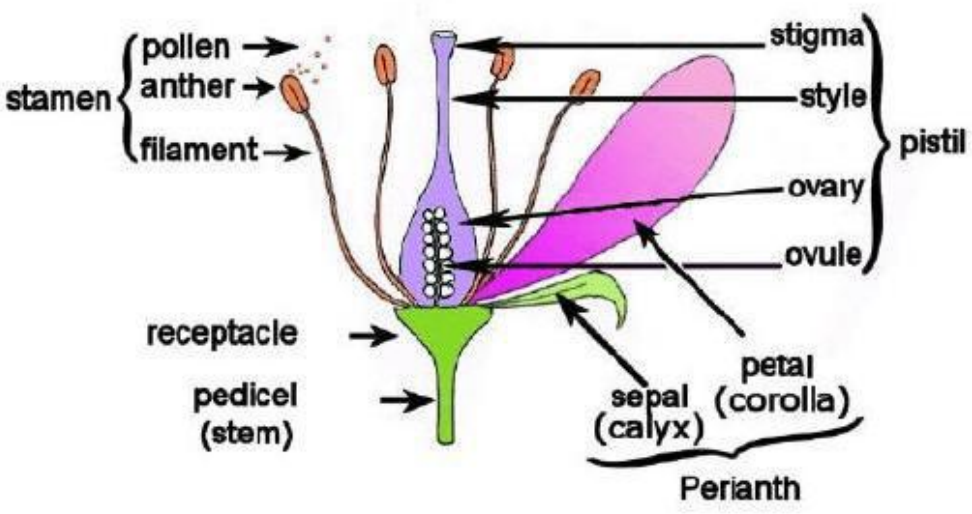
Bio 112: Angiosperm Life cycle



Part III Angiosperm Diversity and Structure: Monocot vs. Eudicots (Dicots)
A. There are two major divisions in the Angiosperma- the Monocots (e.g. corn, grasses, palm trees) and the Eudicots (Roses, Oak trees, Maple trees). Compare and draw the following structures of plants from each group:

1. Number of cotyledons (embryonic leaves)
2. Leaf venation
3. Stem vascular tissue (How are the xylem and phloem arranged?)
4. Root structure and cross section
5. Numbers of flower organs.

B. Flower anatomy: Exam the flower you brought it. Before you cut it count the flower parts. Is a moncot or a eudicot? Include the name of the plant and the size of the flower You must draw and label your dissected flower as well as diagrams of the lily anthers and stamen from prepared slides.



1. Flower Dissection

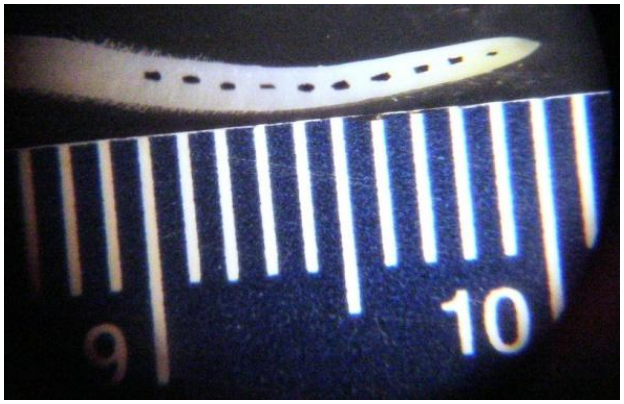
Cut your flower down the center into the stem with the scalpel to open it up. Make a drawing of your flower. Label all of the parts you can identify using your text as a guide. Label the following parts: Carpel (Stigma,Style, Ovary); Stamen (Anther, Filament); Sepal, Petal, Ovule. NOTE: Not all flowers have all of the parts listed, if the flower you brought in lacks some of these parts, look at more flowers. Why might some individual flowers lack some of these parts?

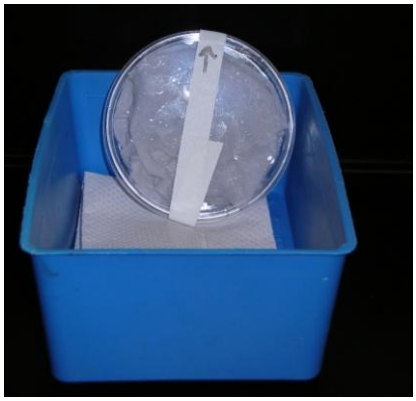
Part IV. Localization of Plant Growth (set up week two, measure week three)
To determine how a root grows, we will mark the last centimeter of pea root tips with ink at 1 millimeter intervals. We will pack these seedlings in a growth chamber for one week and then in the next lab, we'll measure the distances between each marked interval to see if root growth is localized in one area of the root or consistent throughout the root.

A. Select six germinating pea seeds, each having a fairly straight root about 1.5 – 2 cm long.

B. Blot the roots with a Kim wipe to remove any excess moisture and lay them on the stage of a dissecting microscope against a millimeter ruler. Or you may mark the root tips by eye without a microscope if you can.

C. Starting from the tip of each root, carefully mark off 10 – 1 mm intervals with India ink using the tip of a toothpick. Don't put a mark on the very tip; see picture below. Make the marks as small as possible.





D. Lay the seedling down on the Kim wipe for 5 minutes to allow the ink to dry.

E. You will use a plastic Petri dish as a moist chamber to conduct this experiment. Line the Petri dish with paper towels and spray them with water. Align seeds at the top of the dish allowing the roots room to grow.

Cover with wet paper towels. Have the paper towels saturated with water but not soaking in pools of water. Tape the dish shut; mark the top of the dish where the seeds are with an arrow pointing up and your group name. Stand the plate on its side with the seeds up with the others in a plant box.

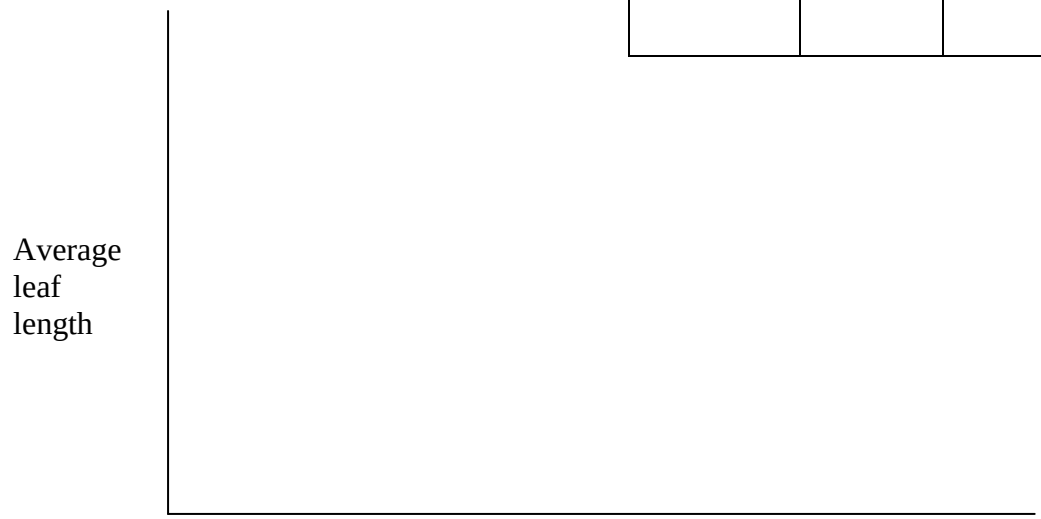
Name_____ Pre-Lab: Plant Structure and Growth

1. Explain the difference between determinate and indeterminate growth. What regions in plants have the perpetual embryonic tissue that allows for continuous growth?

2. What is an epiphytic plant?

3. Graph the average leaf length with age. Put the values in the appropriate space on the axes. Connect the data points with a line.

Length (mm)			Age (days)
Leaf 1	Leaf 2	Leaf 3	
5.0	4.0	3.0	0.5
4.0	4.0	4.0	3
6.0	11.0	11.0	7
98.0	83.0	92.0	14
121.5	148.5	137.5	21
143.5	139.0	136.0	28



Plant Structure and Growth

Purpose

Dissect and examine seed structure
Distinguish between determinate and indeterminate growth
Observe the localization of cell growth in pea root tips
Compare development of moncot and eudicot plants

Introduction

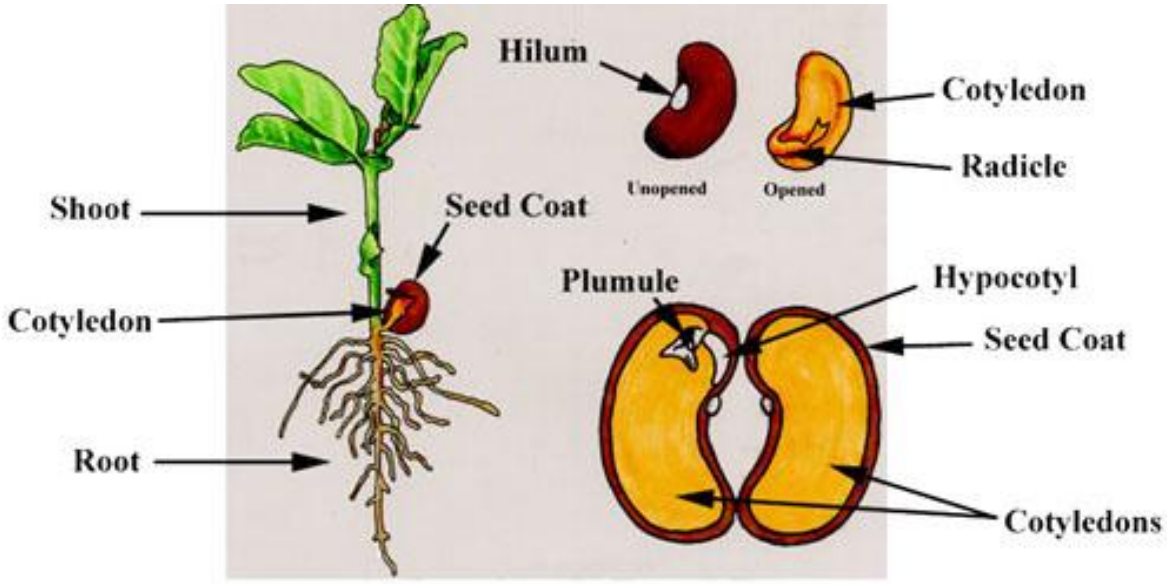
From zygote to mature form, a plant develops through a series of changes involving both **growth** and **differentiation**. For any organism, the term growth refers to quantitative and irreversible changes that take place during the life cycle. (What is the fundamental process of growth in plants?) Differentiation applies to qualitative differences between cells, tissues, and organs that occur during the process of development.

A very young plant embryo consists of a spherical mass of rapidly dividing cells. However, when the embryo reaches a certain size, cell division becomes restricted to a few regions of the embryo, such as shoot and root meristems. Within an embryo, development proceeds by predictable and sequential steps that lead ultimately to the normal growth and development of the plant.

A significant difference between plants and most animals is that plants have **indeterminate growth** while animals have **determinate growth**. Animals grow during a juvenile period until the adult form is reached and growth ceases. Cells are replenished and energy is generated by the organism but its size is limited. Plants, however, can grow in size throughout their lifespan. Regions in stems and roots have perpetually embryonic tissues called **apical meristems** and this is where primary growth occurs. Secondary growth appears in **lateral meristems** which causes thickness in woody plants. Certain parts of plants have determinate growth such as leaves, flowers and fruit.

There are 4 parts to today's lab:

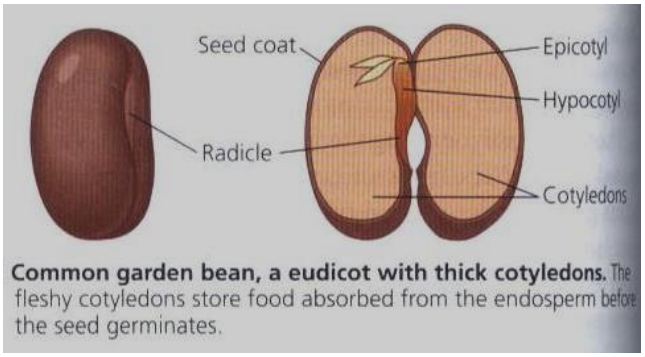
1 – Dissection – bean seed observe early growth stages
2 – Dissection –corn kernal observe early growth stages
3 – Localization of Plant Growth – measure growth in pea roots
4 – Determinate Growth – measure bean leaves of various ages



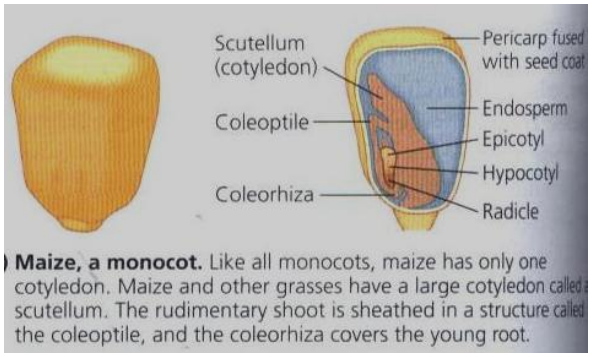
Cotyledons

Procedure

1. **Structure of Bean Seeds and Corn Kernals**
   Obtain 2 or 3 soaked bean seeds and corn kernals. Observe the scar on the seed coat, the **hilum**, where the seed was attached to the wall of the pod. Beside the hilum there is a small opening, the **micropyle**, through which the pollen tube enters the ovule. Remove the seed coat (testa) and notice the number of **cotyledons**. The embryonic axis lies between the two cotyledons. Locate the **hypocotyl** with the **plumule** (the parts that develop into the shoot and the first foliar leaves, respectively) and the **radicle** (the region that becomes the primary root). Sketch the bean seed and corn kernal and label the parts in bold above. Use the dissecting microscope to get a closer view. Save these beans to use later in Part 3.



Common garden bean, a eudicot with thick cotyledons. The fleshy cotyledons store food absorbed from the endosperm before the seed germinates.



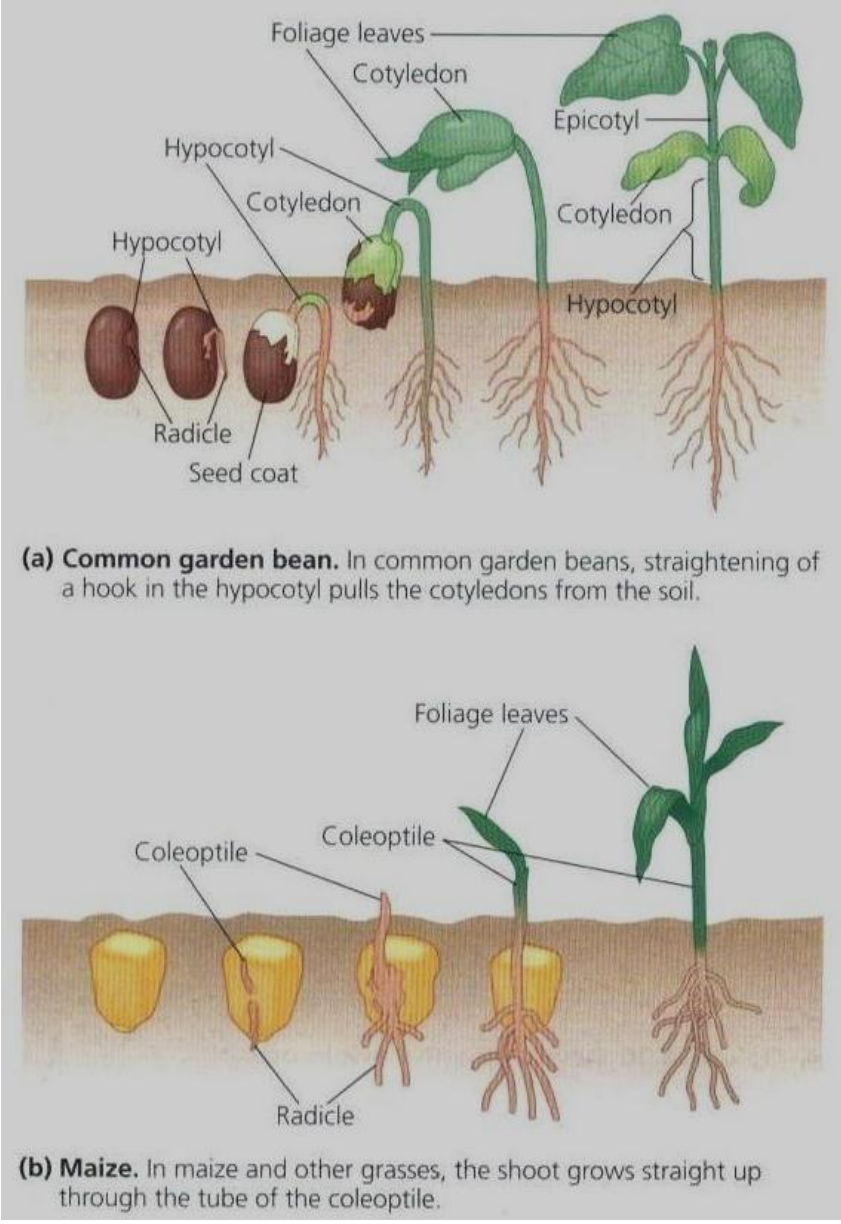
Maize, a monocot. Like all monocots, maize has only one cotyledon. Maize and other grasses have a large cotyledon called the scutellum. The rudimentary shoot is sheathed in a structure called the coleoptile, and the coleorhiza covers the young root.

2. Early growth of monocots and eudicots; look at series of germination bean and corn plants. Answer the following questions for both corn and bean growth:

a. Which part emerges first?

Corn_____ Bean _____

b. How does early growth differ between the two plants?



3. Indeterminate Growth – Pea Root Tips - Localization of Growth

A. In the previous lab you marked the last centimeter of 6 pea root tips at 1 millimeter intervals. Today you will open your growth chamber, observe the ink marks and measure the distance between each millimeter mark after 2 days growth. If any of the ink marks cannot be seen on a root tip and you know which marks are missing, you can list the interval distances that you know and leave blank the ones that are missing. Calculate the average length of growth for each interval, adjusting the total number of seeds you divide by if there are missing values in that column. Record your data in a tabular form (below) and graph the results with the root tip intervals on the X-axis and the average root tip growth on the Y-axis.

Root Tip Growth (mm)

	Intervals									
Root number	1	2	3	4	5	6	7	8	9	10
1										
2										
3										
4										
5										
6										
Average										

Is growth in the pea root tip consistent throughout the length of the root or more pronounced in one area? What is that one area called (hint: see section 3B)?

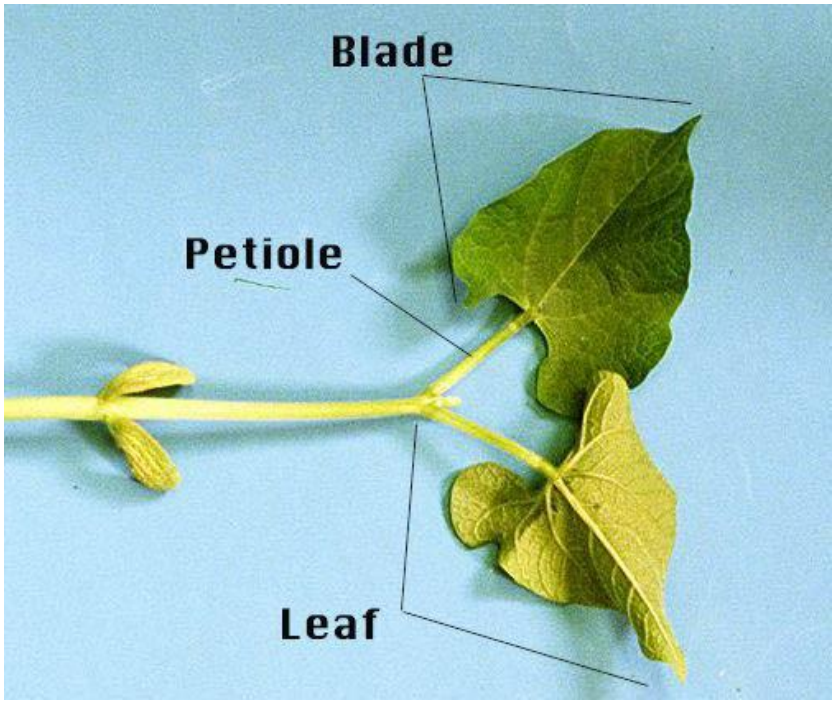
3 B. Examine a prepared slide of a longitudinal section through an onion root tip. The nuclei in these cells are stained dark and chromosomes in the nuclei can be seen in various stages of mitosis (use the 40x lens to see this). Where mitosis is occurring constantly, new cells are being produced. This is the apical meristem. The root cap covers and protects the apical meristem from the soil. These cells are relatively larger and have less mitosis taking place. Behind the apical meristem is the zone of elongation. Here mitosis slows and the cells lengthen. Behind the zone of elongation is the zone of differentiation. Here cells specialize their structure and function becoming root hairs, transporting tissue cells and other types of cells. Make a labeled sketch below that includes the following: root cap, apical meristem, zone of cell elongation and zone of cell differentiation. Show different phases of mitosis in the apical meristem cells.

4. Determinate Growth - Length of Bean Leaves Over Time

Bean leaves grow until they reach their mature size and stop. Cells in these leaves carry on metabolic functions and continue to reproduce but the *size* of the leaf does not increase once it reaches maturity. To observe this characteristic we will measure the length of leaves from bean plants and bean seeds as they develop from a few hours old to a few weeks old.

Procedure

Select three bean seeds that have been soaking in water for several hours. You can reuse the bean seeds that you opened earlier to see the structures in the seeds if the leaves are still intact. Split them open and measure the length of the first foliar embryonic leaves. Record the length in the table below. Measure the length of the first foliar leaves on 3 of the other 2 sets of beans seeds which have been soaking for 1 day and 5 days. Assume that both foliar leaves up to 5 days old are the same length. Measure and take the average of the first two foliar leaves on three plants from each group that were planted on different dates. When measuring leaves, include the length of the petiole and the length of the blade in the total length. List all leaf measurements in the following table. Calculate the age of the plants you are measuring; the day each flat was planted is written on tape on the side of the plant box. Present your data in a graph with the average length of leaf on the y-axis and age of leaves in days on the x-axis as you did in the pre-lab. Why are we averaging the length of 2 leaves on 3 plants? Why not just measure one leaf per plant?



Discuss the nature of the curve obtained. Compare and contrast the growth of roots with the growth of leaves in terms of determinate versus indeterminate growth.

Length of Bean Leaves (mm)

date planted	age (days)	plant 1			plant 2			plant 3			av length all 3 plants
		leaf 1	leaf 2	av plant 1	leaf 1	leaf 2	av plant 2	leaf 1	leaf 2	av plant 3	

Lab assignments: Be sure to complete the following by the end of lab today so you can study them later on. Have the TA check them off before you leave lab and you should go over the graphs and greenhouse questions as a group before you leave lab.

Drawings:
labeled dissected seeds
labeled root tip from the prepared slide

Graphs:
root tip length
leaf growth

Name: _____ Pre-Lab: Animal Diversity

1)Name one coelomate, one pseudocoelomate, and one acoelomate phylum from the lab on animal diversity.

Coelomate:

Pseudocoelomate:

Acoelomate:

2) Name one phylum from the lab on animal diversity that has a complete digestive tract and one with a gastrovascular cavity (same opening for mouth and anus).

Complete:

Gastrovascular cavity:

3) Name one protostome and one deuterostome phylum from the lab on animal diversity.

Protostome:

Deuterostome:

4) Name one phylum lacking symmetry, one with radial symmetry, and one with bilateral symmetry.

No symmetry

Radial symmetry:

Bilateral symmetry:

Animal Diversity I

Note: Today you will study and compare eight animal phyla. If you still have time, you will also be able to start dissecting either a squid or trout: Animal Diversity II. Next week you can finish the dissections. Keep in mind that this material will be on the laboratory practical exam.

You need to bring copies of your textbook for reference, this is extremely important for this lab.

Objectives:

Describe similarities and differences in the anatomy of representative animals.
Discuss how these similarities and differences may indicate phylogenetic relationships.
Discuss the relationship between body form and lifestyle of the organism.
Understand the relationship of cross sections to the whole organism and the terms used to describe anatomical direction.

Introduction

Phylogeny is the evolutionary history of organisms: their lines of descent, the branching of these lines, and thus the relationships between organisms. Much of our understanding of animal phylogeny has come from comparative studies of the anatomy and embryology of present-day animals. Our concepts concerning their ancestral history and relationships have been extended, refined, and sometimes changed as a result of physiological, cellular, or molecular studies.

Just as our understanding of animal phylogeny benefits from a study of anatomy, our understanding of anatomy is enhanced by an understanding of evolutionary principles. The form and function of all features of an organism are determined by: 1) the selection imposed by the organism's environment, and 2) the genetic/morphological/physiological constraints imposed by the general architecture that the organism's lineage has developed over the course of its evolutionary history. Regardless of their particular phylogenetic group, all living animals have the same basic requirements and must perform the same basic functions. Animals may meet these problems in different ways because of differences is size, structure, and environment.

Taxonomists divide the Kingdom Animalia into two subkingdoms: Parazoa which includes sponges and Eumetazoa which includes all other animals (with a couple of controversial exceptions). Parazoa differ from Eumetazoa in that the former lack true tissues and most have an indeterminate form. Taxonomists further divide Eumetazoa on the basis of morphological characteristics such as body symmetry, type of body cavity (known as the coelom), and basic

embryological difference including the number of germ layers and development of the digestive tract. However, results from research using molecular techniques have started to reform the traditional animal phylogeny based on body form. In many ways the phylogenies agree but in many ways they do not.

Before you begin your observations become familiar with the following characteristics:
Symmetry- Radially symmetrical animals have their body parts arranged around a central axis such that any imaginary slice through the central axis would divide the animal into mirror images. These animals have no right/left nor heard/tail; they have an oral (mouth) and aboral (away from the mouth) side.
Bilaterally symmetrical animals have right and left halves which are mirror images of each other. Only one imaginary cut would divide the animal into its mirror-image and posterior (tail) ends, and right and left sides.
Animals with no symmetry cannot be divided into mirror-image halves.

Tissue organization- Are cells organized into well –defined tissue layers (structural and functional units)? If so, how many distinctive layers are present? In Metazoan animals, the process of gastrulation during development results in the formation of concentric layers of tissue called germ layers which give rise to the various tissues and organs of the body. Animals may have up to three layers: ectoderm, mesoderm, and endoderm. Animals with two layers are diploblastic and those with three are triploblastic. Ectoderm, the outermost layer, gives rise to the outer covering of the animal and in some phyla the central nervous system.
Endoderm, the innermost layer, gives rise to the lining of the digestive tract and organs derived from it (e.g., liver). Mesoderm, the layer between the ectoderm and endoderm, forms the muscles and most other organs between the digestive tube and ectoderm.

Coelom (body cavity) – Most triploblastic animals can be assigned to one of three groups depending on characteristics of the body cavity or coelom.
Acoelomate animals have solid, three layered bodies without a body cavity.
Mesodermic tissue completely fills the space between the endoderm (lining of the digestive tract) and the ectoderm (the body wall).
Coelomate animals have a cavity or space between the ectoderm and endoderm that is completely surrounded by the mesoderm. The mesodermal lining of the coelomic body cavity is known as the peritoneum.
Psuedocoelomate animals have a body cavity that is not completely lined by mesodermal tissue; instead, it is bordered by mesodermal tissue toward the outside of the body and endodermal tissue toward the inside of the body.

Digestive tract (gut) – Openings into the digestive tract- Where does food enter and digestive waste leave the body? How many openings are there? Some animals have only

one opening which serves as both mouth and anus. Others have separate openings for the mouth and anus (complete digestive tract), sometimes referred to as a tube within a tube.

Protostome/deuterostome- Coelomates can be further divided based on the developmental fate of the embryonic blastopore (the opening in the gastrula). Protostome ("first mouth") - The blastopore develops into the mouth and anus develops as a secondary opening on the end opposite the mouth. Protostomes also exhibit schizocoelous formation of the coelom in which the coelom forms from splits in the mesoderm. Deuterostome ("second mouth") –The blastopore develops into the anus and the mouth develops as a secondary opening on the end opposite the anus. Deuterostomes also exhibit enterocoelous formation of the coelom in which the coelom forms from outpocketings of the mesoderm.

Cleavage-Pattern of cleavage divisions typically differs between protostomes and deuterostomes (although many exceptions exist).
Spiral cleavage- Most protostomes exhibit spiral cleavage in which cell division results in each tier of cells sitting in the grooves of the adjacent tier of cells.
Radial cleavage- Most deuterostomes undergo radial cleavage in which the tiers of cells sit directly on top of one another.

Where possible also look for:
Type of nervous system- Does the organism have a brain or nerve cord? How many nerve cords and in what location? What types of sensory organs are present? Where and how many?

Circulatory system-Open circulatory system-blood flows through coelomic spaces where it mixes with interstitial fluid and bathes the organs directly.
Closed circulatory system- blood mainly flows through vessels separate from interstitial fluid.

Organs for respiration – Does the organism possess specific organs for the exchange of gases? Where does the exchange occur—skin, gills, lungs?

Organs for excretion- How does the animal get rid of nitrogenous waste?

Support system- How does the animal support its body/ Is there a skeleton present-endoskeleton or exoskeleton? If the animal has no true skeleton, does it use a hydrostatic skeleton for support (fluid within and between cells and in body chambers such as the gastrovascular cavity or coelom)?

Habitat- terrestrial or aquatic

Anatomical glossary

- Anterior or rostral: towards the head end.
- Posterior or caudal: towards the tail end.

  "Your nose is anterior of your belly button. Your chin is posterior of your nose. "
- Dorsal: toward or near the back.
- Ventral: toward or near the belly.

  "Your belly button is ventral of your intestines."
- Medial: in or near the plane in the middle of the body.
- Proximal: near the base or site of attachment
- Distal : near the tip.

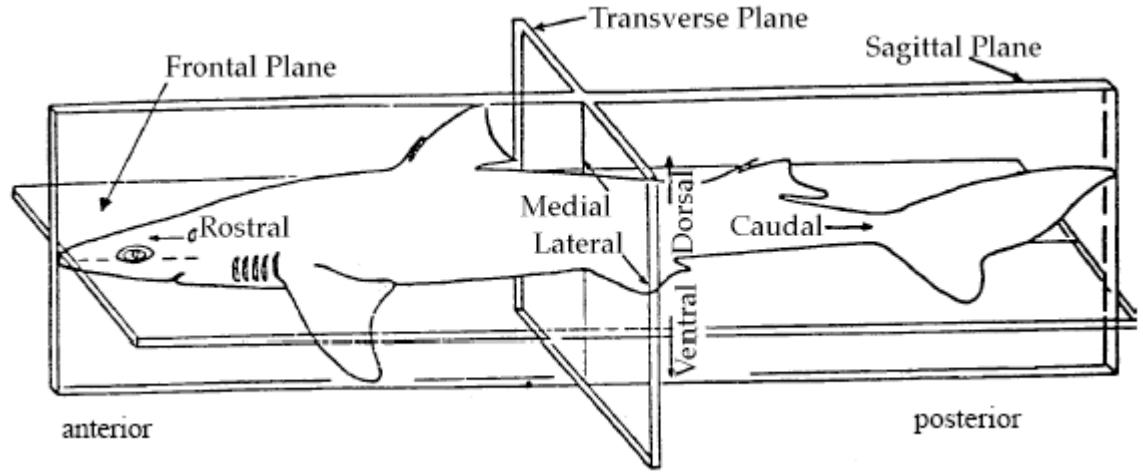
  "Your fingernails are on the distal ends of your fingers."
- Sections through the body are called:

  Sagittal: dividing the animal into left and right sides

  Frontal: dividing the animal into dorsal and ventral parts.

  Transverse: dividing the animal into anterior and posterior parts.

See this figure:



Phylum Porifera

Phylum Porifera (Latin porus, pores; Greek fera, bearing) encompasses the sponges which split early from the main branch of animal evolution and has given rise to no other animal groups. The phylum contains approximately 9,000 species of sponges, all of which are aquatic and most of which are marine. Although adult forms are sessile, the larvae (immature forms) are motile. Sponge bodies consist of two cell layers, an outer epidermal layer and an inner layer of flagellated collar cells, but have no tissues or organs. Between the layers lies an acellular layer called the mesohyl which contains amoeba-like cells (amoebocytes) with various functions such as food storage, digestion, waste elimination, and formation of reproductive cells. Some amoebocytes secrete materials that form an endoskeleton that supports the sponge. These support materials include sponging, a fibrous protein, and spicules, a mineral crystal. Bath sponges, for example, contain fibrous

endoskeletons with no spicules. Sponges generally lack anterior/posterior and left/right symmetry and often grow to fit the space in which they live. Sponges feed by filtering suspended food particles out of the water column (i.e., suspension feeders). Water flows through the numerous pores that perforate the sponge's body into the central opening called the spongocoel and then out of the sponge through a larger opening called the osculum. The central cavity or spongocoel is not a digestive tube or body cavity in the sense of a coelom but is only a channel for water. Moreover, the osculum is not a mouth but an opening used as an outlet for the current of water passing through the sponge. The flagellated collar cells (also called choanocytes) bring water into the sponge through the pores and the collar sieves out food particles such as microscopic algae, bacteria, and organic debris. Most sponges are hermaphrodites and can reproduce both sexually via sperm and eggs and asexually from fragments of a parent sponge.

Examine the section of sponge. Observe the pores for which the phylum is named. Be able to describe how sponges feed.

Examine the spicules. In addition to structure, what other function might these serve the sponge?

What would you hypothesize about the movement of oxygen and waste throughout the sponge body and into and out of the cells?

How would you describe the symmetry of a sponge?

Given that all sponges are filter feeders, why are all aquatic?

Do you see evidence of nervous or circulatory systems?

Do you see evidence of cells organized into tissues or organs?

Phylum Cnidaria

Phylum Cnidaria (Greek Knide, nettle; Latin aria, like) contains approximately 10,000 species, all aquatic and mostly marine. Cnidarians exhibit radial symmetry in two distinct body forms: (1) polyps, a primarily stationary or immobile form (e.g., sea anemones, corals, hydra), and (2) medusa, a free floating, mobile form (e.g., jellies). Some species display both body forms during their life cycle. Cnidarian bodies consist of two tissue layers, the outer epidermis and inner gastrodermis, separated by a gelatinous material, the mesoglea (not to be confused with mesoderm) that helps support the body. The central, sac-like gastrovascular cavity has a single opening which serves as both mouth and anus. Undigested food remains are ejected through the same opening as food is ingested. The water in the gastrovascular cavity also serves as a hydrostatic skeleton. Cells in the two

tissue layers have bundles of microfilaments arranged into contractile fibers (note that true muscle develops from mesoderm, not found in cnidarians). The contractile cells work against the hydroskeleton to perform movement. A non-centralized nerve net coordinates movement of the contractile fibers and simple sensory receptors are distributed around the circumference of the body. Cnidarians use the tentacles around their mouth to capture prey. The tentacles contain specialized cells called cnidocytes which contain organelles called cnidae. Cnidae are capable of everting to entangle or sting a prey item or in defense. The forms of cnidae tipped with a stinging barb or spine are known as nematocysts. Some cnidae can inject poison as well.

Examine hydra whole mount and cross sections.

Sketch the longitudinal cross section and label the gastrodermis, epidermis, mesoglea, mouth, anus, tentacles, and gastrovascular cavity.

Why is radial symmetry adaptive for a sessile animal in an aquatic habitat?

Name a disadvantage of having one opening into the digestive cavity.

Examine the slide of the hydra nematocyst.

How does the hydra use the nematocyst?

Examine the slides of Obelia, a colonial cnidarian with both polyp and medusa stages.

Compare the slides to the diagram provided. Sketch and label feeding polyps, reproductive polyps, and medusa buds.

How does the medusa stage fit into the lifecycle of Obelia?

Which stages are haploid and which are diploid? Label the feeding polyps, reproductive polyps, and medusa buds as such.

Of what advantage is the colonial life adopted by Obelia?

If an animal is stationary what is the purpose of having a motile phase at some point in the life cycle?

Why is it advantageous to have sensory cell encircling the bell of the medusa?

Phylum Platyhelminthes

Phylum Platyhelminthes (Latin platy, flat; Greek helmis, worm) includes about 20,000 species. A dorsoventrally flattened body (i.e., thin between dorsal and ventral surfaces) characterizes these species. Like other bilaterally symmetrical animals, flatworms have three layers of tissue but have no body cavity or coelom. Most Platyhelminthes have a digestive tract with one opening; although the parasitic cestodes or tapeworms lack a digestive tract. Flatworms lack organs specialized for gas exchange and circulation, and most nitrogenous waste diffuses directly out of the cells into the surrounding water. However, they do possess specialized cells, called flame cells that primarily help the organism maintain osmotic balance. Free-living flatworms such as planaria are in the minority, comprising less than ¼ of the species, compared to parasitic forms such as flukes and tapeworms. Flatworms live in marine, freshwater and terrestrial habitats and range in size from microscopic to over 20 meters long in some tapeworms!

<u>Examine the Planaria slide whole mount.</u>

Examine the body for a number of digestive openings (the stained planaria shows the digestive system). Observe the pharynx and mouth. The pharynx lies in a pharyngeal chamber inside the mouth. The proximal end of the pharynx opens into the dark-colored branched intestine. How do planaria rid themselves of solid waste?

Which end is the head? What led you to that conclusion?

Two auricles containing a variety of sensory cells (e.g., touch and chemical receptors) project from either side of the head or anterior end (blunt end.) Two pigmented eyespots, sensitive to light intensity and direction but unable to form images, lie between the auricles. The pigment cups contain the photosensitive end of retinal cells which extend from the brains. Two cerebral ganglia (the brain) lied beneath the eye spots, and two ventral nerve cords extend posteriorly from the brain. Transverse nerves connect the two nerve cords forming a ladder like nervous system.

How might bilateral symmetry be an advantage to a motile organism? What is cephalization? Under what circumstances would it be useful? Under what circumstances would it not be useful?

Look at planaria in cross section. The slide shows sections through three different regions of the body. Do you see a body cavity or coelom? What word describes this condition? (The pharyngeal chamber and spaces in the gut are not a coelom – recall that the coelom is a space surrounded by mesoderm between the body wall (ectoderm) and lining of the digestive tract (endoderm)).

How many tissue layers can be detected?

What provides support for the body?

Diagram the flatworm as seen in a cross section at the level of the pharynx. Label the epidermis, muscle derived from the mesoderm, the lining of the digestive tract derived from endoderm, and the pharynx.

How do flame cells relate to the ability of flatworms to live in freshwater and terrestrial habitats?

Examine the tapeworm slide.

Adult tapeworms live in the intestine of numerous animals including humans. The scolex at the anterior end of the worm allows the animal to anchor itself to the intestinal wall to avoid being swept out by digestive movements. Posterior to the scolex is a chain of reproductive structure called proglottids each containing male and female reproductive organs. Mature proglottids loaded with thousands of eggs detach from the worm and leave the host's body with the feces. Tapeworms lack a digestive system and must absorb nutrients across their body surface directly from their hosts.

Sketch the tapeworm proglottid and label the uterus, ovary, testes, and genital pore.

Compare tapeworm and planaria anatomy in relation to their lifestyles (especially consider the proglottids, anterior structures, and lack of a digestive tract in tapeworms).

How does the flat body shape help with gas exchange and circulation in an animal without organs specialized for such functions?

Phylum Nematoda

Phylum Nematoda (Greek *nema*, thread,; *eidos*, form) contains about 90,000 identified species but may contain up to a million; indeed, nematodes may be the most abundant animals on earth. They live in virtually all habitats, including moist soils, beach sand, slat flats, ocean, hot springs, and lakes, and exhibit a great diversity of lifestyles. Although some of our most familiar parasites are nematodes (e.g., dog and cat heartworm, hookworm, roundworm, pinworm) most species are harmless or beneficial. These worms range from 0.1mm to 9m (a parasite in sperm whales) in length. These animals exhibit bilateral symmetry with three germ layers. Nematodes have a complete digestive tract with two openings but no circulatory system or organs for excretion of gas exchange. Their nervous system consists of a ring of nervous tissue around the anterior end of the worm with one dorsal and one ventral nerve cord extending posteriorly from the ring.

Examine the slides of the longitudinal sections of male and female Ascaris.

Identify the digestive tract. How is food taken in and undigested wastes expelled? What are the advantages of a complete digestive tract (i.e., a gut with two openings)?

Look at the cross section of male and female Ascaris.

Note that the body wall is made up from the outside inward of the cuticle (noncellular), epidermis (cellular), and muscle fibers. The muscle derived from the mesoderm lies at the outer boundary of the body cavity. Locate the intestine (derived from endoderm).

Can you detect muscle tissue adjacent to the endodermal layer? What do we call a body space that is lined by mesoderm laterally and endoderm medially?

Most of the body cavity is filled with reproductive organs. Identify male and female reproductive organs.

Identify the nerve cords. How many are there and where are they located?

Sketch a cross section of a male Ascaris and label cuticle, epidermis, muscle fibers, intestine, body cavity (give specific name), testis, dorsal and ventral nerve cords.

Phylum Nemertina

Phylum Nemertina (Greek Nemertes, a sea nymph) contains about 900 identified species ranging form less than 0.5mm to 30m in length, one of the longest invertebrates known. Their common name of ribbon worms refers to their flat bodies and often vibrant color patterns. They are characterized by a long anterior proboscis used to capture prey, explore their environment, and defend themselves. They can rapidly evert their proboscis which may extend up to three times their body length. Nemertines possess a blood vascular system through which blood pumps and a digestive tract with two separate openings, one for the mouth and one for the anus. Some species may promote gas exchange through a vascularized foregut but most occurs through the epidermis. Like flatworms, these species also possess flame cells that regulate ions and water and perhaps dissolved waste. Their nervous system resembles that of flatworms with cerebral ganglia and longitudinal nerve cords with connecting nerves. They also possess numerous anterior sensory organs for chemical, tactile, auditory, and light reception. Nemertines possess three body layers but their categorization as to the status of a coelom remains somewhat controversial. However, molecular data and ultrastructural evidence strongly suggest that they indeed possess a coelom but one that has undergone significant modification into the blood vascular system, gonadal sacs, and cavity that houses the proboscis.

Examine the slides of *Cerebratalus* embryonic development.

Identify the type of cleavage. Sketch the 8-cell stage. Is this animal a protostome or deuterostome?

Identify the blastula and gastrula. Sketch the gastrula and label the blastopore and archenteron. Will the blastopore become the anus or mouth? What does the archenteron become?

Phylum Annelida

Phylum Annelida (Latin *annellus*, little rings) contains approximately 15,000 species including marine, freshwater, terrestrial members and both free-living and parasitic species. Earthworm and leeches are the most familiar examples and a quick examination of their bodies illustrates why they are referred to as segmented worms. However, the most specious group of annelids is the polychaete class, most of which are marine. The outer body segments coincide with internal compartments containing serially repeated nervous, muscle, and excretory systems. Body segments are filled with a fluid that serves as a hydrostatic skeleton against which the muscles contract to allow movement. In contrast to the relatively unspecialized nematode digestive tract, the earthworm digestive tract is divided into several different organs. At the anterior end are the mouth and pharynx, a muscular organ used to draw food into the mouth. The esophagus, a passageway between the pharynx and the crop (a temporary storage organ for food), follows. From the crop, food passes to the gizzard which grinds the food into smaller pieces. The intestine follows posteriorly and runs the remainder of the worm. Enzymatic digestion occurs in the intestine followed by absorption of nutrient molecules. Undigested material is expelled through the anus. A pair of cerebral ganglia (brain) lies anterior to the pharynx and connects to a ventral nerve cord with segmented ganglia. The closed circulatory system consists of dorsal and ventral blood vessels connected by segmental pairs of vessels some of which around the esophagus are muscular and pump blood. The excretory system consists of segmentally –repeated pairs of metanephridia that remove nitrogenous waste from the blood and coelomic fluid. Individual earthworms possess both male and female reproductive organs (i.e., hermaphroditic). Mating worms lay head to tail side by side and transfer sperm to each other. On each worm the clitellum, a specialized swollen section of segments, produces a mucous cocoon that surrounds the eggs and transferred sperm. The cocoon then slides off each worm's head depositing the mass in the soil. The embryos develop inside the protective cocoon.

Observe the living earthworms under the stereoscope. Note the segmentation, mouth, anus, clitellum (a structure specialized for reproduction), and setae (small bristles on each segment used for locomotion).

Examine the cross section posterior to the clitellum. Sketch and label the intestine, two muscle layers (one inside the skin and one on the surface of the intestine), coelom, ventral nerve cord, dorsal and ventral blood vessels, epidermis, and metanephridia. Which parts are derived from the endoderm, mesoderm, and ectoderm? Label these.

Gas exchange must take place across thin wet surfaces. How do you think gas exchange occurs?

Examine the composite cross sectional slide of the earthworm. Locate the brain and pharynx.

Examine the slide of the leech whole mount. Note the segmentation.

What structures adapt it to a parasitic (external blood sucker) life style?

Locate the mouth and anus.

Examine the cross section of the leech. Identify the ectoderm, mesoderm, endoderm, and coelom. What does the coelom look like?

Phylum Echinodermata

The phylum Echinodermata (Greek echin, spiny, Greek derma, skin) includes about 7,000 species. All live in marine habitat, and as adults all exhibit a radial, five-part appearance. Some sources refer to them as radially symmetrical while other sources cite certain anatomical features that render them not truly radially symmetrical. However, this radial appearance (symmetrical or not) is believed to be secondarily derived from a bilateral ancestor-the larvae of echinoderms are in fact bilateral. This phylum includes sea stars, sand dollars, sea urchins, and sea cucumbers. Echinoderms possess many unique adaptations. For example, they locomote by means of a water vascular system, a network of canals connected to the outside by pores through the epidermis. By using specialized contractile organs, echinoderms vary the water pressure in certain portions of the water vascular system causing hundreds of tube feet that project through the body wall to extend or retract; this allows the echinoderm to move, capture food, or cling to surfaces. Many sea stars represent important predators of clams and mussels and have an unusual way of feeding on them-the sea star wraps its tube feet around the shell and pulls constantly with them until it fatigues the muscles that hold the shells together. This causes a small opening in the shell (less than 1mm) that allows the sea star to evert its stomach and insert it into the shell where it digests the mollusk's soft body parts. Sea urchins and sand dollars have no arms; all are spherical or disk shaped. The body surface consists of

epidermis covering an endoskeleton composed of calcareous ossicles, and all urchins and sand dollars are covered with movable spines. The digestive tract of most echinoderms have an oral surface or mouth which faces the ventral side of the animal (i.e., toward the seafloor or substrate) and an aboral surface with an anus that opens in the opposite direction. Echinoderms achieve circulation through hemal and perihemal coelomic systems, an array of canals and rings derived from the coelomic space. Nitrogenous waste diffuses from the coelom through the tube feet which, in some species, also serve respiratory functions. The nervous system consists of a nerve ring encircling the esophagus (in the central disc of the body) with nerve nets extending into the body. Echinoderms also have sensory receptors for light, chemical, touch, and balance. Many echinoderms can regenerate missing body parts.

Examine the pictures or samples of echinoderms.
Note the radial appearance and five-part body plan. Understand why despite this they are included in phylogenies with bilateral rather than radial animals.
Do you see any evidence of a head or anterior end and a tail or posterior end?

Examine the cross section of a sea star arm.
Identify the coelom, tube feet, radial canal (part of the water vascular system), and gonads.

Examine the slides of sea star cleavage, blastula, and gastrula.
Identify the type of cleavage. Sketch the 8-cell stage. Is this animal a protostome or deuterostome?

Identify the blastula and gastrula. Sketch the gastrula and label the blastopore and archenteron. Will the blastopore become the anus or mouth? What does the archenteron become?

Phylum Chordata
Your textbook includes two subphyla of invertebrate chordates (animals without a vertebral column) and one subphylum of vertebrate chordates within the phylum Chordata (your textbook is a lumper). In contrast, the Five Kingdoms book splits the two groups of invertebrate chordates into their own separate phyla (Phylum Urochordata and Phylum Cephalochordata) and likewise places the vertebrate chordates into a separate phylum (Phylum Craniata, animals having a brain encased in a skull or cranium). The Urochordata includes tunicates and sea squirts, and all are marine. The Cephalochordata include lancelets or Amphioxus, and all live in aquatic habitats. The vertebrates or craniata comprise the largest group of chordates with about 45,000 species. This group includes animals most familiar to humans-fish, birds, amphibians, reptiles, and mammals. All chordates, vertebrate or invertebrate, share common characteristics: a single dorsal hollow nerve cord, a notochord, gill structures and a post-anal tail.

For this part of today's lab, you will view images of cross sections of the human body. These images came from a project in which human bodies were preserved, frozen, cross sectioned in more than 150 locations along the body, and photographed. The images for this lab exercise were selected from this enormous undertaking. Note that all sections are viewed from below, looking toward the head. This corresponds to the way radiologists view sections of the body but not to the usual anatomical view of looking down toward the feet. Also, the left side of the body usually appears on the right of the photograph and vice versa.

On the outline of the human body (to be provided during lab for inclusion in your write up), identify the approximate location of the selected cross sections. Where appropriate identify the images as male or female. Use figures from the Photographic Atlas for reference.

Using these images, identify and provide evidence for the following:

Type of body symmetry
Layers of embryonic tissue
Presence of coelom
Number of Digestive tract openings
Open or closed circulatory system
Organs of respiration
Organs of excretion
Type of support system
Position and complexity of nervous system

Write-up: as the case with the plant diversity labs, your answers are for you to review as you learn and study this material. Your TA will require a check off, prior to the start of the practical exam. Hand-drawn and labeled drawings are fine. All drawings must indicate size.

PART ONE:

There are 5 organisms. You need to have at least two drawings of each, one of the whole animal, and one (or more, see below) cross section drawing of that animal, with features labeled:

1) ***Hydra***

Whole body (name of organism, phylum, scale)
Longitudinal cross section
Name of organism, phylum, scale, label the gastrodermis, epidermis, mesoglea, mouth, anus, tentacles, and gastrovascular cavity

2) ***Planaria***

Whole body (name of organism, phylum, scale)
Cross section at the level of pharynx

Name of organism, phylum, scale, label the epidermis, muscle derived from the mesoderm, the lining of the digestive tract derived from endoderm, and the pharynx.

3) ***Ascaris***

Whole body (name of organism, phylum, scale)

Cross section of male

Name of organism, phylum, scale, label cuticle, epidermis, muscle fibers, intestine, body cavity (give specific name), testis, dorsal and ventral nerve cords

Cross section of female

Name of organism, phylum, scale, label cuticle, epidermis, muscle fibers, intestine, body cavity (give specific name), ovaries, uterus, dorsal and ventral nerve cords.

4) Earthworm

Whole body (name of organism, phylum, scale)

Cross section posterior to clitellum

Name of organism, phylum, and scale. Label the intestine, two muscle layers (one inside the skin and one on the surface of the intestine), coelom, ventral nerve cord, dorsal and ventral blood vessels, epidermis, and metanephridia. Label parts derived from the endoderm, mesoderm, and ectoderm

5) Sea star

Arm (name of organism, phylum, and scale)

Cross section

Name of organism, phylum, and scale. Identify the coelom, tube feet, radial canal (part of the water vascular system), and gonads.

PART TWO:

Draw 8-cell stage and gastrula of embryo development for:

Cerebratalus

Sea star

For each drawing, provide the name of the organism, the phylum, and an indication of scale. Label each according to the type of cleavage pattern exemplified and whether it is a protostome or deuterostome.

On the gastrula drawings, label the blastopore and indicate whether it will become a mouth or anus.

PART THREE:

Indicate on the human outline where each of the cross sections are located. State what aspects of the cross section helped you to make those conclusions for each one. Indicate whether the cross section is from a male or female. Turn in the labeled human outline page with your write up.

PART FOUR:

Brief answers to the following questions (asked already in the manual):

a) Compare and contrast the manner in which sponges and anemones acquire oxygen and rid themselves of carbon dioxide and the way they acquire food and rid themselves of food wastes.

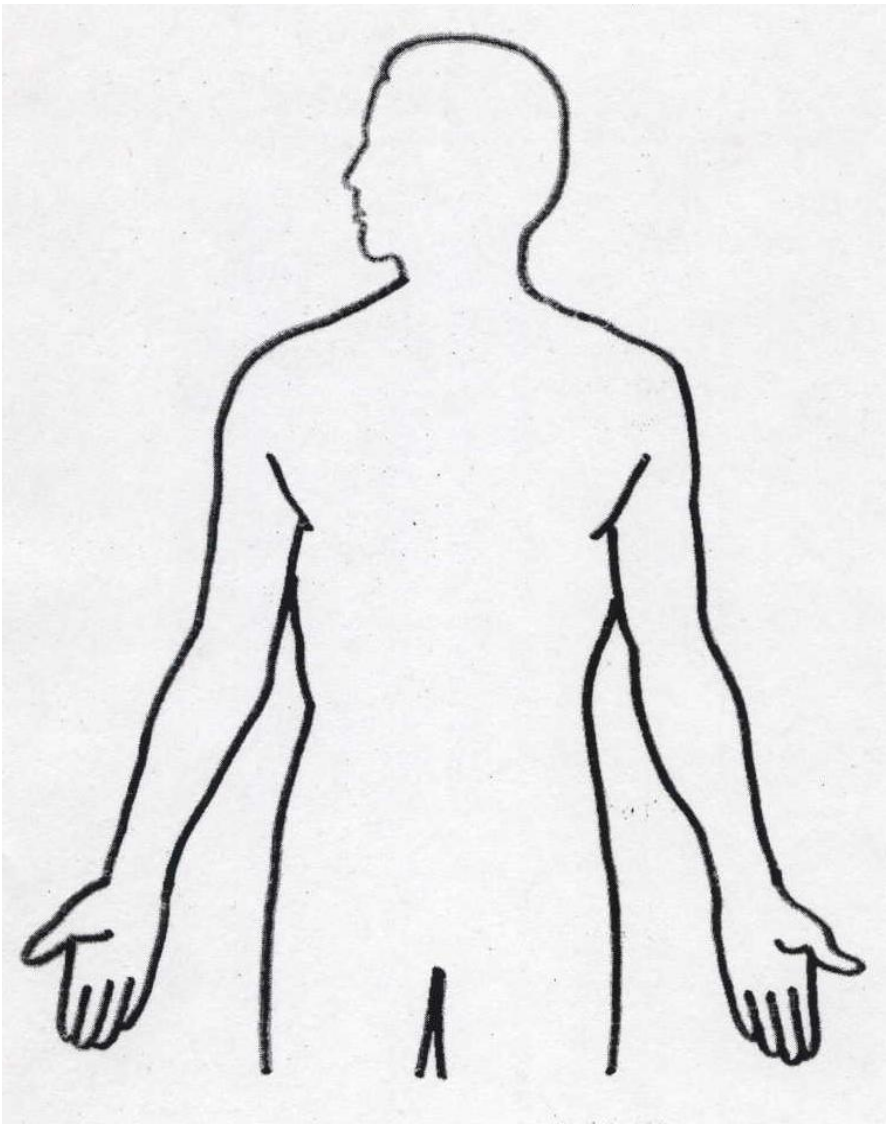
b) Name two organisms that you saw in lab that have one opening into the digestive tract and two organisms with a separate mouth and anus. What is the disadvantage of a single opening over a complete digestive tract?

c) Jellies have sensory organs encircling the bell of the medusa. Why would it be advantageous for jellies to have this?

d) Compare tapeworm and planarian anatomy in relation to their lifestyles (especially consider the proglottids, anterior structures, and lack of a digestive tract in tapeworms).

e) Platyhelminthes derive their name from their flat body shape. How does the flat body shape help with gas exchange and circulation in an animal without organs specialized for such functions?

f) Compare and contrast how earthworms and humans accomplish gas exchange.



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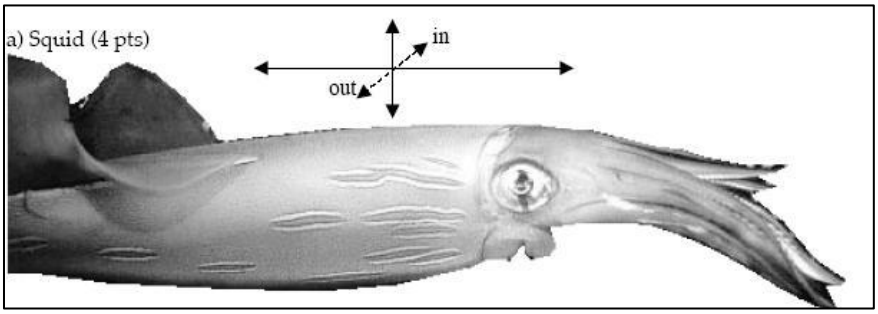
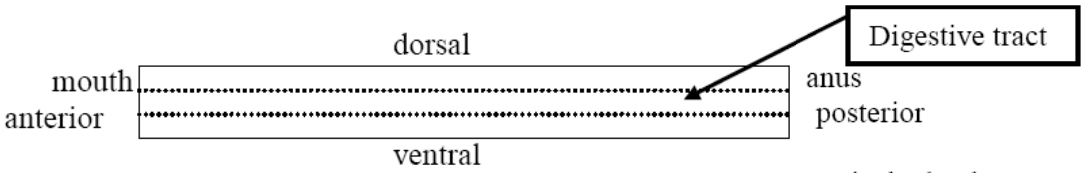
Name_____ Pre-Lab: Animal Diversity II

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.



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, dorsal, ventral, mouth, anus, and digestive tract) it into an animal with a body plan like the squid?

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Animal Diversity : Dissection of the Trout
(*Salvelinus fontinalis*)

Objectives

- Examine the internal and external anatomy of trout.
- Compare and contrast the trout and the squid

Introduction

Phylogeny is the evolutionary history of organisms: their lines of descent, the branchings of these lines, and thus the relationships between organisms. Much of our understanding of animal phylogeny has come from comparative studies of the anatomy and embryology of present-day animals. Our concepts concerning their ancestral history and relationships have been extended, refined, and sometimes changed as a result of physiological, cellular or molecular studies.

Just as our understanding of animal phylogeny benefits from a study of anatomy, our understanding of anatomy is enhanced by an understanding of evolutionary principles. The form and function of all features of an organism are determined by: 1) the selection imposed by the organism's environment, and 2) the genetic/morphological/physiological constraints imposed by the general architecture that the organism's lineage has developed over the course of its evolutionary history. Regardless of their particular phylogenetic group, all living animals have the same basic requirements and must perform the same basic functions.

Animals may meet these problems in different ways because of differences in size, structure and environment. Within a single class, for example Mammalia, one may find animals as different in appearance as a mouse and a whale, although internally much of their machinery will be similar. You will also see examples of "convergence", where animals from different phylogenetic backgrounds and different basic architecture appear similar in many ways. As you work through the two lab periods devoted to phylogeny, keep examining animals with a view to both their phylogenetic history and the selection pressures exerted by their environments and try to build up a fuller picture of why animals are what they are today. Reading appropriate sections of your textbook will help guide the way.

We will have available in lab for dissection two different animals, the brook trout (*Salvelinus fontinalis*) and the squid (*Loligo pealii*), representing two major phyla: Chordata (Craniata in Five Kingdoms) and Mollusca, respectively.

GENERAL HINTS AND INSTRUCTIONS FOR DISSECTIONS

Preparation: Wash your animals in cold running water to remove slime and/or reduce fumes from the preservatives. Spray preserved specimens (squid) with humectant periodically, and rinse whenever fumes become annoying. KEEP ANIMALS MOIST WITH WATER DURING DISSECTIONS — dried out organs and tissues are impossible to dissect and maneuver. You should wear gloves to protect your hands.

Tools:

• **Scalpel:** This is the first tool that most people grab. It is the most dangerous one -both to the user and to the animal. The danger is, if you have a sharp scalpel, you can easily cut through

important structures before you realize what you've done. Thus, you should only use it when the scissors don't work. You should also be sure the blade is sharp; change it frequently.

• **Scissors:** These are the best tools for cutting through skin, etc. You can feel the different tissues better and are less likely to cut something important than you are with the scalpel. Be sure these are sharp; trade in dull ones immediately.

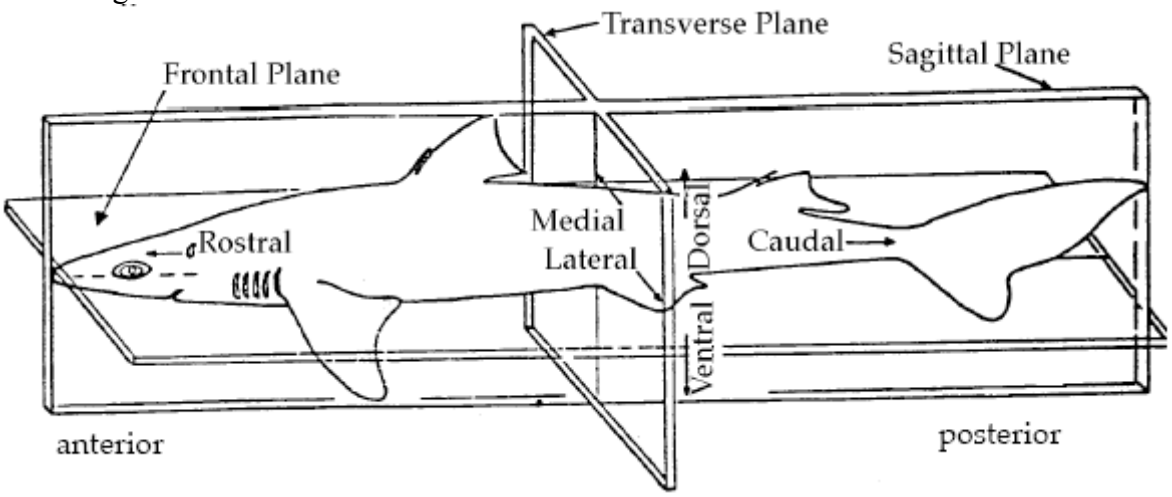
• **Pick:** Your 'best friend' once inside the animal. This can easily be used to pull apart and cut the connective tissue that holds organs to each other, but it is unlikely to break anything important unless you push really hard. You should use this most of the time.

All dissection instructions must be left in lab. **The lab must be left really clean**—rinse your pans, pins, and instruments, dry them carefully, and return them to designated places; clean up benches and sinks.

ANATOMICAL GLOSSARY

• **Anterior** or **rostral**: towards the head end.
• **Posterior** or **caudal**: towards the tail end.
"Your nose is anterior of your belly button. Your chin is posterior of your nose."
• **Dorsal**: toward or near the back.
• **Ventral**: toward or near the belly.
• **Median**: in or near the plane in the middle of the body.
"Your belly button is ventral of your intestines."
• **Proximal**: near the base or site of attachment.
• **Distal**: near the tip.
"Your fingernails are on the distal ends of your fingers."
• **Sections** through the body are called:
Sagittal: dividing the animal into left and right sides
Frontal: dividing the animal into dorsal and ventral parts.
Transverse: dividing the animal into anterior and posterior parts.

See this figure:



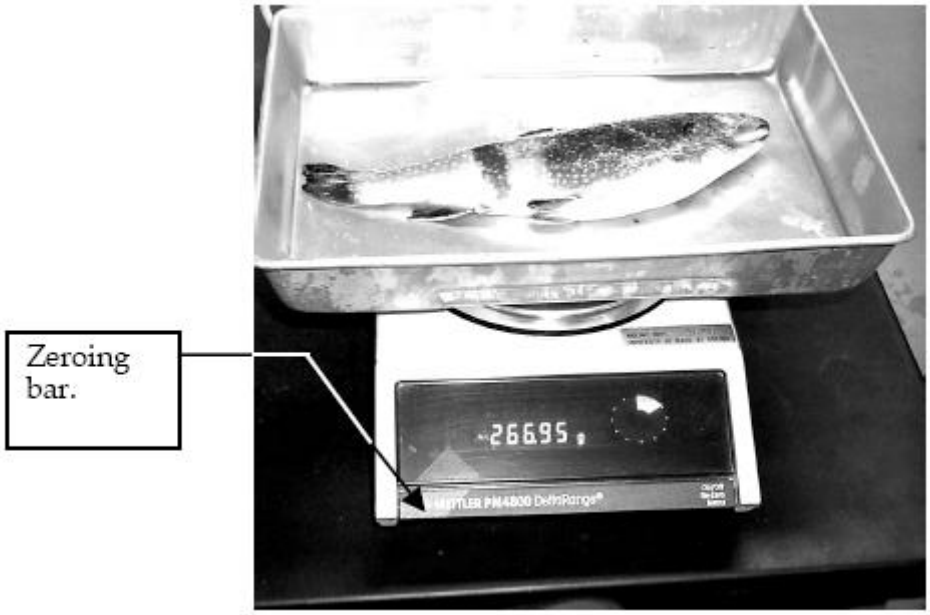
Part I Dissection of the brook trout (*Salvelinus fontinalis*)
This dissection should take you one lab period.

Note: Since later steps may destroy some structures, you should take notes and draw sketches as you go.

1) Put on gloves so that your hands won't smell of fish.

2) Obtain a fresh brook trout from your TA. These were raised at a fish hatchery in western Massachusetts and shipped fresh to UMass. Rinse it gently in cold water to remove any slime.

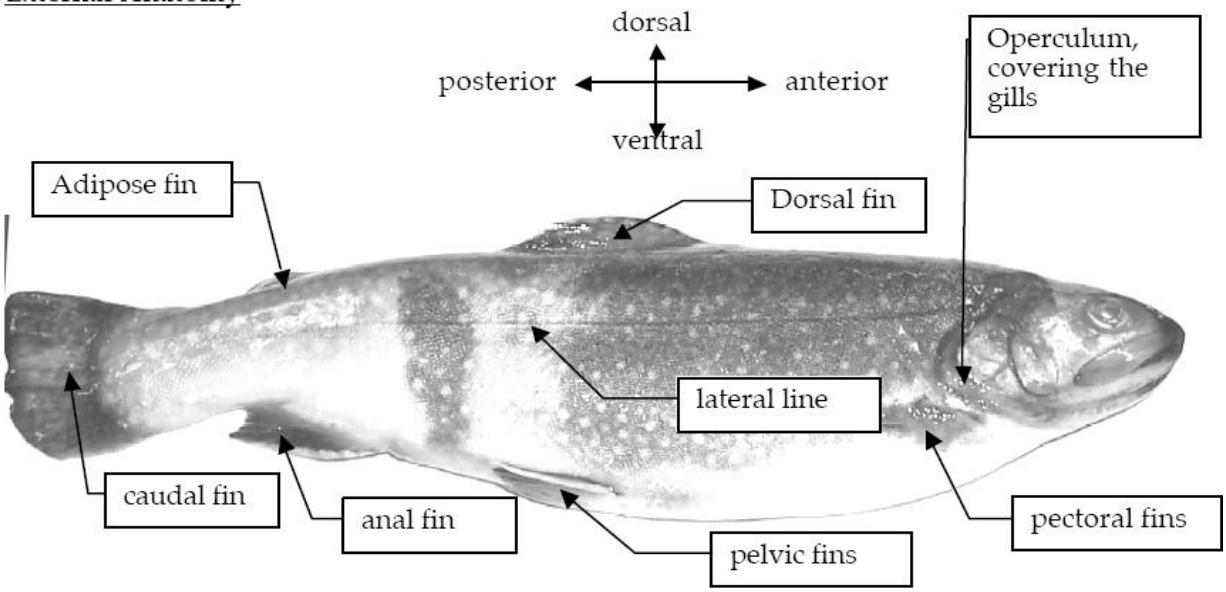
3) Bring your fish to the scale. Make sure there is a paper plate on the scale to keep the scale from getting wet. Zero the scale before putting the fish in the tray by pushing down on the big bar at the front of the scale. This is shown below:



4) After zeroing the scale without your fish on it. Put the fish on the tray and record the weight.

Weight of fish in grams _____

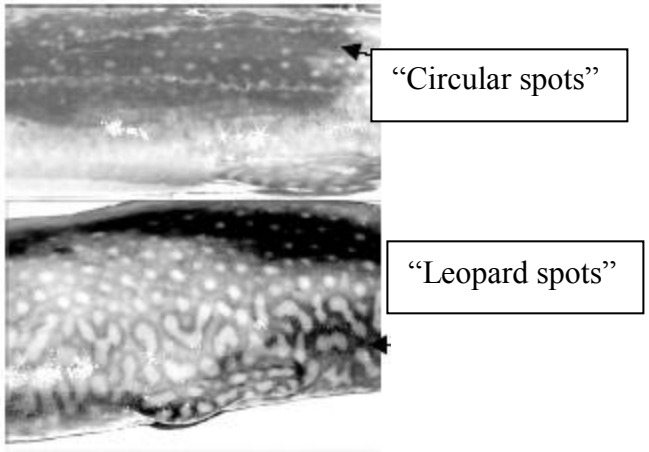
External Anatomy



a) the lateral line is a sensory organ that the fish uses to sense vibration as well as to feel objects and other fish along its sides. In some fish, this plays a crucial role in schooling.
b) the pectoral and pelvic fins are homologous to human arms and legs, respectively and are used in turning and stopping the fish.
c) the dorsal and anal fins are used to keep the fish from rolling.
d) the caudal fin provides most of the forward movement of the fish and controls direction.

Count the number of rays (bone-like spikes) in the dorsal fin. _____

5) Note the pattern of spots on the fish, especially on the dorsal side. Briefly describe them, using the photo below as a guide.



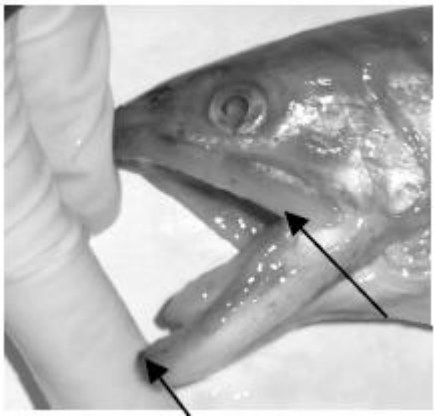
Spot pattern _____

6) Measure the length and girth of your fish as shown below. Use a string to measure the fish and then measure the string and record the value in your notebook.

– length (from tip of 'nose' to tip of tail along lateral line) _____cm

– circumference (all the way around fish at anterior end of dorsal fin) _____cm

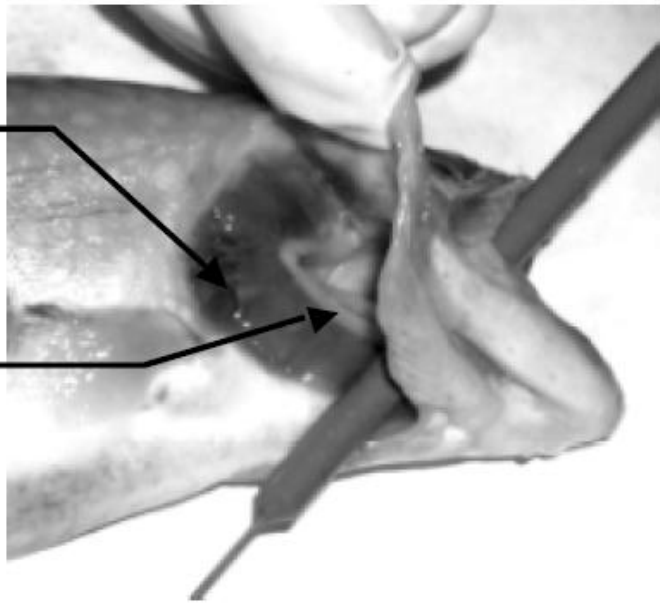
7) Measure the length of the lower (ventral) jaw as shown below. Be sure to open the jaw as wide as you can (be careful of the teeth).



Jaw length _____cm

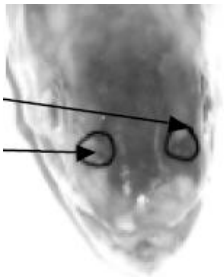
<u>Respiratory System</u>:

1. Lift open the operculum and look in at the gills and gill rakers as shown below.



2) Stick a blunt probe through the mouth as shown on the previous page to determine the flow of water over the gills and rakers. Observe the live fish in the tanks in the lab to see how the mouth and operculum function in respiration. What are the steps in a fish's "breath"?

3) The nares are circled in the picture below. They are used in smell sensation in the fish and bear a superficial resemblance to nostrils on a human. In a human, the nostrils are also used in breathing. As best you can, carefully dissect the area around the nares and determine if these can function in respiration in the fish.

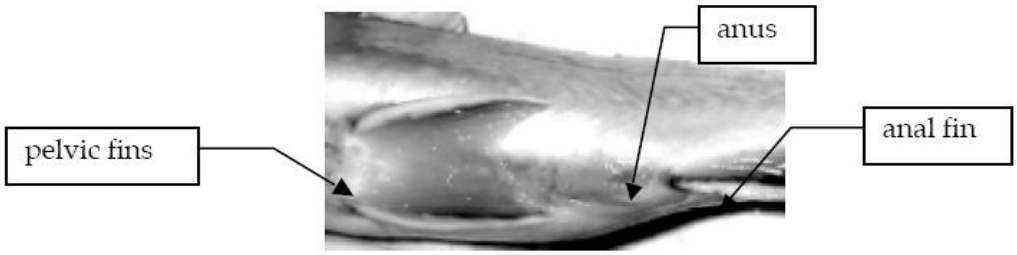


**Stop and draw the respiratory system; consult the requirements for the lab notes for details.

Internal anatomy

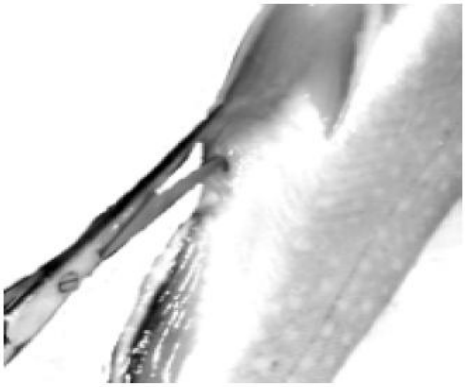
4) Open the body cavity of the fish. You must do this very delicately or you will make it impossible to study the internal organs. This is the most critical part of the dissection. Hold the fish with its ventral side up and locate the anus - it is the opening just anterior of the anal fin.

This is shown below:



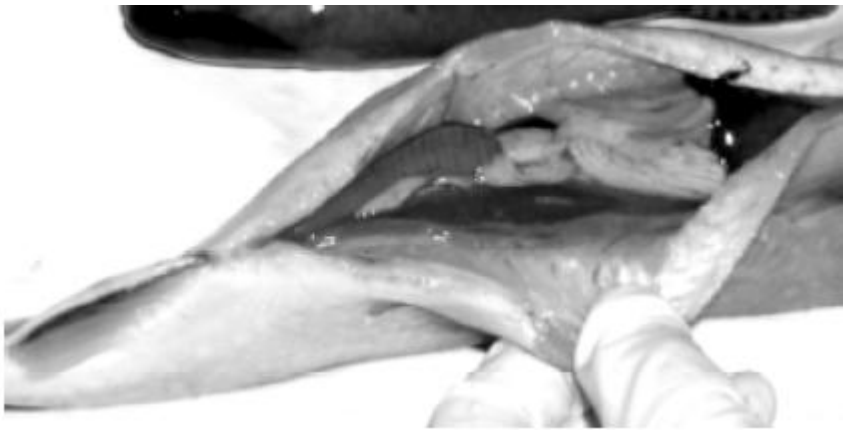
Insert the tip of a pair of scissors into the anus and cut towards the head; DO NOT USE A SCALPEL HERE! Be sure to cut as shallow a cut as you can so as not to disturb the internal organs. Cut as far anteriorly as you can - at least as far as operculum. You may have to go back over your cut to be sure it is deep enough. When it is deep enough, you should be able to open the sides of the body cavity to expose the internal organs.

Starting to cut:

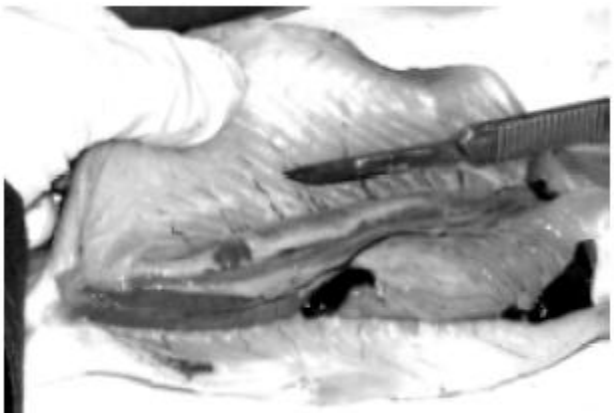


Cutting:

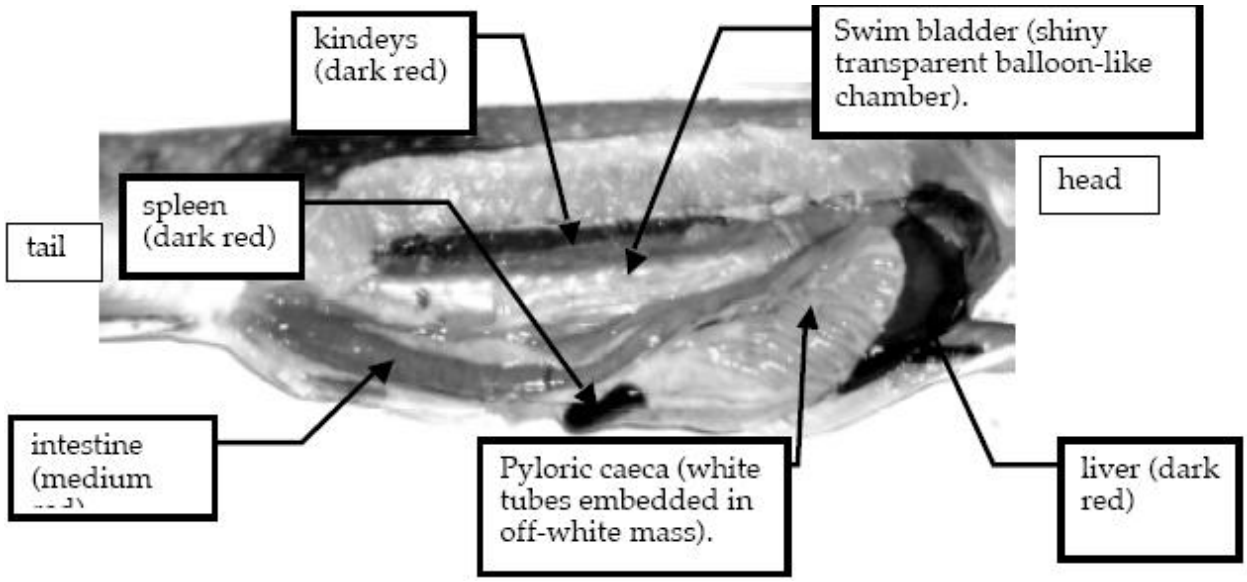
When you open it up, it should look like this:



5) On one side, carefully cut dorsally from the anterior and posterior ends of your first cut all the way to the lateral line. Lift open the 'filet' you have cut, carefully scraping any internal organs free with a scalpel.



Cut across the dorsal side of this 'filet' and remove it, completely exposing the body cavity. It should look like this:



6) The kidneys lie along the dorsal end of the body cavity as shown in the previous diagram.

7) If the body cavity contains eggs, then you have a female; if not you have a male. Eggs are spherical and vary in size and color - the smallest are 0.5 mm and white, the mature large eggs are 3-5 mm and clear with a white spot. Record the sex of your fish.

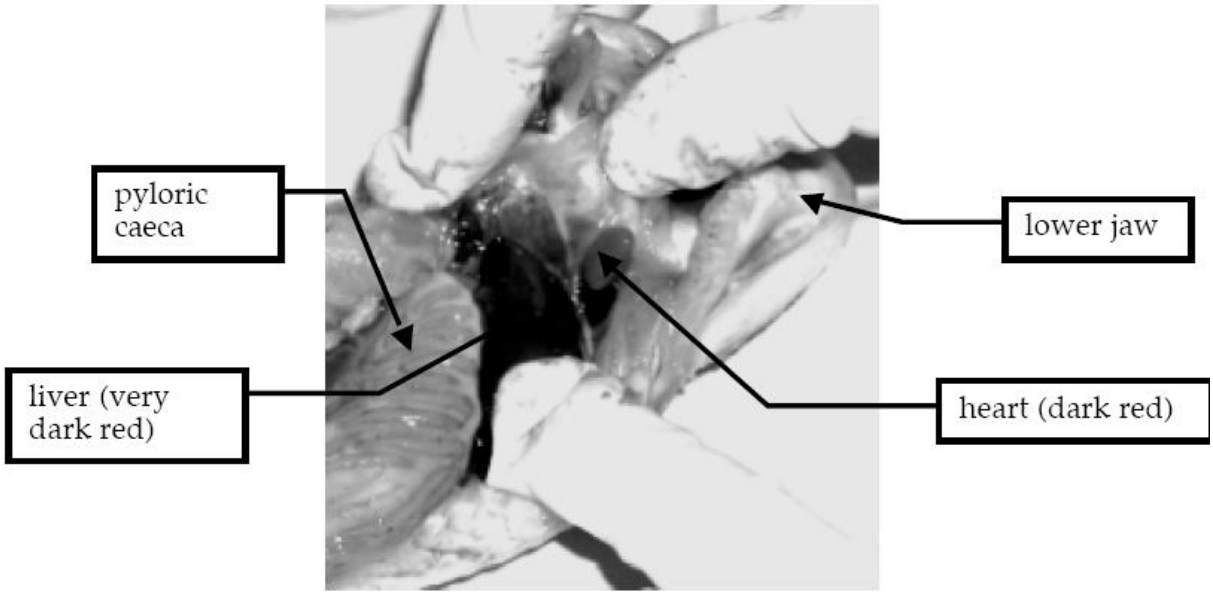
8) In some fish, the swim bladder is filled with air that the fish swallows and forces from the intestine to the swim bladder (this is a physostomous swim bladder). In other fish, the gas in the swim bladder is produced from the blood by a specific organ and the swim bladder is not connected to the intestine (this is a physocleistous swim bladder). By careful observation, try to determine whether the trout has a physostomous or physocleistous swim bladder.

9) Carefully remove the gills from the side of the fish that is facing up. Take a small fiber from one of the gills and look at it under low power in the compound microscope. Sketch the pattern of blood vessels in one of these gill fibers. Estimate the diameter of the smallest vessel you can see.

Approximate diameter of smallest vessel _____ μm

<u>Circulatory System</u>

10) Locate the heart - it is the dark red object about the size of the fingernail on your smallest finger and is located right in the 'throat' of the fish (anterior to the liver and right between the gills); it looks like a heart-shaped kidney bean.



11) Carefully remove the heart and weigh it. Heart weight _____g

** Stop and draw the circulatory system; consult the lab notes section for more details.

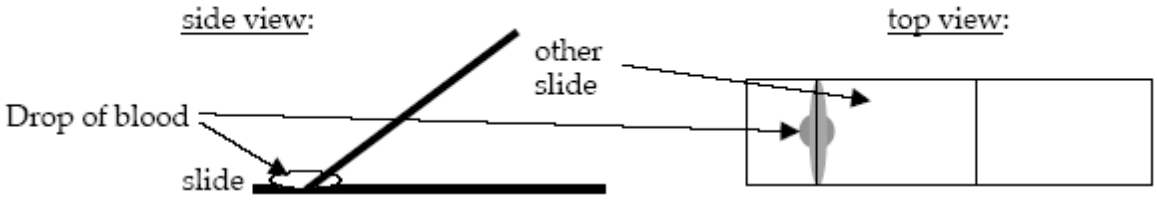
12) Make a smear of the blood cells. This is a tricky procedure; it may take more than one try.

a) Clean off 2 microscope slides with ethanol and dry them. Squeeze a drop of blood from the heart onto a slide, near one end of the slide.

side view:

b) Take another slide and touch it to the drop until the drop spreads out along the edge of the slide:

the slide:



c) Quickly drag the second slide across the first to smear the blood out to a thin layer.

d) Let the smear dry completely.

You will now stain the smear with Wright-Giemsa Stain to make the white blood cells more visible.

You must wear gloves when staining.

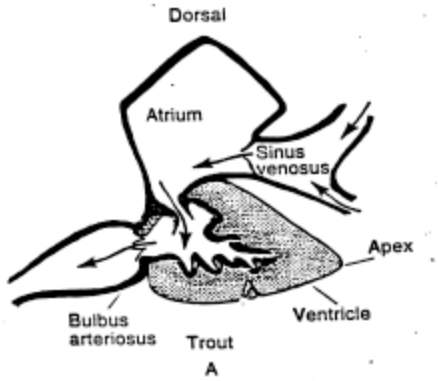
e) Dip blood smear in Fixative Solution 5 times, one second each time. Drain excess fixative with a paper towel.
f) Dip into Solution I, 5 times, one second each time. Drain excess solution with a paper towel.
g) Dip into Solution II, 5 times, one second each time. Drain excess solution with a paper towel.
h) Rinse slide in beaker of water.
i) Allow slide to air dry.
j) Examine under the compound microscope.

Look for red blood cells under the microscope. They will be visible as very faintly red dimpled discs (like a cookie with a dent in the center) at high power. Note that, unlike human red blood cells, these have nuclei. Estimate their diameter.

minimum red blood cell diameter_____ μm

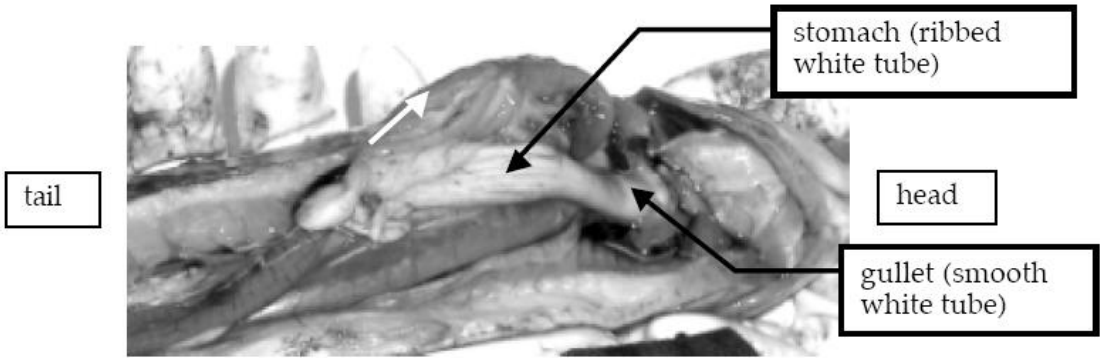
Look for the white blood cells under the microscope. They will look like figure 1.31 in the Lab Atlas. Draw one of the white blood cells you see and use figure 1.31 to identify it as best you can.

13) Shown below is a diagram of a fish heart. Carefully cut the heart open across its length; which part of the diagram below does the muscular part you found correspond to?



Digestive system

14) With a blunt probe, find the gullet (the tube that connects the pharynx to the stomach; the 'throat' in a human) and trace the path of food into the stomach. You can identify the stomach because it has a ribbed texture visible from the outside (the ribs run the long way along the length of the stomach).



15) Carefully trace the digestive tract. Be sure to note the following:

e) the positions along the tract of the gullet, intestine, mouth, pyloric caeca, anus, and stomach.

f) are the pyloric caeca all one long tube, a set of parallel tubes, or a set of 'dead end' branches of the digestive tract?

g) the internal textures of the various parts of the digestive tract

16) Carefully remove the digestive tract from the fish.

** Stop and draw the digestive system; consult the lab notes section for details.

Tissue Structures

In general, it will be easier to observe internal structures in *thin* sections. With a fresh razor blade, try to cut the thinnest pieces you can so that you will be able to observe them clearly.

17) In all fish, digestive enzymes are secreted into the pyloric caeca. In some fish, nutrients are absorbed from the pyloric caeca into the blood. Any organ that is involved in absorbing nutrients into the blood will be highly vascularized (have lots of blood vessels in it). Look at a sample of the pyloric caeca and the surrounding tissue under the microscope. Do you see many blood vessels (they would be red like those you saw in the gills)? Based on this, are the pyloric caeca in the trout likely to be involved in nutrient absorption? Observe the pattern of the blood vessels if you find any. How does this pattern compare to the pattern you found in the gills?

18) Similarly, take a sample of the intestine. Is it likely to be involved in nutrient absorption? Sketch the pattern of the blood vessels if you find any. How does this pattern compare to the pattern you found in the gills?

19) Pool the data you have taken with your TA. Your TA will help you with this and will check off that you have done this.

For the lab practical, identify the following parts: Lateral line, dorsal fin, pectoral fin, anal fin, caudal fin, adipose fin, pelvic fin, gills, gill arches, gill rakers, operculum, nares, mouth, anus, kidneys, swim bladder, intestine, liver, pyloric caeca, heart, spleen, stomach and gullet.

Be able to draw the respiratory system, the circulatory system and the digestive system of the trout including the flow of food, blood or oxygen. Also be able to answer all questions in the lab manual.

Lab Notes Assignments: Have you TA check off your drawings for the exterior, the respiratory, circulatory and the digestive systems for the trout. Take these home as part of your study guide for the lab practical.

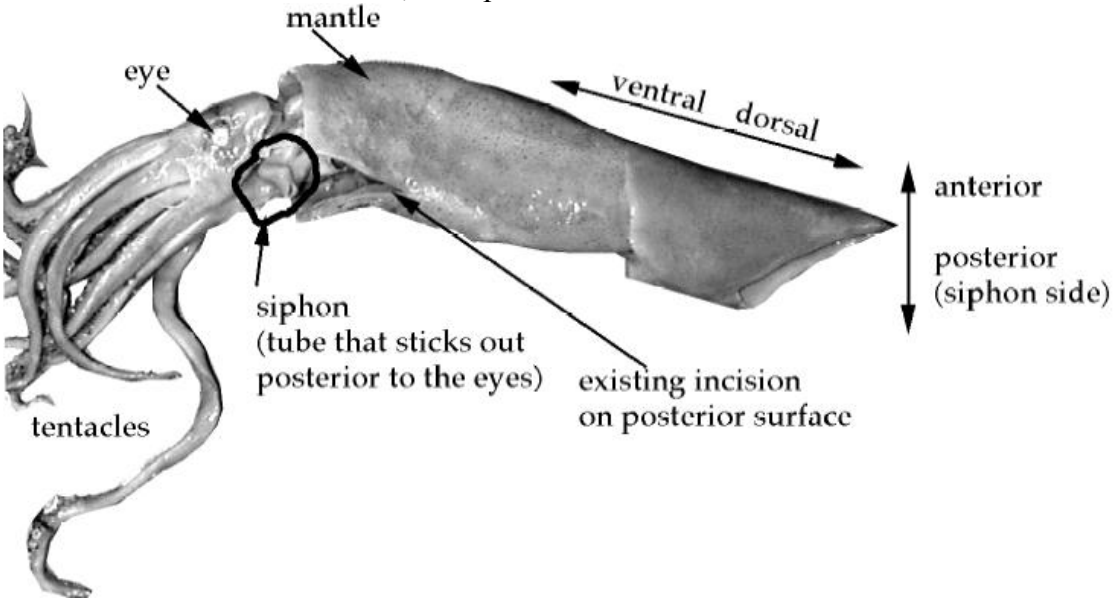
Clean up

Place your fish and any fish parts into the bag labeled "fish". These will be recycled. Other trash goes into the barrel. Wash all of your dissection tools and tray out well with soap and water and leave to dry on the counter.

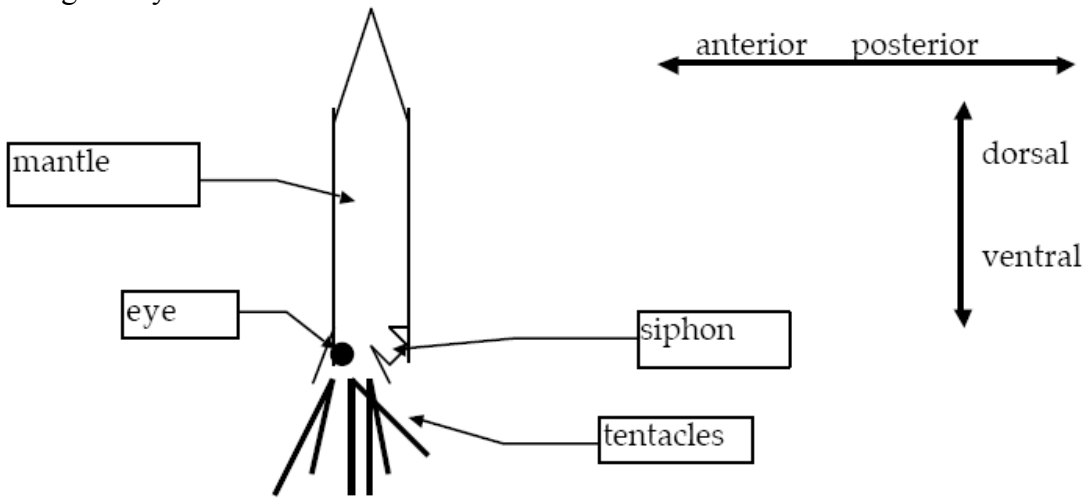
Animal Diversity : Dissection of the squid (*Loligo pealii*)

1) Obtain a preserved squid from your TA.

2) Find the siphon. It is a fleshy tube at the base of the head. The side with the siphon is the posterior side. The tentacles are the ventral side; the tip of the tail is the dorsal side.

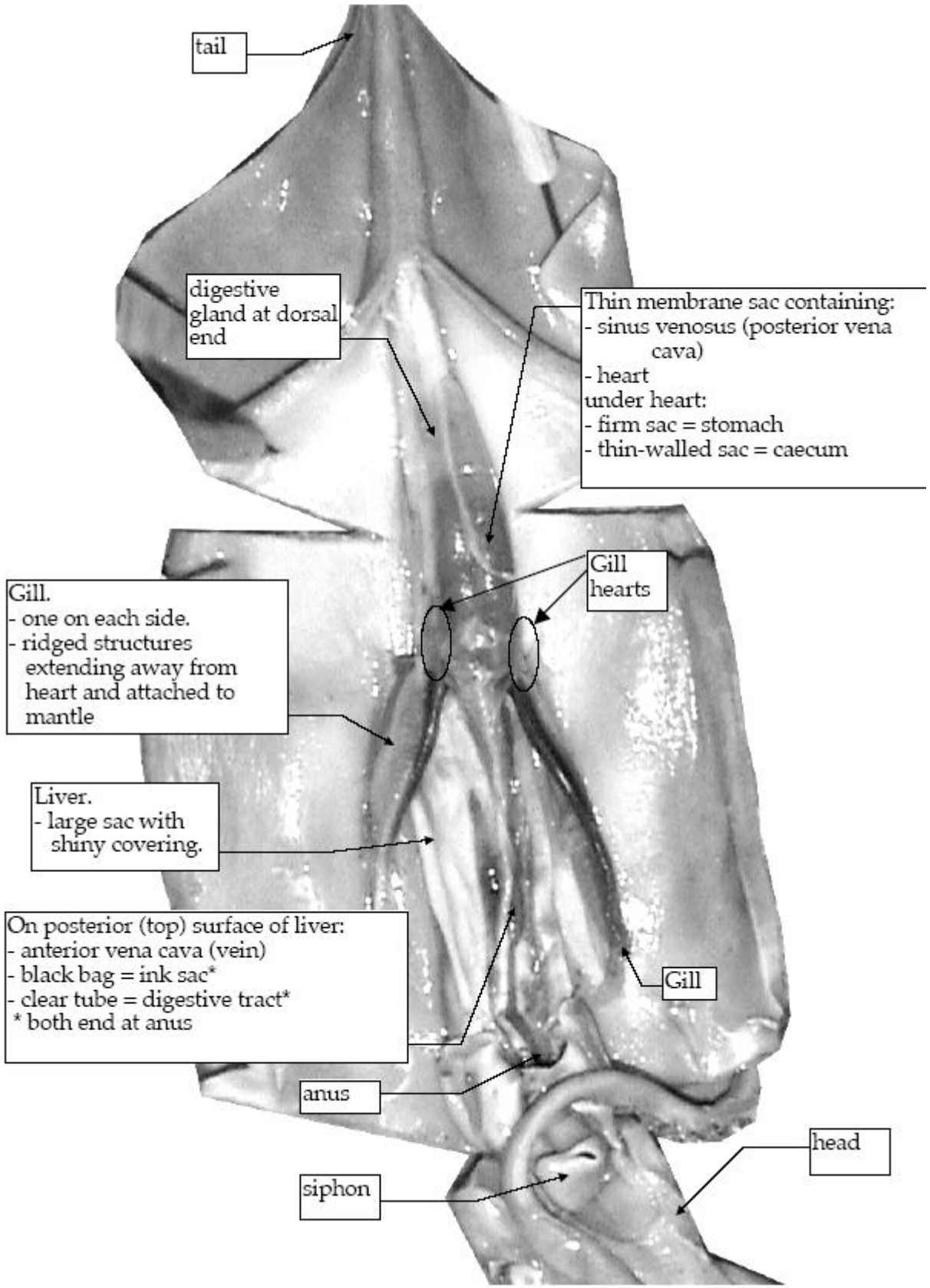


Thus, the squid would look like this if it were in the same orientation as the shark shown under the "anatomical glossary":



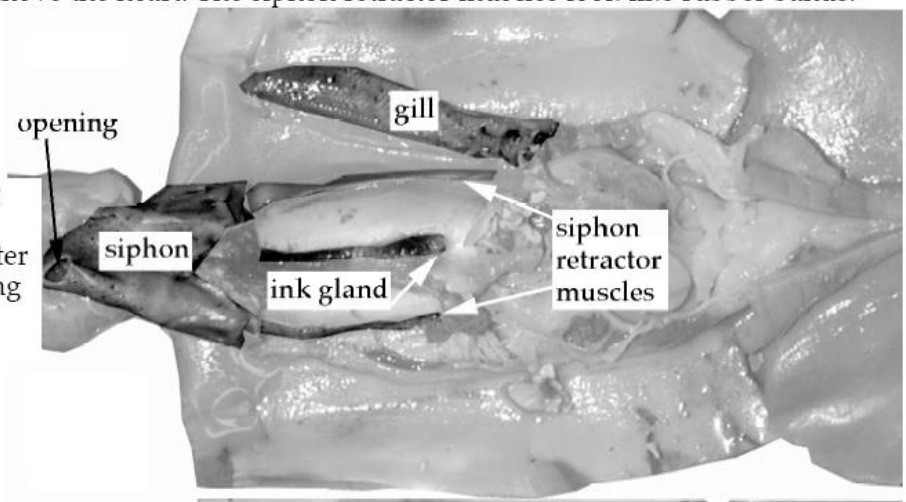
3) Lay the squid on its anterior side - siphon up.

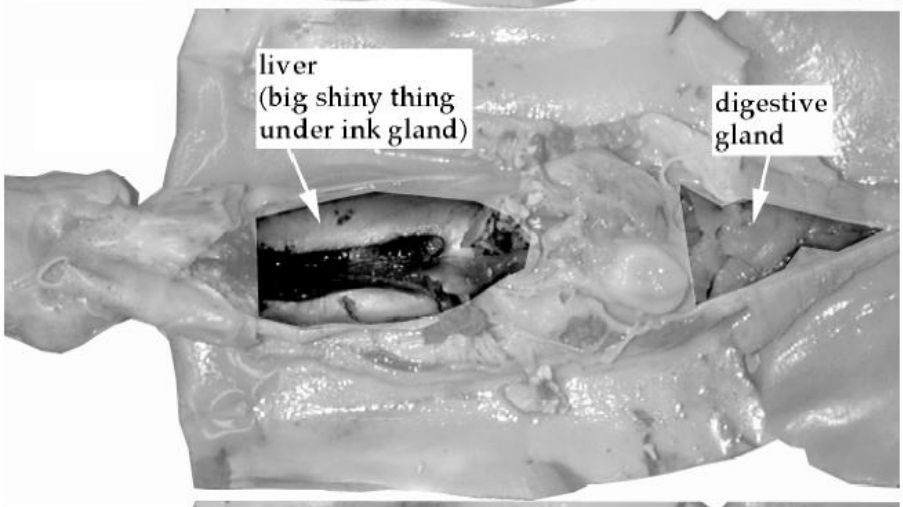
4) You will see a started incision in the mantle, continue this to the dorsal end and peel the sides of the mantle apart. You will see something like the following page.


tail
digestive gland at dorsal end
Thin membrane sac containing:
- sinus venosus (posterior vena cava)
- heart
under heart:
- firm sac = stomach
- thin-walled sac = caecum
Gill hearts
Gill.
- one on each side.
- ridged structures extending away from heart and attached to mantle
Liver.
- large sac with shiny covering.
On posterior (top) surface of liver:
- anterior vena cava (vein)
- black bag = ink sac*
- clear tube = digestive tract*
* both end at anus
Gill
anus
head
siphon

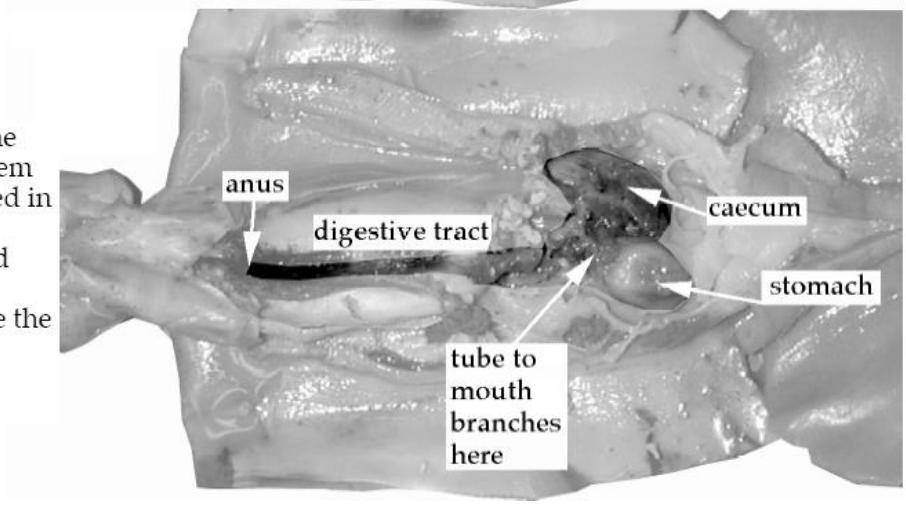
5) Trace the red and blue blood vessels as best you can.
6) Using a pick, gently pick open the sac that contains the heart, etc. The stomach and caecum are very fragile. Remove the heart. The siphon retractor muscles look like rubber bands.

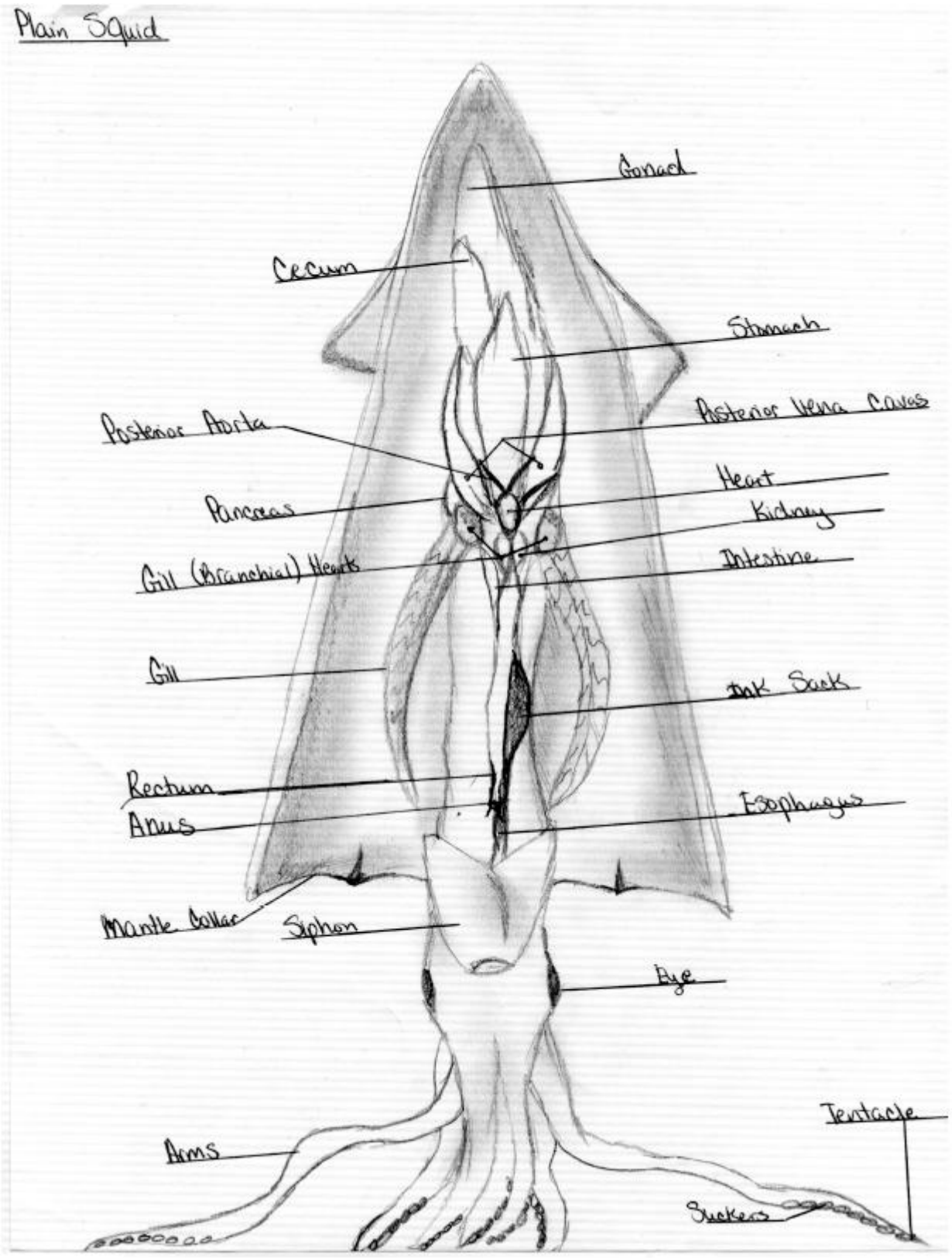
Look carefully at the siphon. The squid squirts water out of the opening in it.



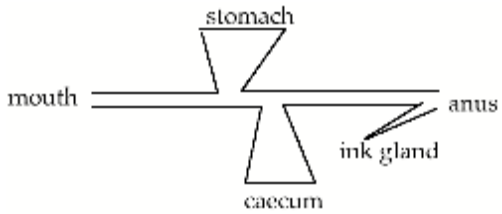


Try to trace the digestive system as it is arranged in the squid.
Slit it open and examine the textures inside the stomach and caecum.




Plain Squid
Gonad
Cecum
Stomach
Posterior Aorta
Posterior Vena Caves
Heart
Pancreas
Kidney
Gill (Branchial) Hearts
Intestine
Gill
Ink Sack
Rectum
Anus
Esophagus
Mantle Collar
Siphon
Eye
Tentacle
Arms
Suckers

If the digestive system were stretched out straight, it would look like this:

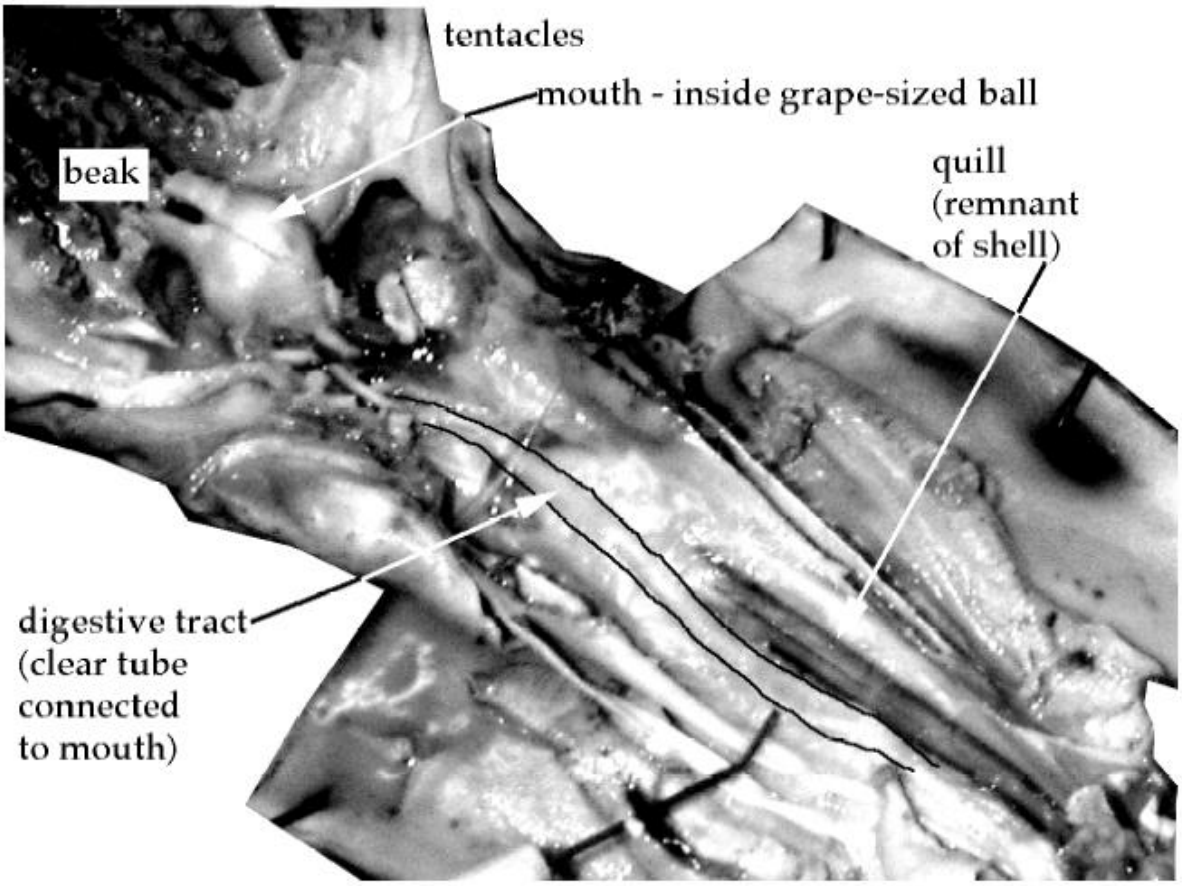


caecum = a blind pouch in the digestive system where food is digested and/or absorbed

ink gland = squids squirt black ink when threatened. It blinds the attacker and inhibits its sense of smell.

7) Carefully remove the liver. Lift it starting from the dorsal (tail) end. Using a pick, detach the connective tissue and the digestive tract (clear tube) as you lift out the liver.

8) Cut through the middle of the head from the posterior side. Do this slowly and spread the sides apart to reveal the mouth and beak. Trace the digestive tract. Cut open the mouth to examine the beak and 'teeth' (radula).



9) Cut open the eyes and look at the lens (it will be firm & clear - you can look through it) and retina (black light-sensitive membrane at the back of the eye).

10) Try to look at the gills of the fresh squid under a microscope. How do they differ from the fish gills?
11) If you have a fresh squid, try to make a blood smear from the heart or sinus venosus. What kinds of blood cells do you see?

12) Clean up. You are done with the dissection.

Clean up
Place your squid and any squid parts into the bag labeled "squid". These will be disposed of separately. Other trash goes into the barrel. Wash all of your dissection tools and tray out well with soap and water and leave to dry on the counter.

For the lab practical, be able to identify: parts of the eyes, tentacles, mouth, beak, siphon, mantle, heart, stomach, caecum, gills, liver, ink sac, anus, anterior vena cava, posterior vena cava.

Be able to draw the respiratory system, the circulatory system and the digestive system of the squid including the direction of the flow (of food, blood or oxygen, etc). Also be able to answer all questions in the lab manual.

Lab Notes Assignment: Have you TA check off your drawings for the exterior, the respiratory, circulatory and the digestive systems for the squid. Take these home as part of your study guide for the lab practical.

Name: _____ Pre-Lab: Animal Behavioral Diversity and the Scientific Method

1) (3 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) (7 pts) Suppose that you are trying to determine if a coin you have is biased towards flipping more or less heads than the expected 50%. Thus,

H_0 = the coin is 'fair', that is #Heads = #Tails

H_A = the coin is biased, that is #Heads ≠ #Tails

Take a coin and flip it 16 times and record the number of heads and tails you flip. Using these data and the graph on BehDiv-10, determine the probability that your coin is biased. Show your work.

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 which 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.

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 **ultimate** cause, 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.

- **Antagonistic behavior**. 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.
- **Reproductive behavior.** 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?

<u>The Scientific Method</u>

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 which is male and one of which 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 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 <u>all</u> 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 <u>proven</u> 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 <u>before</u> 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).

<u>Experimental Organisms</u>

In today's lab, you will work with two different animal species from two different phyla: red worms (*Eisenia foetida*), and Betta or fighting fish (*Betta splenda*). You will investigate taxis in red worms, and, as befitting its name, agonistic behavior in fighting fish. For the Betta fish, 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 the Betta fish. After you complete your Betta fish 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 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 five 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 their intensity).

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.**

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.**

Red worms

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.

Hypothesis testing with red worms

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:

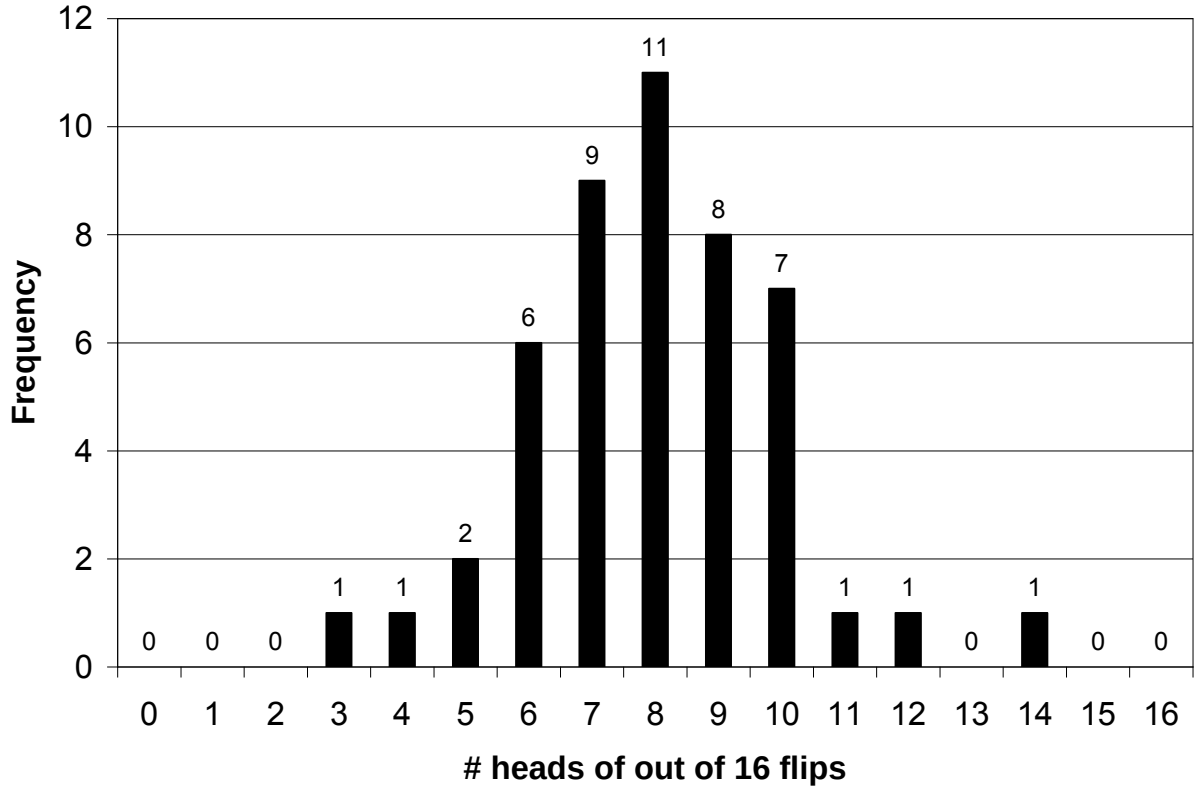
Subject #	Worm on warm side after 3 min	Worm on cool side after 3 min
1	X	
2		X
3		X
4		X
5		X
6	X	
7		X
8		X
9		X
10		X
11		X
12		X
13	X	
14	X	
15		X
16		X
Total	**4**	**12**

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 <u>not</u> 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(≥11 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 the two extremes or tails of the distribution. In other words, what is the probability <u>due to chance alone</u> that 12 of 16 worms would move either to the cooler side or warmer side? Based on the above distribution,

P(≤4, ≥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; photographs are not acceptable. 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

(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.

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 16 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 course website 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 20 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)

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

Number of phyla	**Points**
1 – 16	1.25 points per phylum

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 – your course instructor
the WWW (I have put relevant links on the course website 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.

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.
The class will pick 10 different organisms from 10 different phyla and:
-construct a table of their properties, as described later
-make a phylogenetic tree of all (as best you can) the organisms. This tree will be based on the kingdom, phylum, etc. for each organism.

<u>The 10 Organisms you have chosen to explore in detail</u>: Write their names and a brief description below:

1)

2)

3)

4)

5)

6)

7)

8)

9)

10)

A) Describing the Organisms

As a wrap-up of the course material (especially the Themes, Plants, and Animals material) and a review for the final exam, the class will discuss the answers to the following questions. These are based on the Themes, Plants, and Animals sections of the course. Write the numbers of the organisms from the previous page in the blanks as appropriate.

1) Life cycles

a) Which of the *samples* you have contains:

i) Sporophyte

ii) Gametophyte

iii) Gamete

iv) Adult

v) Spore

vi) Seed

vii) Diploid cells

viii) Haploid cells

b) Which of the *organisms* in your collection includes the following anywhere in its *life cycle*?

i) Sporophyte

ii) Gametophyte

iii) Gamete

iv) Adult

v) Spore

vi) Seed

vii) Diploid cells

viii) Haploid cells

2) Nutrition

Complete the following table as best you can:

Organism	Carbon Source	Nitrogen Source	Energy Source
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

3) Miscellaneous details

a) Which of the organisms have a flow-through digestive system?
b) Which of the organisms have a nervous system?
c) Which of the organisms have an excretory system?

4) Size and Scale

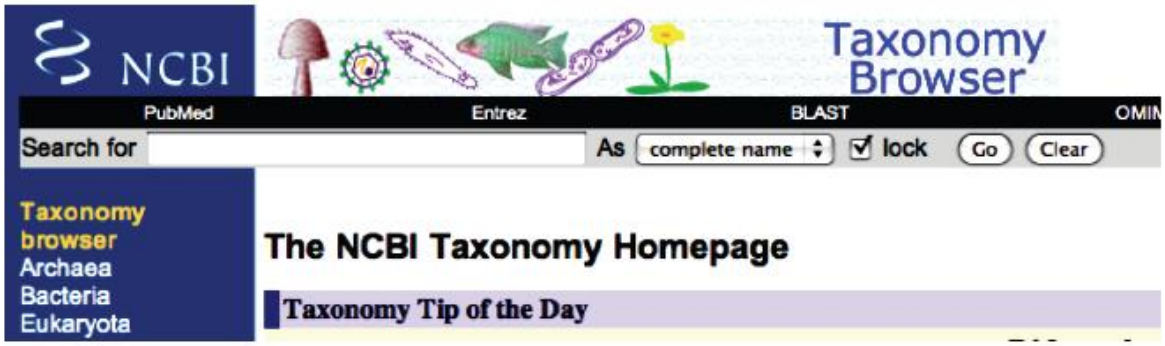
Which of the organisms have specializations required for life at larger than microscopic size? List the organisms and one different specialization for each.

B) Making the phylogenetic tree:

1) For each of the organisms in your class's collection that you can, you need to find its abbreviated taxonomic classification. This should include the levels of classification listed in *Campbell*:

kingdom
phylum
class
order
family
genus
species

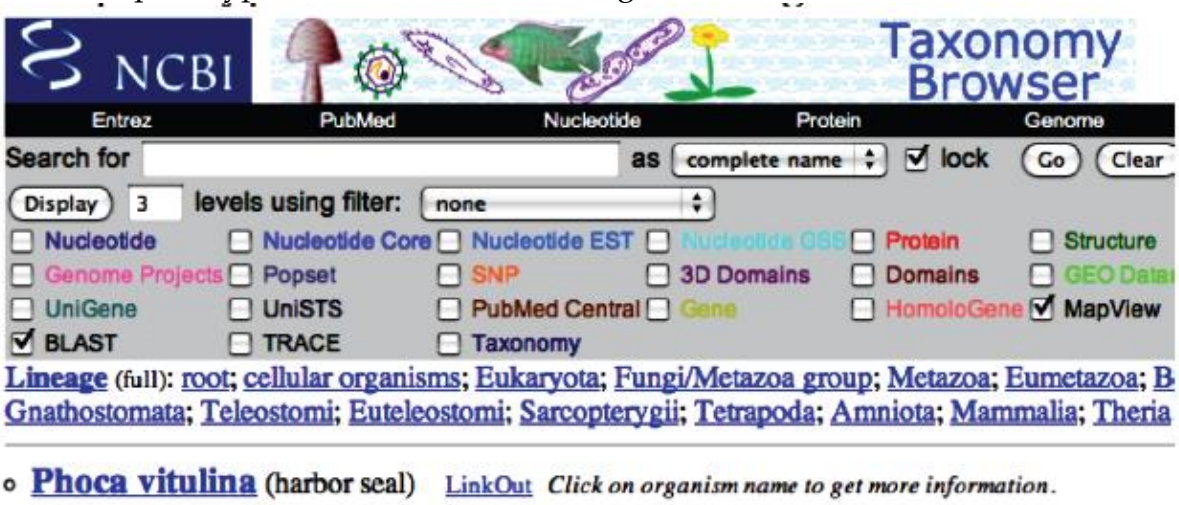
Since taxonomists disagree on some classification, it is important to use one single source for this information. We will use the Taxonoomy Browser at the National Center for Biotechnology Information. Their website is:
http://www.ncbi.nlm.nih.gov/Taxonomy/
When you go to that site, you will see:



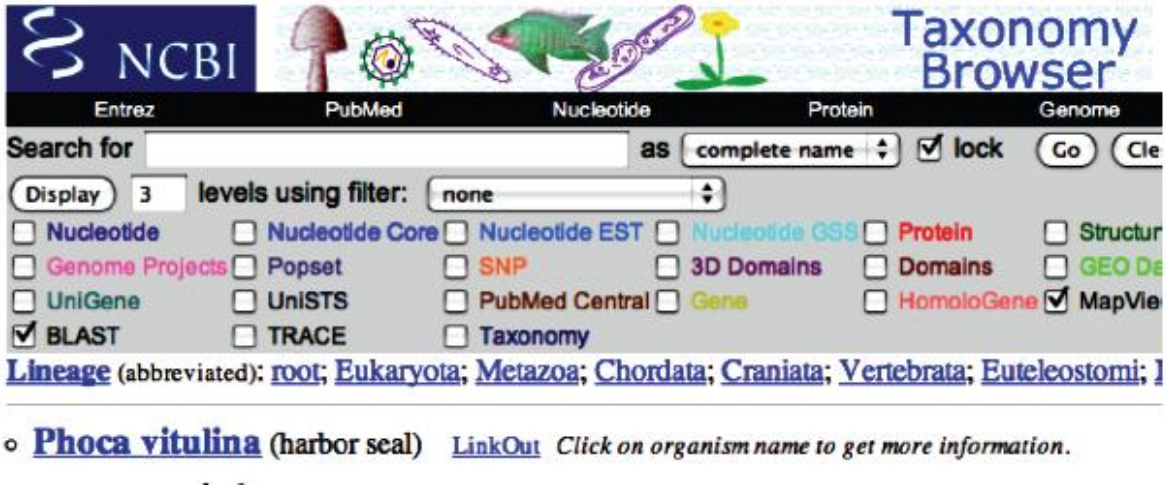
WARNING Taxonomy is a field that is subject to lots of disagreement and debate. As a result, the names of phyla, orders, etc. on the NCBI site may be different from those we use in Bio 112 lectures and other labs. When there is a conflict, **always** use the names used in lecture and other labs when you are giving answers on an exam. In this lab, you should use the NCBI names, but **only for this lab**. Sorry for the confusion; welcome to the complex and contentious world of taxonomy...

2) Type the name of the organism into the "Search for" blank and click "Go". You might want to play around with some of the other choices under "complete name".

As an example, I typed in "harbor seal" and I got this:



This gives the full lineage for the harbor seal -this is too much information. 3) Click on the blue "Lineage (full)" link and it will change to "Lineage (abbreviated)" with



This is more useful.

Note the line that says: Lineage (abbreviated): root; Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia;

If you put the cursor over the terms, a little window will pop up showing the taxonomic level of that term. In this case, you'd get:

- Eukaryota - superkingdom (ignore this one)
- Metazoa - KINGDOM
- Chordata - PHYLUM
- Craniata - subphylum (ignore this one)
- Vertebrata -no rank (ignore this one)
- Euteleostomi -no rank (ignore this one)
- Mammalia - CLASS

•Eutheria -no rank (ignore this one)
•Laurasiatheria - superorder (ignore this one)
•Carnivora - ORDER
•Caniformia - suborder (ignore this one)
•Phocidae - FAMILY
•Phoca - GENUS

What about the species? Look down to where it says "Phoca vitulina" - the species is "vitulina".
4) Use the kingdom, phylum, class, order, family, genus, and species to draw a phylogenetic tree of the organisms in your class using a format similar to that described on page MolPhyl-2. Label the branch points with the appropriate names.

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, your text book, *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 30 points (Collection is worth 20pts). 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.

4. Using examples from the phylum you chose, explain 'what it takes to be a phylum'. That is: How *similar* must organisms be to be in the *same* phylum? How *different* must organisms be to be in *different* phyla?

This page intentionally left blank.

						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						

						Where it lives	
TA checkoff	Sample #	Phylum	Page	Name	Where you found it	Geography	Habitat
	13						
	14						
	15						
	16						

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