Skulls & Evolution

Objectives
• 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.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Details</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Brain case</td>
<td>size?</td>
<td>The bigger brain case allows a bigger brain which, in general, allows greater intelligence.</td>
</tr>
<tr>
<td></td>
<td>cranial ridge?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>brow ridge?</td>
<td></td>
</tr>
<tr>
<td>2 Teeth</td>
<td>size?</td>
<td>See under “Snout”</td>
</tr>
<tr>
<td></td>
<td>canines - large and sharp or more like incisors?</td>
<td></td>
</tr>
<tr>
<td>3 Palate</td>
<td>U-shaped or rectangular</td>
<td>See under “Snout”</td>
</tr>
<tr>
<td>4 Forehead (compared to face)</td>
<td>size?</td>
<td>Related to size of brain case.</td>
</tr>
<tr>
<td></td>
<td>height?</td>
<td></td>
</tr>
<tr>
<td>5 Location of eye sockets (orbits)</td>
<td>sides/front of skull</td>
<td>Eyes in front allows binocular vision (seeing most objects with both eyes at once) which allows depth perception and 3-d vision.</td>
</tr>
<tr>
<td>6 Snout</td>
<td>present?</td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>length?</td>
<td></td>
</tr>
<tr>
<td>7 Cheekbones (zygomatic bones)</td>
<td>width of face</td>
<td>Wider face correlates with shorter snout.</td>
</tr>
<tr>
<td>8 Foramen magnum (where the backbone attaches)</td>
<td>location - rear or bottom of skull?</td>
<td>Foramen magnum at bottom of skull allows walking erect, as opposed to walking on 4 legs.</td>
</tr>
</tbody>
</table>

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 upper jaw, and 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 to be more ancestral than any of the other samples; the orangutan is the most ancestral, followed by the gorilla, then the chimp.

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

3) To the best of your ability, try to determine when, on the chart on the first page of this lab section, humans first walked upright.
Part II: Comparing skulls of other mammals

4) Each group will be given three skulls, one from a carnivore (exclusively meat-eating: leopard, or cougar), one from an omnivore (eats both meat and plants: raccoon or wolf), 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.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Details</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Canine teeth</td>
<td>• present?</td>
<td>Used for cutting and tearing of food.</td>
</tr>
<tr>
<td></td>
<td>• large or small</td>
<td></td>
</tr>
<tr>
<td>2 Molars</td>
<td>• flat</td>
<td>Used for grinding food.</td>
</tr>
<tr>
<td></td>
<td>• pointed</td>
<td></td>
</tr>
<tr>
<td>3 Eye Sockets (orbits)</td>
<td>• allow for overlapping fields of vision?</td>
<td>Overlapping fields of vision allow for better depth perception; more visual field allows better observation.</td>
</tr>
<tr>
<td></td>
<td>• allow for greater visual field coverage</td>
<td></td>
</tr>
<tr>
<td>4 Masseter muscle attachment points (see next page for description)</td>
<td>• large</td>
<td>Used for moving jaws when grinding food.</td>
</tr>
<tr>
<td></td>
<td>• small</td>
<td></td>
</tr>
<tr>
<td>5 Temporalis muscle attachment points (see next page for description)</td>
<td>• large</td>
<td>Used for moving jaws when biting and tearing food.</td>
</tr>
<tr>
<td></td>
<td>• small</td>
<td></td>
</tr>
</tbody>
</table>

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.

Assignment: Type the answers to the following questions and hand them to your TA at your next lab meeting. Remove the pages of the dolphin pictures, label them and attach them to your questions.

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 Table 1. Describe the trend, not just the individual observations. Please format your answer as a table.

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

Part II: 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.

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.
• Dolphin skull; left side view:
Rear view:

Top (dorsal) view:
Molecular Phylogeny

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

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

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

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

There are two main methods for comparing protein sequences from different organisms in order to determine their phylogenetic relationships:
• **Sequence Divergence** This compares the sequences and counts the number of differences between them. The longer since their common ancestor, the more differences expected. This is the simplest method. You will do this ‘by hand’ to see how it works and then let the computer do the hard work. This method is best for finding approximately how long it has been since two species had a common ancestor. It works fairly well for finding out which creatures are related to which. In studies of cytochrome c from many organisms, it has been found that (very approximately) one amino acid change occurs every 21 million years. The rates of change of other proteins are different.

• **Parsimony** This is a more sophisticated method that also takes into account the particular differences between the sequences. It is described in detail in Campbell, 9th edition, pages 544 – 547 (Campbell, 8th edition, pages 540-550). Although it can not tell you how long ago two organisms had a common ancestor, it is much better at telling which creatures are most closely related to which than the Sequence Divergence method.

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

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

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

Given organisms 1 through 5 with the following classifications:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Kingdom</th>
<th>Phylum</th>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>F</td>
<td>K</td>
<td>U</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>F</td>
<td>K</td>
<td>V</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>F</td>
<td>M</td>
<td>X</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>G</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>5</td>
<td>B</td>
<td>H</td>
<td>O</td>
<td>Z</td>
</tr>
</tbody>
</table>

Thus:

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

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

Notes:
• since all species listed are currently alive, they must line up vertically, like this:
• the only distances that matter are the horizontal ones; vertical positions don’t matter
• since all the species listed are currently alive, the distance from any one to the common ancestor must be the same.
* If given.
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”, start Safari from the Dock.

2) Click on the link to the OLLM and then the link for the “Phylogenetic Tree Constructor”.

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

**Main Tree Organisms**
Select several organisms here to construct the tree.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Plants</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>allanthus silkmoth</td>
<td>buckwheat</td>
<td>chloroplast</td>
</tr>
<tr>
<td>American alligator</td>
<td>corn</td>
<td>green alga</td>
</tr>
<tr>
<td>Arabian camel</td>
<td>calfiflower</td>
<td></td>
</tr>
<tr>
<td>brown garden snail</td>
<td>China jute</td>
<td></td>
</tr>
<tr>
<td>bullfrog</td>
<td>corn</td>
<td></td>
</tr>
<tr>
<td>California gray whale</td>
<td>ginkgo</td>
<td></td>
</tr>
<tr>
<td>carp</td>
<td>hemp</td>
<td></td>
</tr>
<tr>
<td>chicken</td>
<td>lark</td>
<td></td>
</tr>
<tr>
<td>chimpanzee</td>
<td>mung bean</td>
<td></td>
</tr>
<tr>
<td>cow</td>
<td>nasturtium</td>
<td></td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Plants</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>bullfrog</td>
<td>buckwheat</td>
<td>Desulfovibrio vulgaris</td>
</tr>
<tr>
<td>California gray whale</td>
<td>carp</td>
<td>Aspergillus nidulans</td>
</tr>
<tr>
<td>carp</td>
<td>calfiflower</td>
<td>Aspergillus niger</td>
</tr>
<tr>
<td>chicken</td>
<td>China jute</td>
<td>Neurospora crassa</td>
</tr>
</tbody>
</table>

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) 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:

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

![Image of a 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.

![Image of JalView]

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

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

![Image of amino acid sequences]

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

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

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

7) Using this technique, find the number of substitutions between your two closely-related organisms. Save this number for later.
8) Choose a third, more distantly-related organism and find the number of substitutions between it and your two original organisms. This will take two separate runs of the program.

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

• corn vs. cow:

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

• corn vs. donkey:

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

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

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

\[
\begin{array}{c}
\text{cow} \\
\text{22} \\
\text{22 corn}
\end{array}
\]

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:

\[
\begin{array}{c}
\text{donkey} \\
\text{1} \\
\text{X} \\
\text{22} \\
\text{Y} \\
\text{X corn}
\end{array}
\]
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:

\[
\begin{array}{c}
\text{donkey} \\
| \\
\text{cow} \\
| \\
\text{corn}
\end{array}
\]

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:

\[
\begin{array}{c}
\text{donkey} \\
| \\
\text{cow} \\
| \\
\text{corn}
\end{array}
\]

- 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 don’t add up properly. This is what we call “close enough for government work”.

10) Check the tree you made by having the program calculate it for you.
   a) Go back to the “Tree constructor” page.
   b) Select your three creatures and click “Calculate Tree”.
   c) Click “JalView”.
   d) When the window appears,

   e) From JalView’s “Calculate” menu, select “Calculate Average Distance Tree using PID”. Again, be patient. Set the “Font Size” to 12 and check “Show Distances” (these controls are near the bottom of the window). You will get a tree like this:
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 x 112 = 22 (which is close to the 20.5 in my tree)
- the bottom fork = 0.89\% = 0.0089. The number of substitutions would be 0.0089 x 112 = 1 (which exactly matches my tree)

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

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

b) Select the 4 “Main Tree Organisms” as you did previously. Use shift-click to select more than one organism at a time. If you want to select non-adjacent organisms in a list, use 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:
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 this:
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 rough rule of “1 change in 21 million years”.

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: Each student should pass their phylogenetic tree of 5 organisms into their TA. Make sure all group members’ names are on the paper.