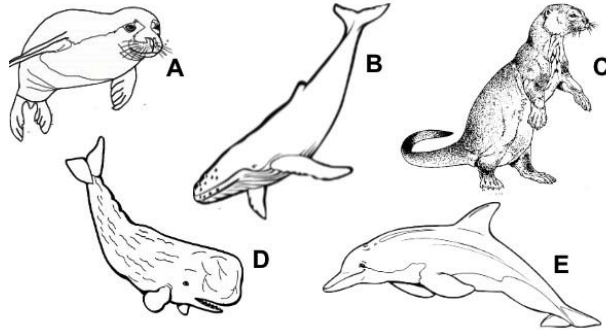


Name _____

Pre-Lab: Phylogeny

Note read the entire lab to answer the following questions (pg Phylogeny 1-24)

- The following are images of Marine Mammals. WITHOUT RESEARCHING THEM, put them into groups representing how YOU THINK they could be related (NOTE: It's not about grouping them correctly; We just want you to experience grouping things based on physical appearance, as you would out in nature).

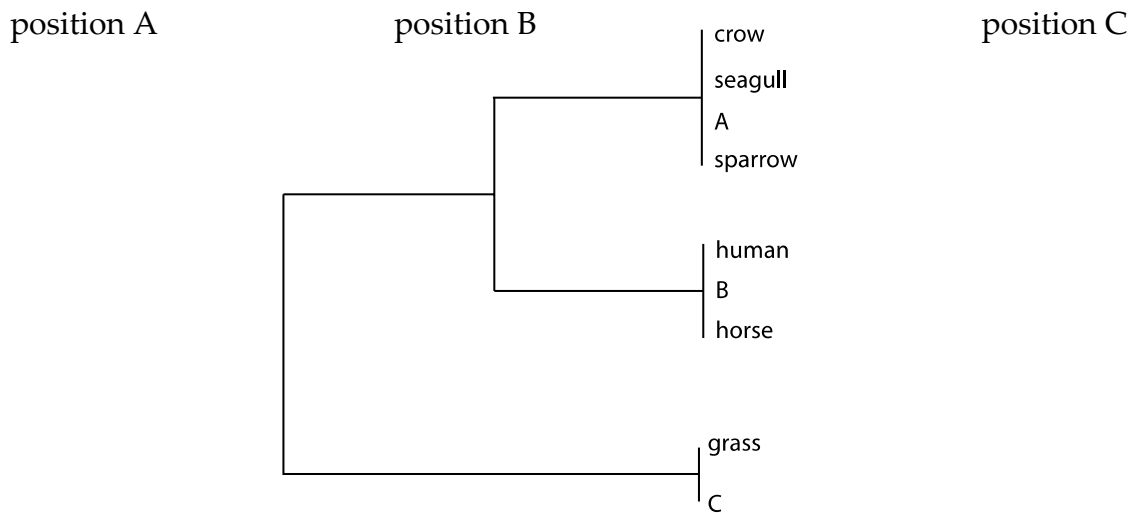


- Given the following data:

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.

- 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.



Phylogeny-1

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PHYLOGENY

Today we will look at how evolution is studied using 2 different types of data. In the first part you will use comparative morphology to examine mammal skulls including hominids. In the second part you will use molecular data to construct phylogenetic trees.

A. SKULLS

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

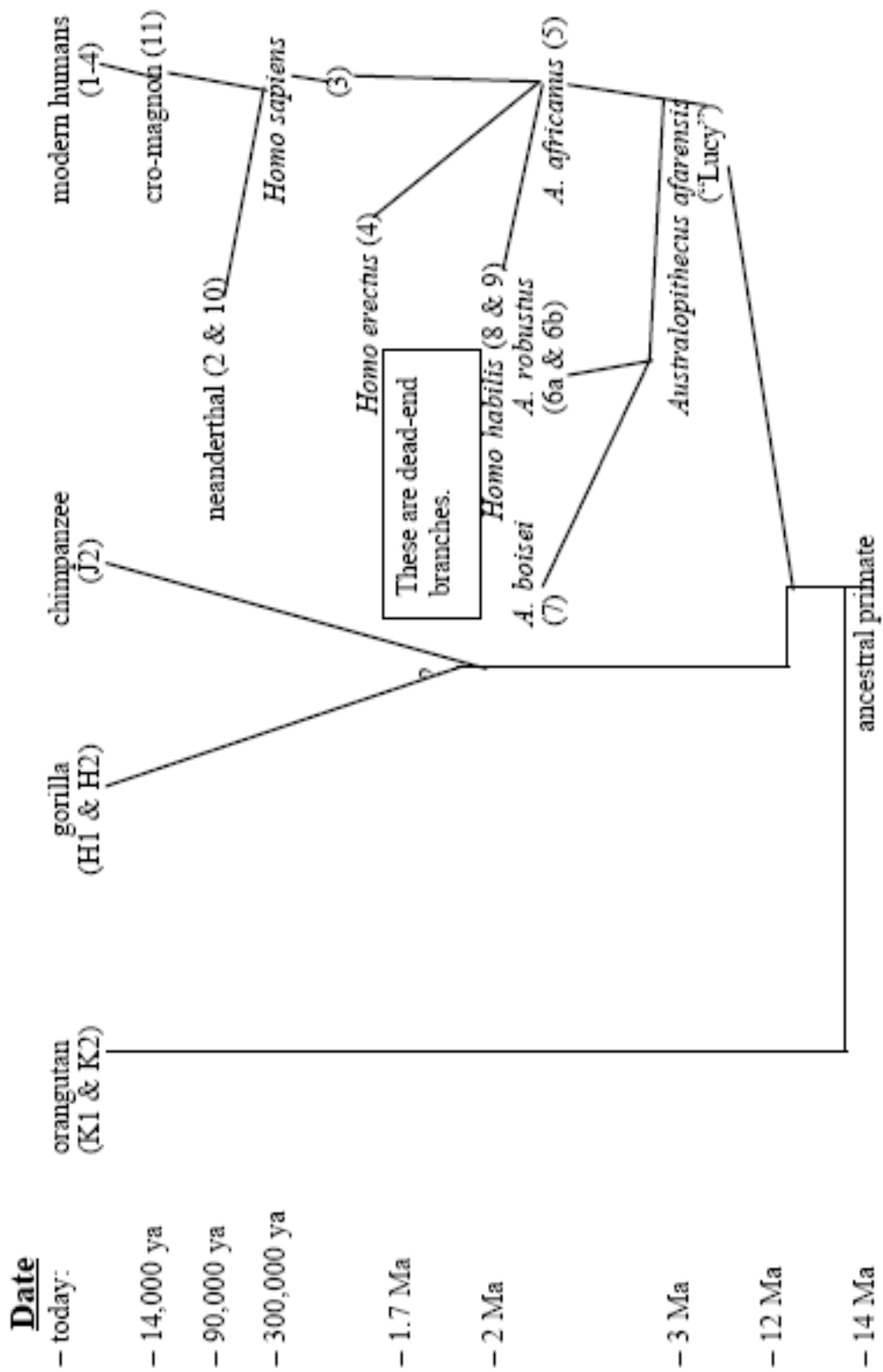
Much of what we know about evolution comes from the study of comparative anatomy. In many cases, bones (either as fossils or skeletons) have been useful in these studies. Bone and skeletal structures can reveal how an animal moves, eats, reproduces, etc. In this lab, we will look at the skulls of various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

Procedure

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

Part I: Human Evolution

Shown on the next page 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.



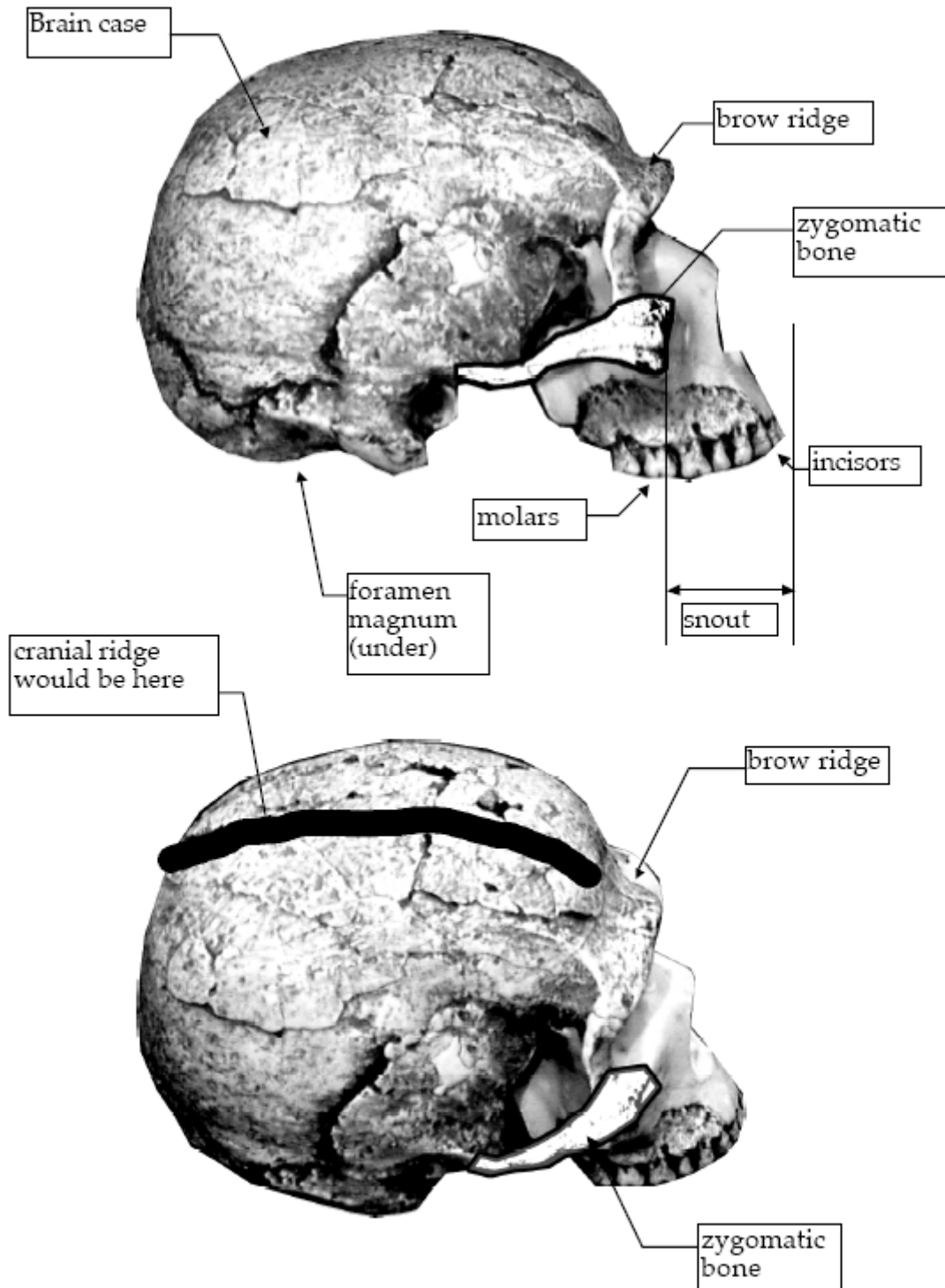
From the comparison of skulls from different primates, eight (somewhat overlapping) trends in the evolution of humans have been found. Note that not all traits in a given skull will be equally 'human' – that is, you will likely find skulls where one feature is ancestral and others are modern. This chart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

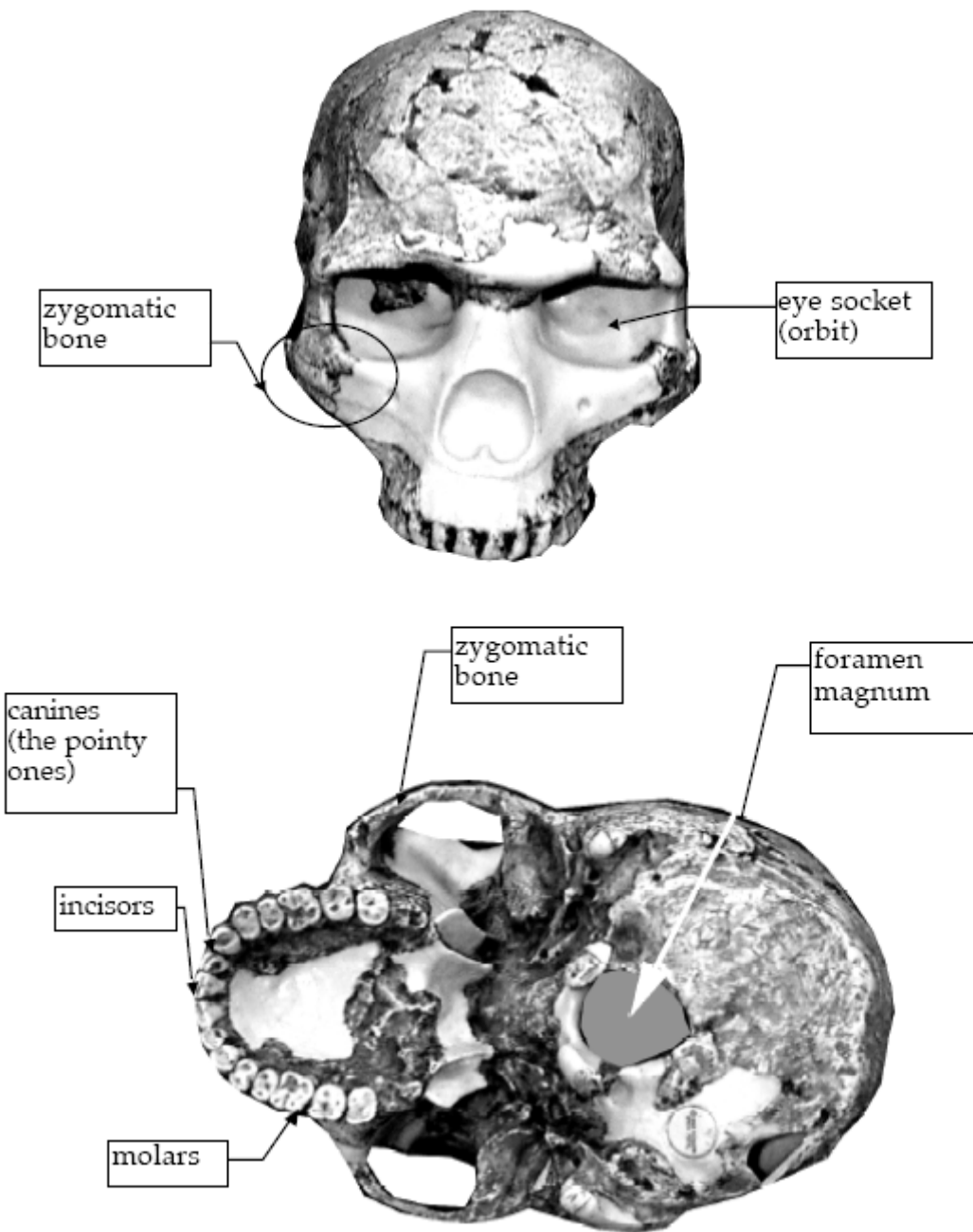
Table 1.

	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 table of skull features found in the previous pages, 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 evolutionary diagram 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.

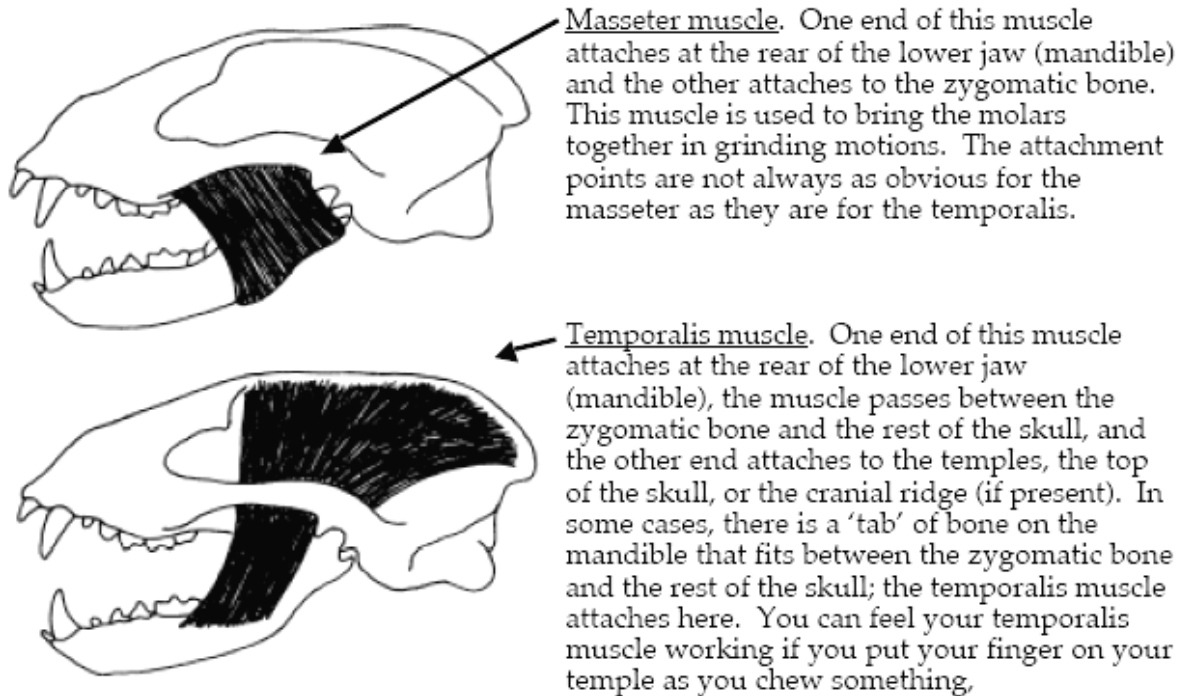
5) Consider the following features and determine the trends in these features as you go from carnivore to omnivore to herbivore.

Table 2.

	<u>Feature</u>	<u>Details</u>	<u>Explanation</u>
1	Canine teeth	<ul style="list-style-type: none"> • present? • large or small 	Used for cutting and tearing of food.
2	Molars	<ul style="list-style-type: none"> • flat • pointed 	Used for grinding food.
3	Eye Sockets (orbits)	<ul style="list-style-type: none"> • 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)	<ul style="list-style-type: none"> • large • small 	Used for moving jaws when grinding food.
5	Temporalis muscle attachment points (see next page for description)	<ul style="list-style-type: none"> • 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.



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

Dolphin
Gray Seal
Harp Seal
River Otter
Sea Otter

Terrestrial Mammals

Sheep
Dog/Wolf
Raccoon
Leopard
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 (part A):

- 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 Phylogeny-5.

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 Phylogeny -10.

Part B: Molecular Phylogeny

Turn in a copy of your trees generated in the second part of this lab. Include the names of the members of your lab group.

Dolphin Worksheet (attach to your write up)

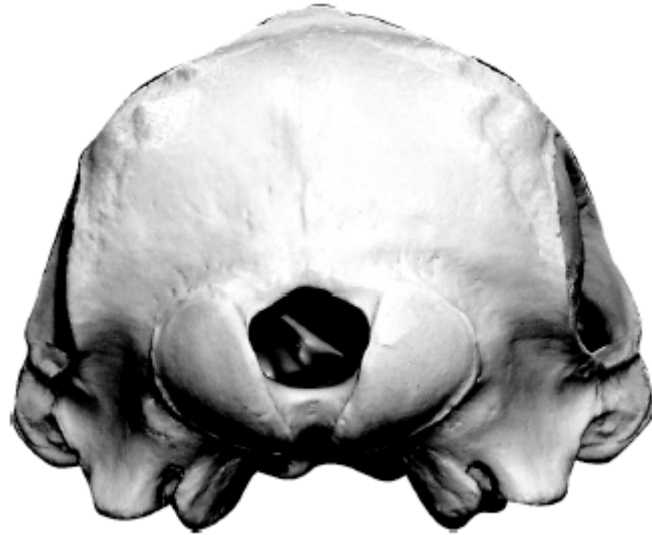
An intact dolphin;
left side view.



• Dolphin skull; left side view:



Rear view:



Top (dorsal) view:



PART B:

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 differences in the amino acids coded 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 to examine the evolutionary connections between species. 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 very few differences in the amino acids of their cytochrome c molecules. The longer the time since two organisms had a **common ancestor**, the more different the cytochrome c molecules will be. We will compare the amino acid sequences of cytochrome c from various organisms to determine their degree of evolutionary relatedness.

There are several methods for comparing protein sequences from different organisms in order to determine their phylogenetic relationships. Some of them are complex and take longer to compute than the given class time. We will focus on only two of the methods because they are the faster to compute and easier to understand.

1. **Sequence divergence:** This method compares the sequences and counts the number of differences between them. It is the simplest method. You will do this 'by hand' to see how it works and then let the computer do the hard work. This method can be used for finding approximately how long it has been since the two species had a

common ancestor. 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.

2. **Parsimony:** This method is more sophisticated. Unlike the sequence divergence method, which builds a tree, this method searches through all possible trees (ways of connecting the species). The method ignores positions where all species have the same amino acid and where only one species has a difference. It is based on finding trees that represent the most **shared derived** amino acids and fewest **convergent** amino acids. In other words if a dolphin and corn have the same amino acid at position at position 52 would it be due to a) most organisms have the same amino acid at position 52 because first respiring organisms had that amino acid (if this is the case the method would ignore this amino acid) b) corn and dolphin share a recent common ancestor that had that trait or c) corn and dolphin had independent mutations that converged on the same amino acid. Parsimony tries to find the tree with the most data that fit the b) explanation while minimizing having c) as an explanation.

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

Procedure

You will work in groups of three per computer in this lab. The instructions in the manual are for the Macintosh computers. You can also access all of the resources for this lab from any computer with www access – no special plug-ins are required.

Part I Sequence Divergence “The hard way” (you do half of the work)

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

- 1) To access the “Tree Constructor”, open a browser (Google Chrome) to use the internet.
- 2) In lab the browser should open to a Bio 112 Fall 2013 website. Find the link for the “New Phylogenetic Tree Constructor” next to the title for this week’s lab. (You can also link to this remotely via your Bio 112 BlackBoard online component)
- 3) You will see a page that looks like this

Main Tree Organisms

Select several organisms here to construct the tree.

Animals	Plants	Other
<input type="checkbox"/> ailanthus silkmoth <input type="checkbox"/> American alligator <input type="checkbox"/> Arabian camel <input type="checkbox"/> brown garden snail <input type="checkbox"/> bullfrog <input type="checkbox"/> California gray whale <input type="checkbox"/> carp <input type="checkbox"/> chicken <input type="checkbox"/> chimpanzee <input type="checkbox"/> cow	<input type="checkbox"/> buckwheat <input type="checkbox"/> castor bean <input type="checkbox"/> cauliflower <input type="checkbox"/> China jute <input type="checkbox"/> corn <input type="checkbox"/> ginkgo <input type="checkbox"/> hemp <input type="checkbox"/> leek <input type="checkbox"/> mung bean <input type="checkbox"/> nasturtium	<input type="checkbox"/> 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 Organism

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

Animals	Plants	Other
<input type="checkbox"/> bullfrog <input type="checkbox"/> California gray whale <input type="checkbox"/> carp <input type="checkbox"/> chicken	<input type="checkbox"/> buckwheat <input type="checkbox"/> castor bean <input type="checkbox"/> cauliflower <input type="checkbox"/> China jute	<input type="checkbox"/> Desulfovibrio vulgaris <input type="checkbox"/> Aspergillus nidulans <input type="checkbox"/> Aspergillus niger <input type="checkbox"/> Neurospora crassa

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.

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 be patient.

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

Main Tree Organisms

Select several organisms here to construct the tree.

Animals	Plants	Other
ailanthus silkmoth American alligator Arabian camel brown garden snail bullfrog California gray whale carp chicken chimpanzee cow	buckwheat castor bean cauliflower China jute corn ginkgo hemp leek mung bean nasturtium	Desulfovibrio vulgaris Asperg nidulans Asperg niger Neurospora crassa smut fungus Crith fasciculata Crith oncopelti Eugl gracilis Eugl viridis green alga

Outgroup Organism

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

Animals	Plants	Other
desert tortoise dog donkey duck earthworm	buckwheat castor bean cauliflower China jute	Desulfovibrio vulgaris Asperg nidulans Asperg niger Neurospora crassa

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

You selected the following creatures:

Main Tree Organisms: cow,

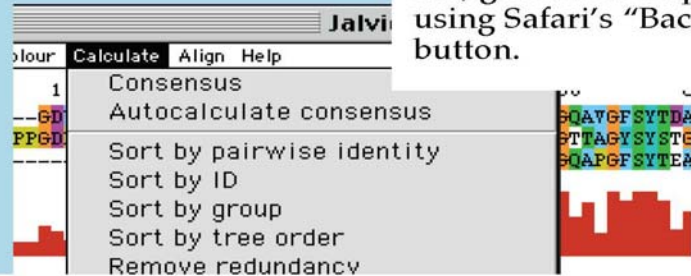
Outgroup: donkey

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

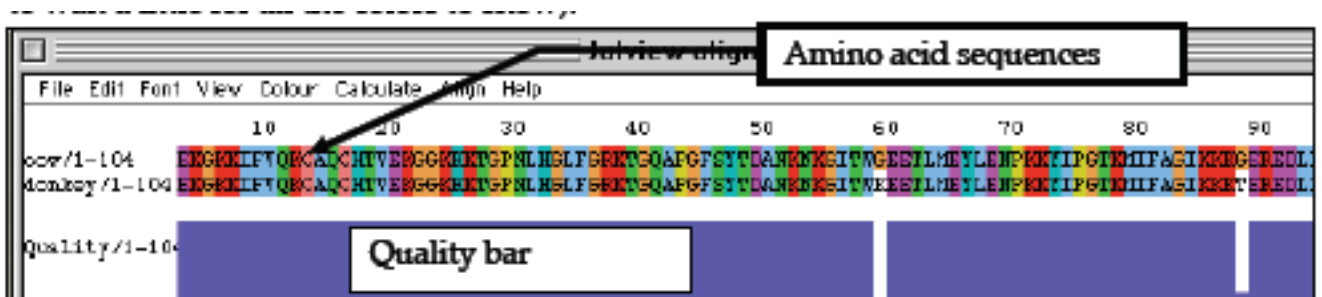
Tree based on number of substitutions

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



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



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

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

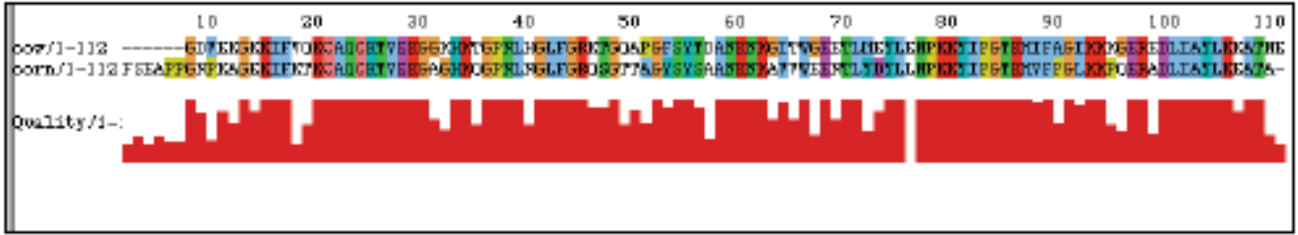
From this, we can conclude that there are two amino acid differences between the cytochrome c’s of cow and donkey. We would then say “cow and donkey differ by 2 substitutions”.

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

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

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

- corn vs. cow

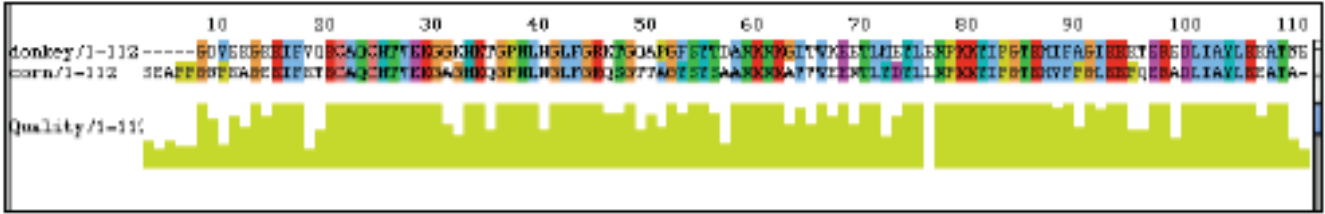


Counting all the places where the sequences don't match:

- Don't forget to count the dashes at the start of the sequence (in this case, there are 5)
- anyplace where the "Quality" bar isn't at its full height (but you can't always see small drops in the "Quality" bar; so you should look at the amino acid sequence carefully)

In this case, there are 44 substitutions out of 112 amino acids.

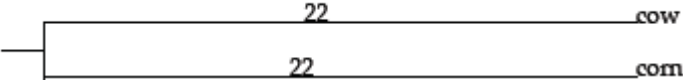
- corn vs. donkey:



Counting all the places where the sequences don't match (see notes above), there are 40 substitutions out of 112 amino acids.

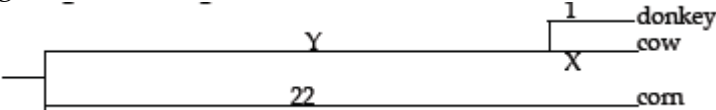
9) Make a phylogenetic tree of your three organisms based on the substitution data. Here is a simple way:

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

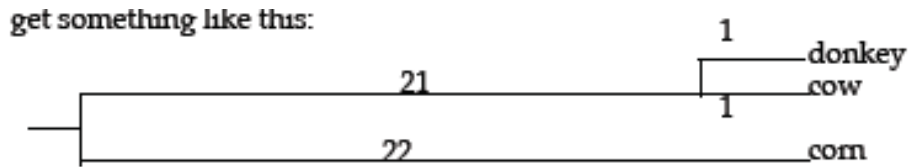


Note that the total distance between cow and corn is 22 + 22 = 44.

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:

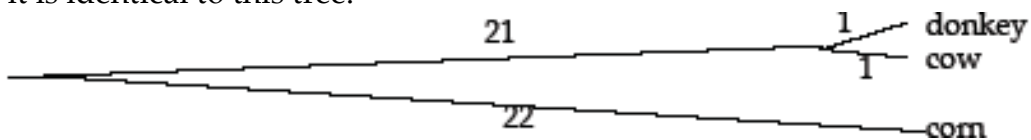


iii) But what about the “X” and “Y”? Since the distance between cow and donkey must be 2, $X + 1$ must = 2. Therefore $X = 1$. Since the total length from the branch at the left to cow must equal 22 and $X = 1$, $Y = 22 - X$ or $22 - 1$, or 21. This gives the final tree:



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

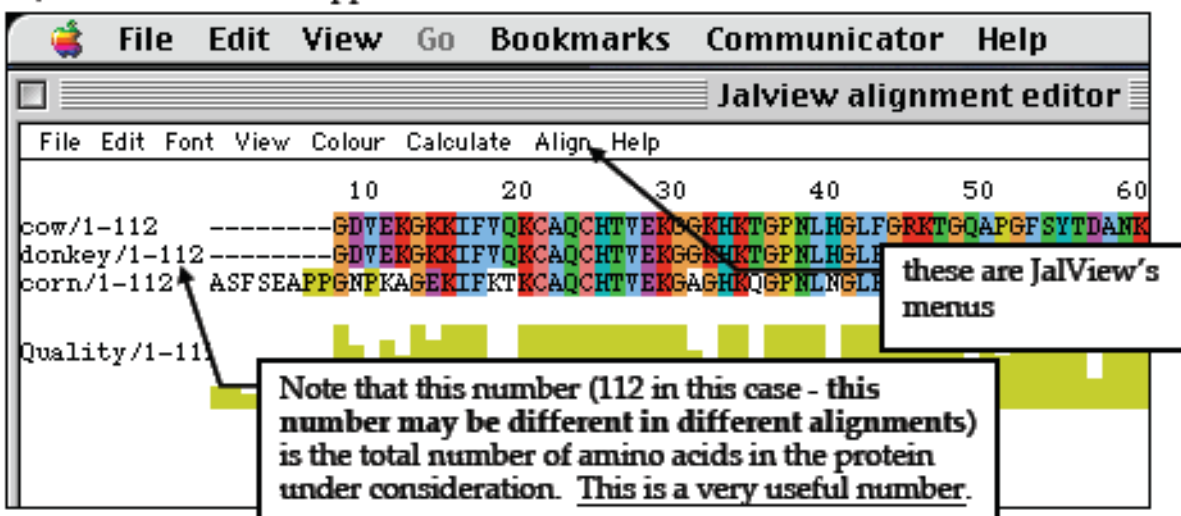
- The lengths of the vertical lines are not counted in the branch lengths. Therefore it is identical to this tree:



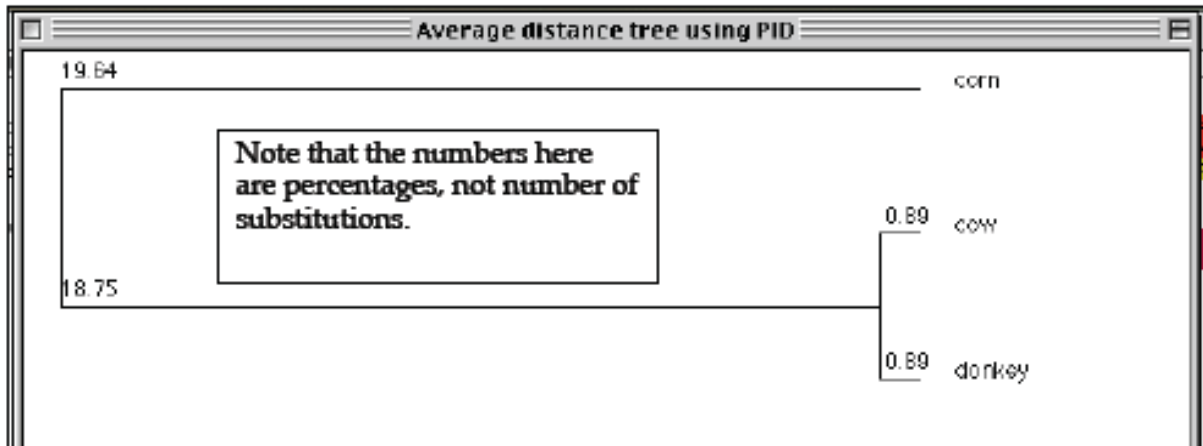
- It is approximate! The distance from donkey to corn should be 41 substitutions (as measured from the sequences) but the tree shows it as 44. Sometimes, it comes out like this and sometimes the numbers add up properly. This is what we call “close enough for government work”.

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

- Go back to the “Tree constructor” page.
- Select your three creatures and click “Calculate Tree”.
- Click “JalView”.
- When the window appears,



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



You can roughly check the numbers using the following calculations. The numbers are % difference, so the actual *number* of substitutions = (% difference) x (total number of amino acids).

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

* This number may be different in different alignments.

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

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

a) Look within the Main Tree Organisms lists to find a total of 5 organisms, 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) Now select the 4 "Main Tree Organisms" as you did previously. Use shift-click to select more than one organism at a time. If you want to select non-adjacent organisms in a list, use apple-click. Once you have made your selections, click the "Calculate Tree" button.

In the example below, I selected:

Main tree: carp
chicken
Chimpanzee
Cow
(all of these are vertebrates)

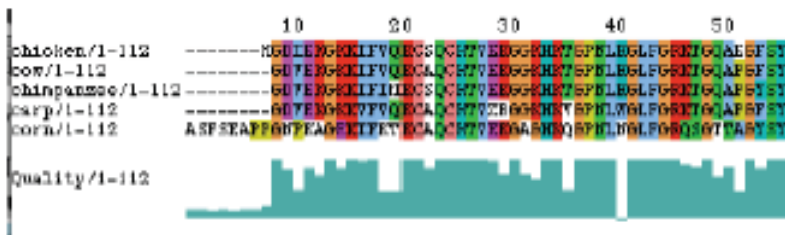
Outgroup: Corn
(this is very different from a vertebrate!)

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



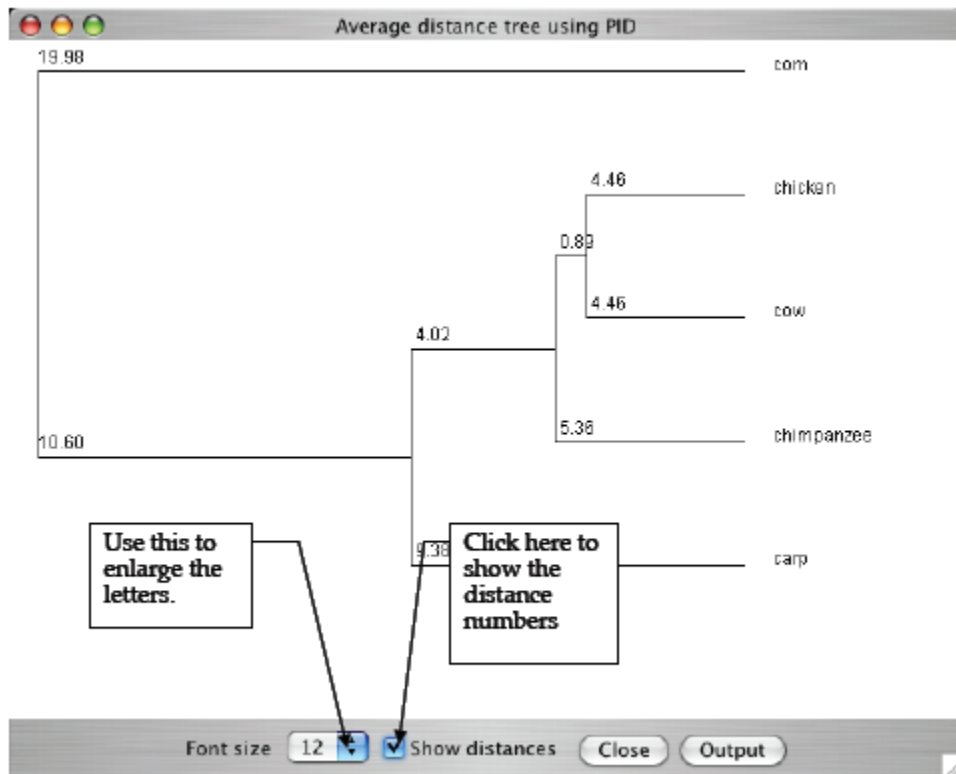
[The only time you should really worry is if you get a message like “server not responding” at this point. In this case, contact your TA.]

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:



**Note that the dashes “-” above are “gaps” in the alignment - places where a mutation resulted in a *missing* or *added* amino acid. One interpretation of the figure above is that a mutation in an ancestor of corn added several amino acids to the N-terminus of the protein.

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:



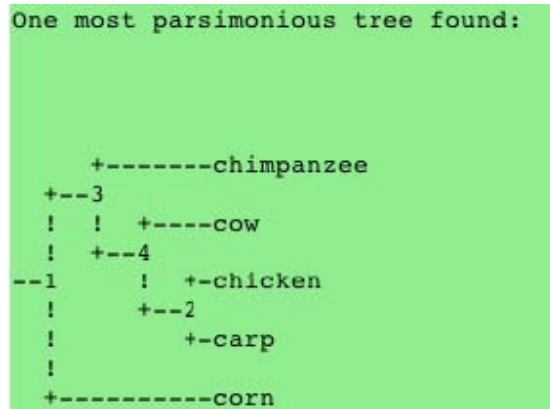
The tree gives the numbers needed to find the approximate date of the last common ancestor of these creatures. You should put approximate dates on your tree using the calculations described on page MolPhyl-21.

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

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

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

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



*** Note that the “!”s don’t mean anything -they are just vertical lines. Also, the numbers (“1” through “4”) also don’t mean anything -they just identify the branch points.

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

So, which is the “right” tree?

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

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

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

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

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