1. How do the brow ridge, cranial ridge, brain case size and forehead size compare between the skulls of *Australopithecus afarensis*, *Homo erectus* 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

–crow

–seagull

–A

–sparrow

–human

–cow

–B

–horse

–grass

–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.
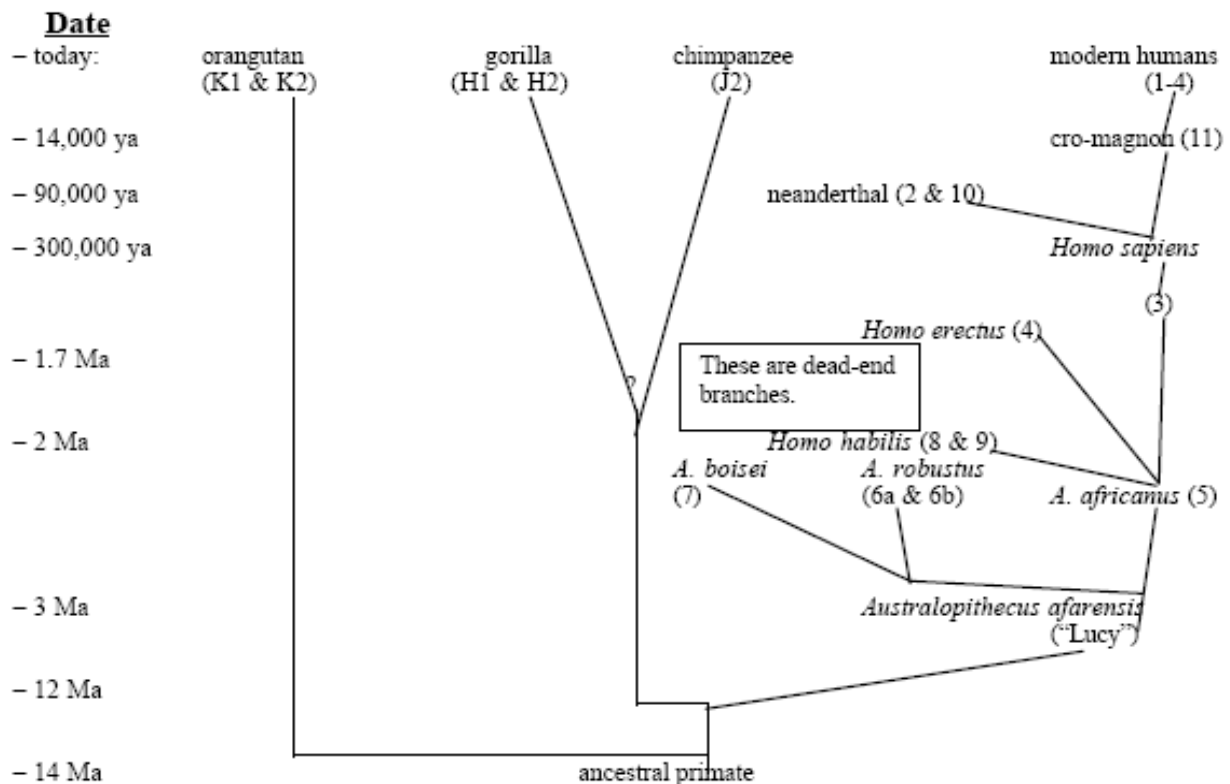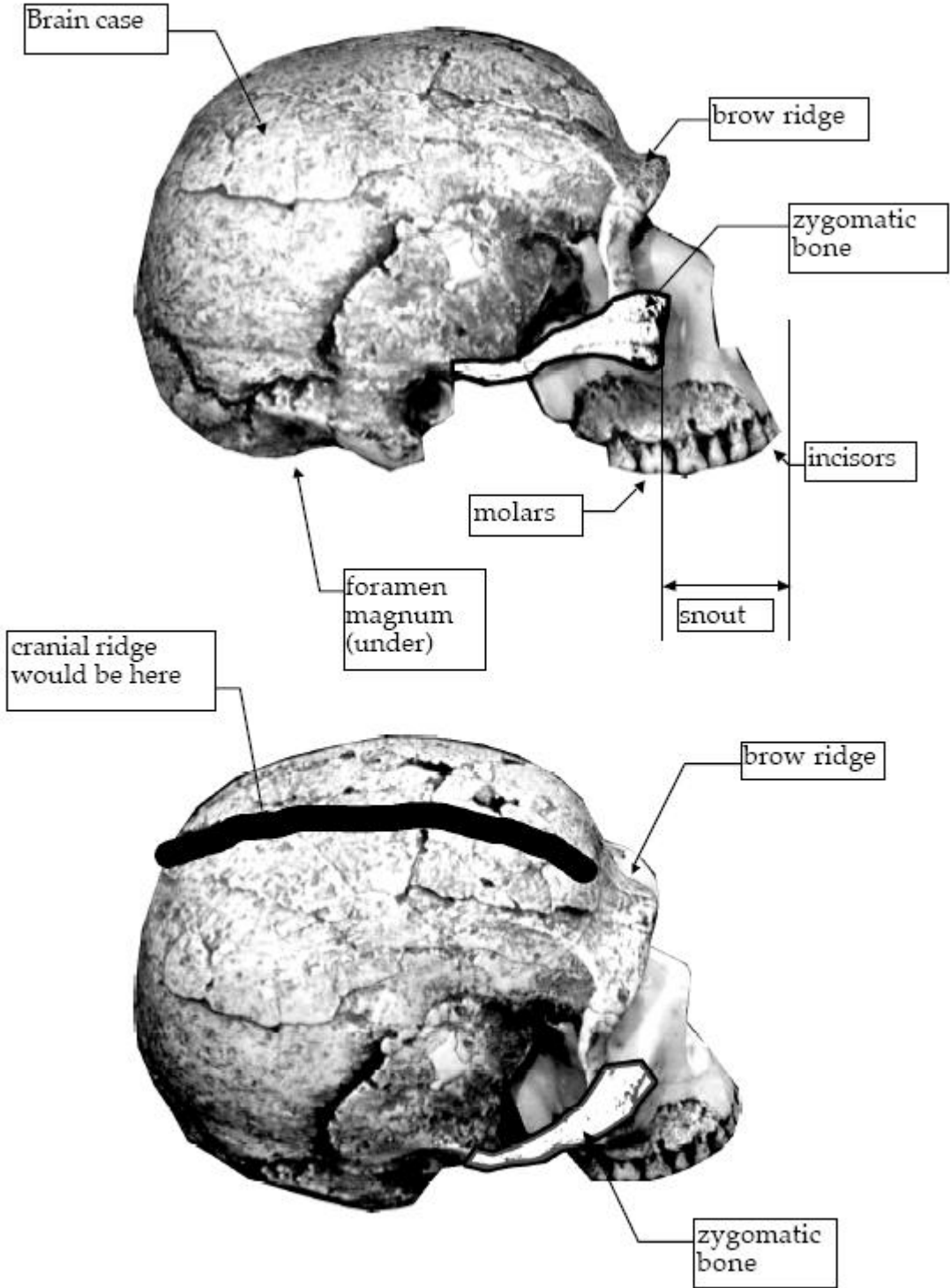
From the comparison of skulls from different primates, eight (somewhat overlapping) trends in the evolution of humans have been found. Note that not all traits in a given skull will be equally 'human' – that is, you will likely find skulls where one feature is ancestral and others are modern. This chart describes these eight trends. The following pages illustrate the skull features described in the table.
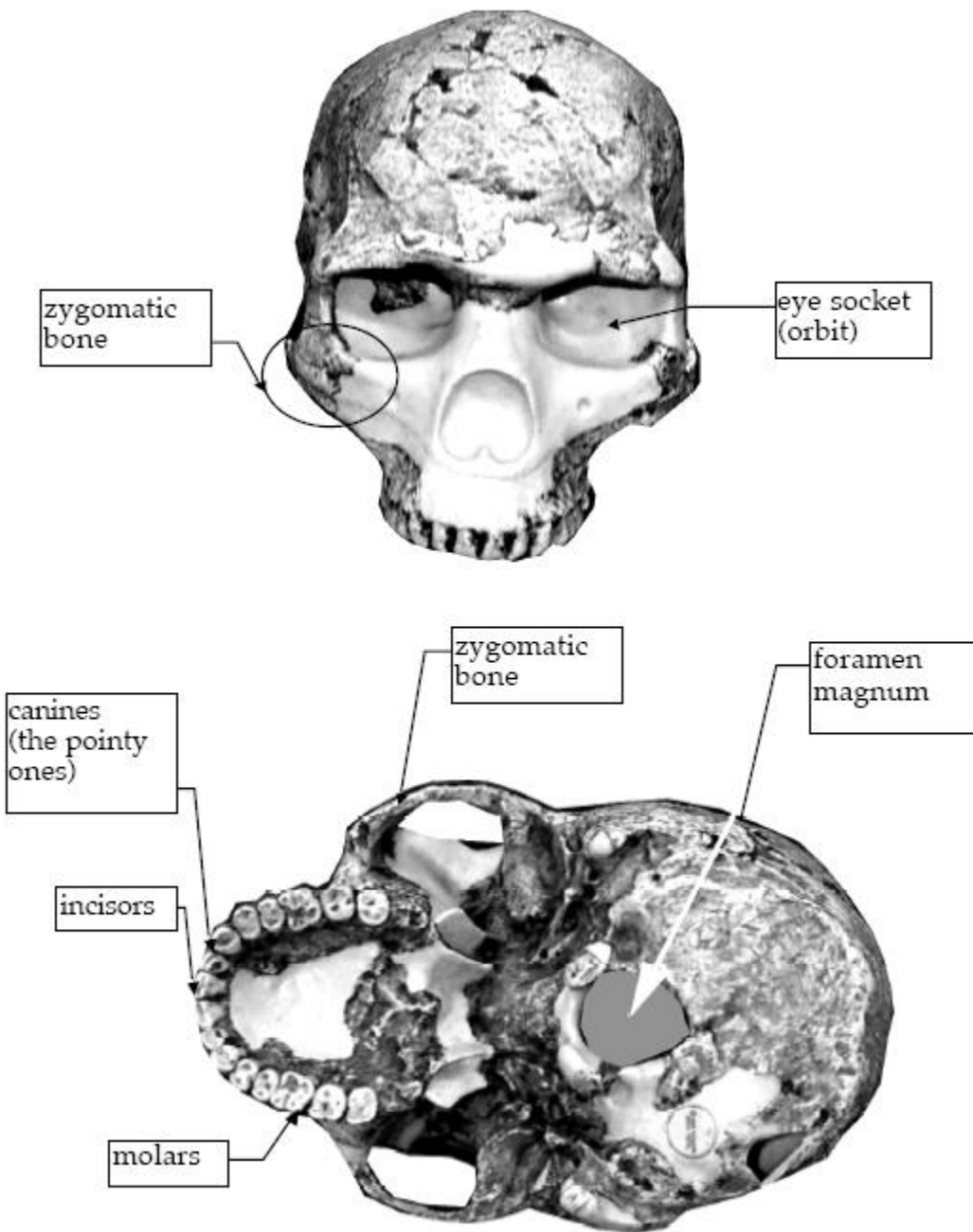
Table 1.

	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:

Brain case

brow ridge

zygomatic bone

incisors

molars

foramen magnum (under)

snout

cranial ridge would be here

brow ridge

zygomatic bone

zygomatic bone

eye socket (orbit)

zygomatic bone

foramen magnum

canines (the pointy ones)

incisors

molars

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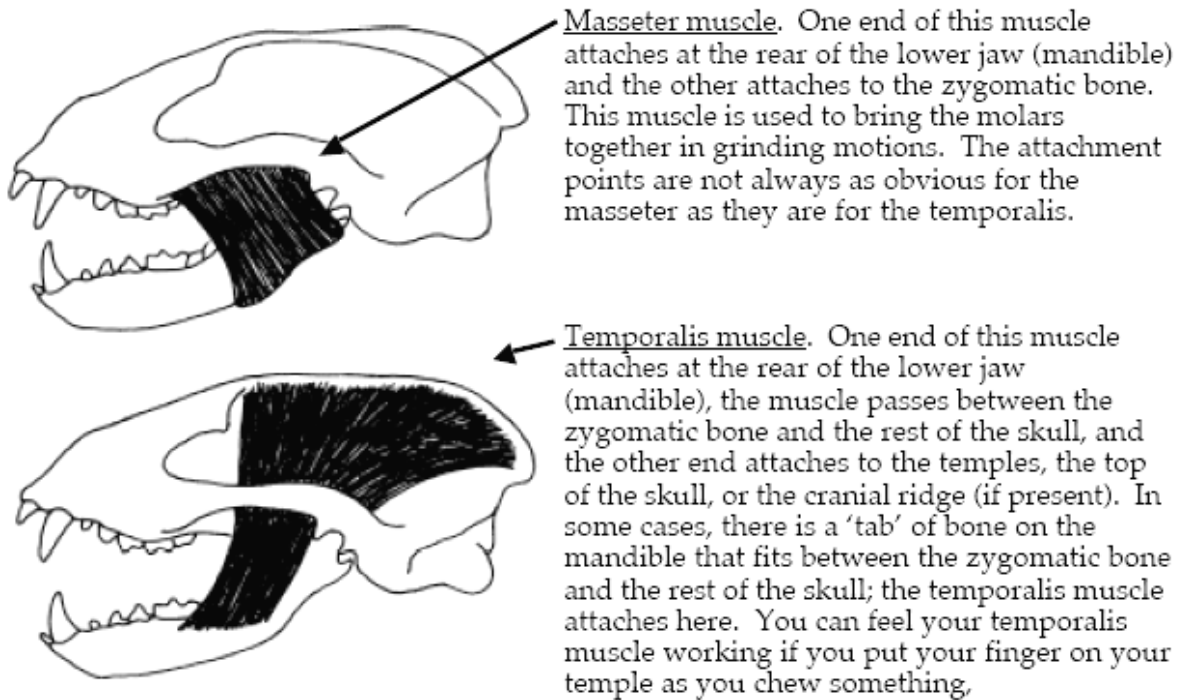
5) Consider the following features and determine the trends in these features as you go from carnivore to omnivore to herbivore.

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.
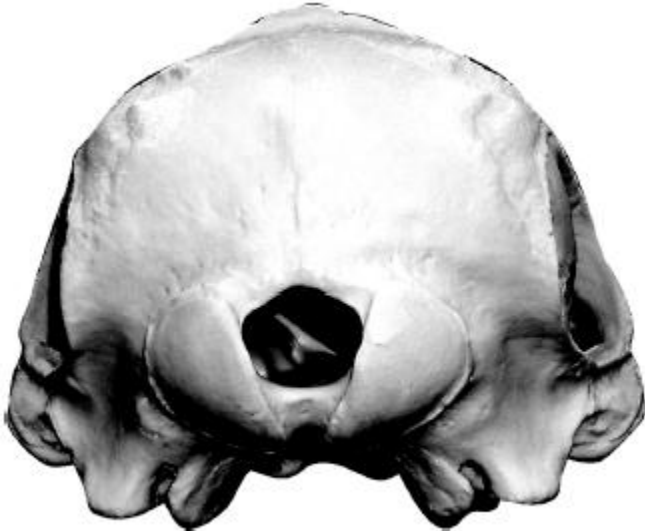
Dolphin Worksheet (attach to your write up)

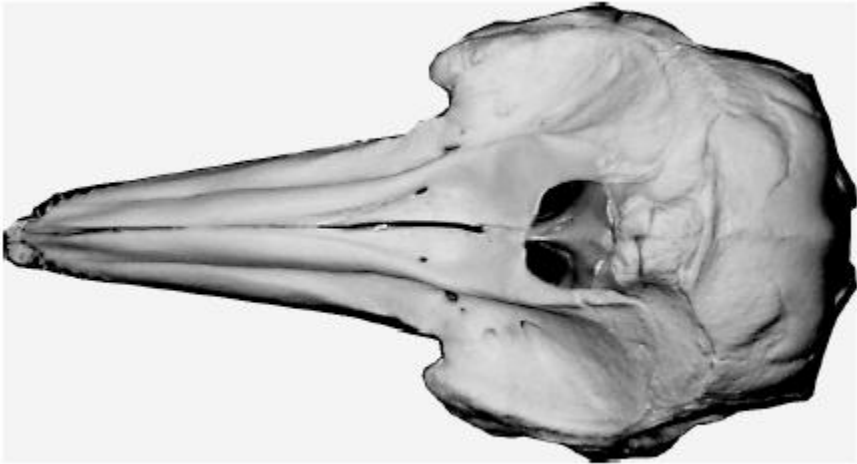An intact dolphin;
left side view.

• 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):

Main Tree Organisms

Select several organisms here to construct the tree.

Animals	Plants	Other
ailanthus silkmoth	buckwhe	
American alligator	castor be	
Arabian camel	cauliflow	
brown garden snail	China jut	
bullfrog	corn	
California gray whale	ginkgo	
carp	hemp	
chicken	leek	
chimpanzee	mung be	
cow	nasturtium	green alga

Select the creatures to include in your tree by clicking on their names in these lists. You can select as many as you want. To select more than one non-adjacent creature in the same list, hold down the apple key. (Click this button to clear & start over)

Outgroup Organsim

Select **one** organism that you did not select above to act as an outgroup. This sh closely-related to the organisms above.

Animals	Plants	Other
bullfrog	buckwheat	Desulfovibrio vulgaris
California gray whale	castor bean	Asperg nidulans
carp	cauliflower	Asperg niger
chicken	China jute	Neurospora crassa

(Calculate Tree) (Clear all selections)

Click here when you are satisfied with your selections. it will submit your creatures to the server computer which will do the calculations.
These calculations may take a few minutes; please be patient.

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

You selected the following creatures:

Main Tree Organisms: cow,
Outgroup: donkey

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

Tree based on number of substitutions

Click on the button below to view the sequences in the JalView multiple alignment editor and to see the tree based on number of substitutions.
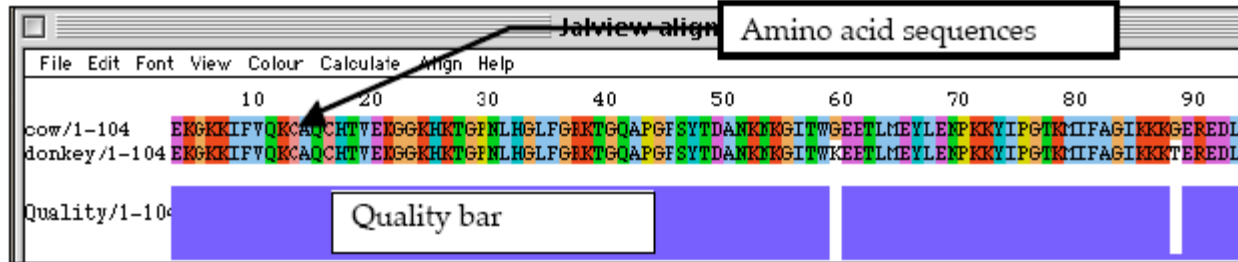
(JalView)

Calculate Align Help
Consensus
Autocalculate consensus
Sort by pairwise identity
Sort by ID
Sort by group
Sort by tree order
Remove redundancy

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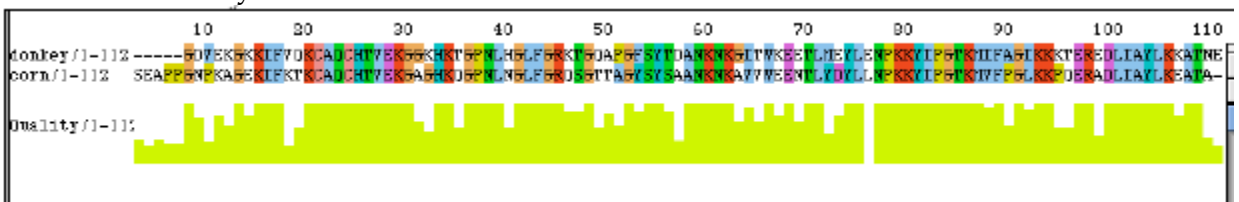
I chose corn as my distantly-related organism. Here are the results I got:

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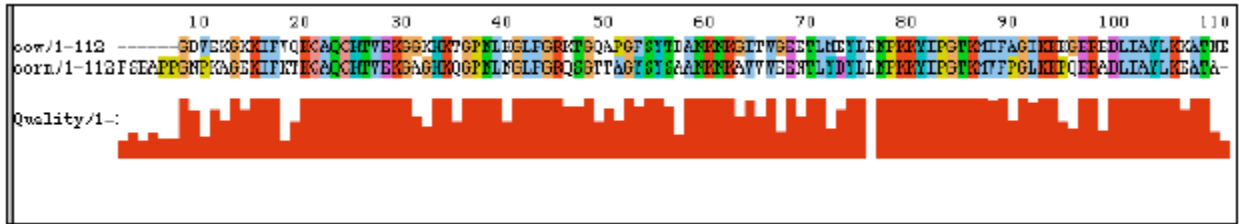
Counting all the places where the sequences don't match (anyplace where the "Quality" bar isn't at its full height), there are 44 substitutions out of 112 amino acids.

• 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.
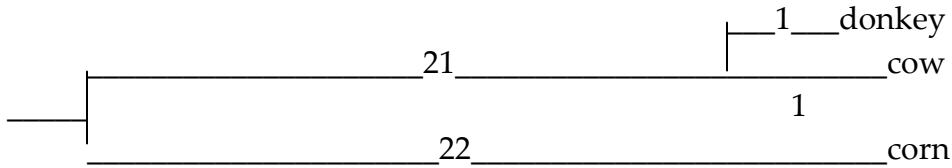
```
 _____22_____cow
|
|_____22_____corn
```
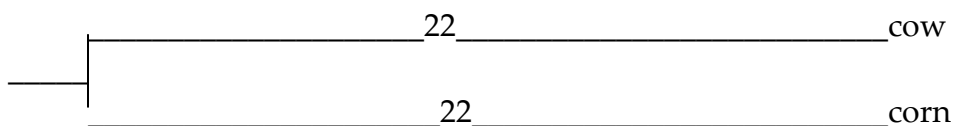
Note that the total distance between cow and corn is 22 + 22 = 44.

  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:
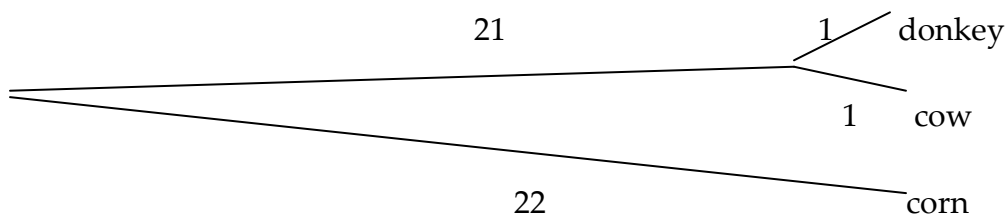
```
                                          ┌1__donkey
 _____Y_____|
|                                         X └_cow
|
|_____22_____corn
```
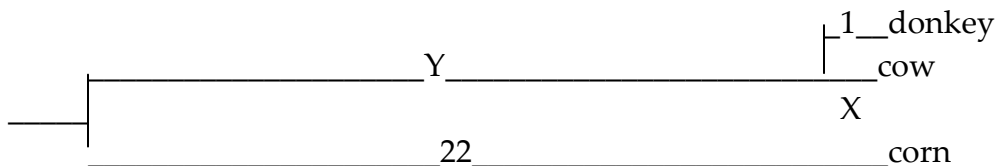
iii) But what about the "X" and "Y"? Since the distance between cow and donkey must be 2, X + 1 must = 2. Therefore X = 1. Since the total length from the branch at the left to cow must equal 22 and X = 1, Y = 22 –X or 22 – 1, or 21. This gives the final tree:

```
                                              ___1___donkey
                          21_____|_____cow
       _____|                                    1
           |_____22_____corn
```

There are a couple of things to notice about this tree:
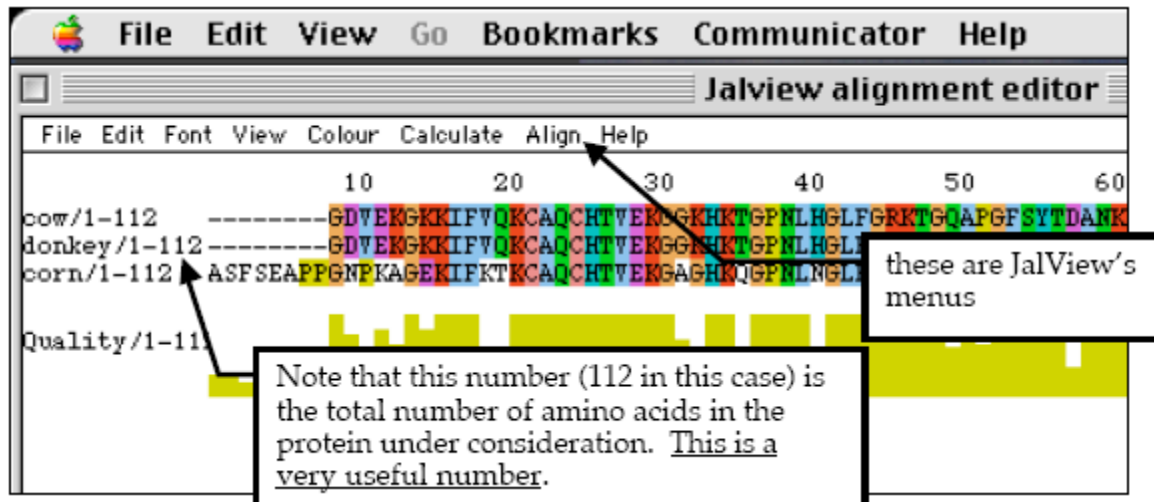   • The lengths of the vertical lines are not counted in the branch lengths.
     Therefore it is identical to this tree:

```
            21                              1    donkey

                                          1    cow

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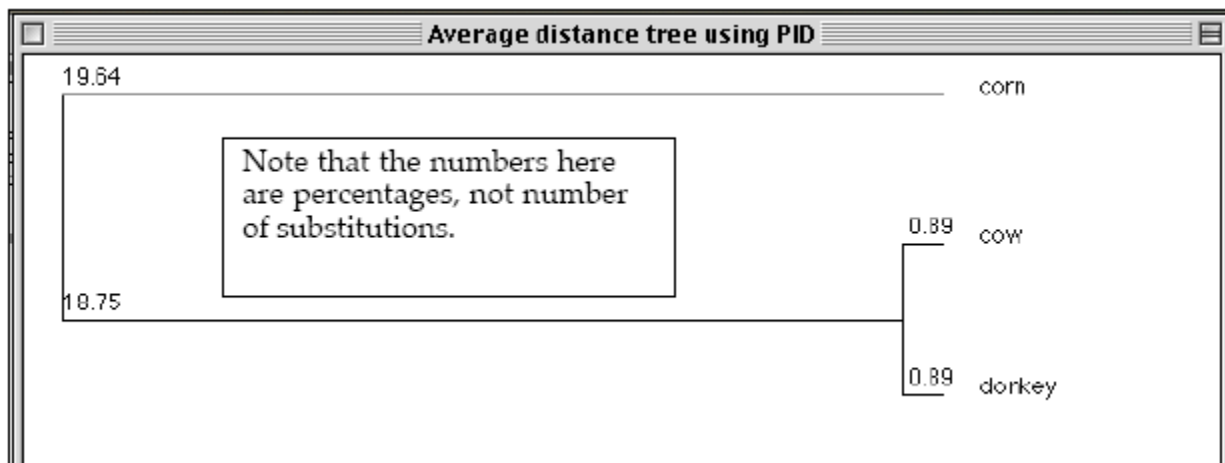
• It is approximate! The distance from donkey to corn should be 44 substitutions (as measured from the sequences) and that is what the tree shows. Sometimes, it comes out like this and sometimes the numbers don't add up properly. This is what we call "close enough for government work".

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,

File   Edit   View   Go   Bookmarks   Communicator   Help

Jalview alignment editor

File Edit Font View Colour Calculate Align Help

```
                10        20        30        40        50        60
cow/1-112     ----------GDVEKGKKIFVQKCAQCHTVEK GKHKTGPNLHGLFGRKTGQAPGFSYTDANK
donkey/1-112  ----------GDVEKGKKIFVQKCAQCHTVEKGGNHKTGPNLHGL
corn/1-112    ASFSEAPPGNPKAGEKIFKTKCAQCHTVEKGAGHKQGPNLNGL

Quality/1-11
```

these are JalView's menus

Note that this number (112 in this case) is the total number of amino acids in the protein under consideration. This is a very useful number.

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:

Average distance tree using PID

```
19.64 _____ corn


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

                                                    0.89  cow
18.75


                                                    0.89  donkey
```

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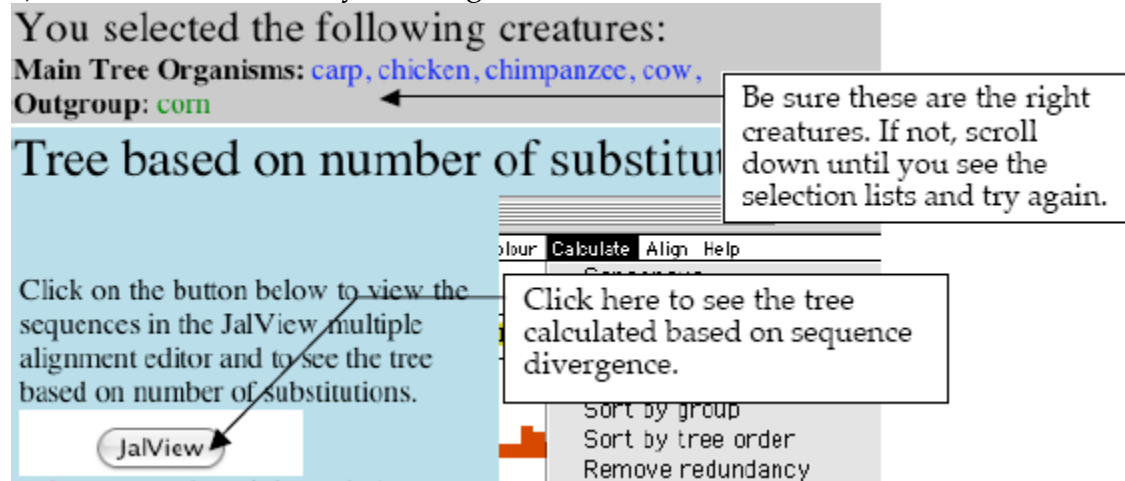
[The only time you should really worry is if you get a message like "server not responding" at this point. In this case, contact TA ASAP.]

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:
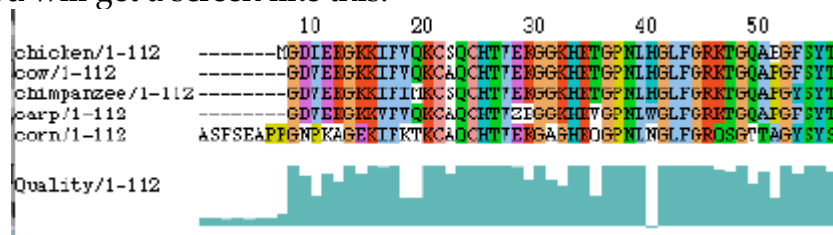
e) From JalView's "Calculate" menu, select "Calculate Average Distance Tree using PID". Again, be patient. Set the "Font Size" to 12 and check "Show Distances" (these controls are near the bottom of the window). You will get a tree like the one on the next page:
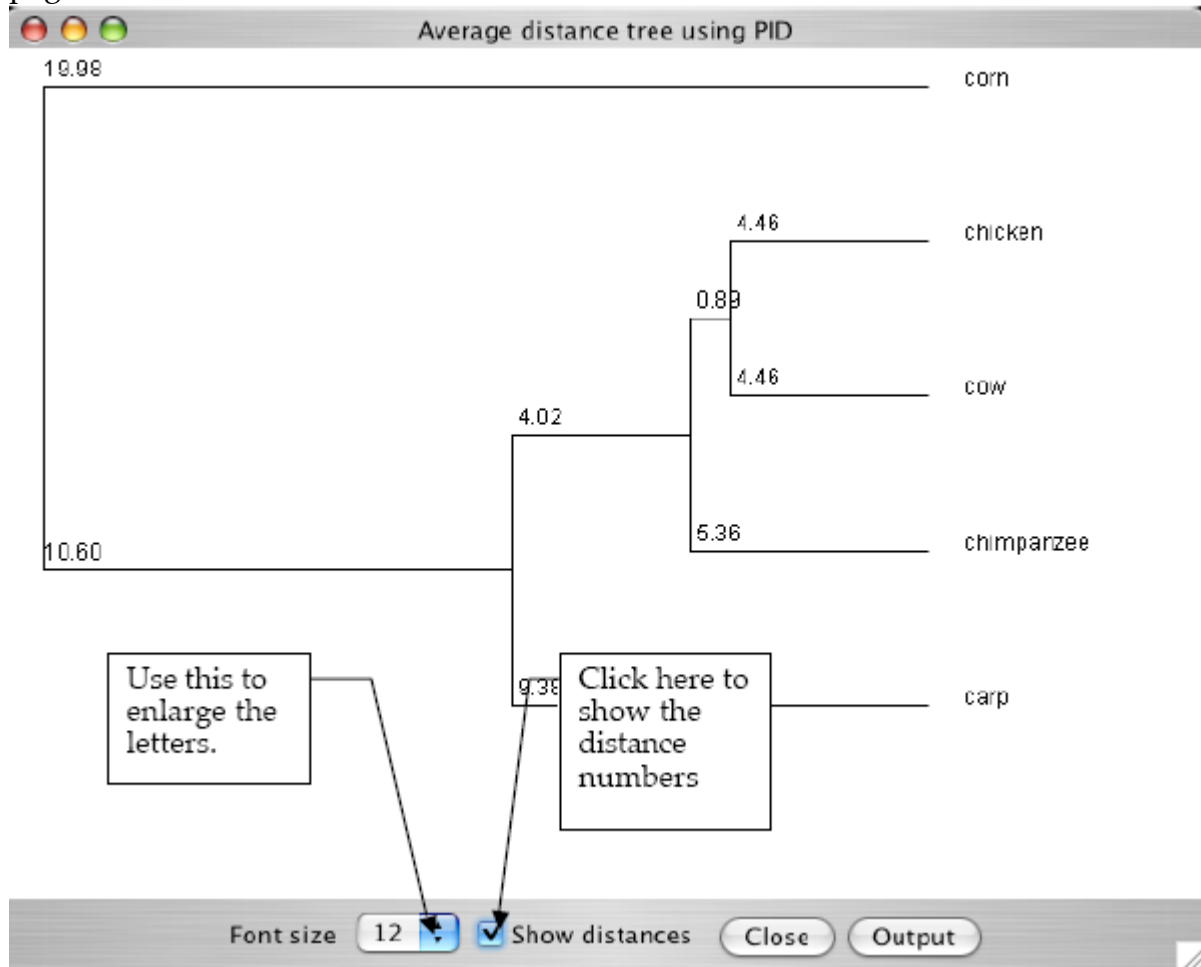
f) Unfortunately, you cannot print this out; you will have to copy it down by hand. Do not have the program mail it to you, that feature does not work.

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.