

Prerequisites

1. You must have the software program "Mesquite" properly installed and running on your computer. Please see the ***Mesquite Download Instructions*** file on Blackboard for instructions.
2. Download and save the files from Blackboard: ***primate-mtDNA.nex*** . This file contains *orthologous sequences* of the NADH dehydrogenase gene from twelve primate species.

Activity and Instructions

Note: All questions here are for thinking, not for submitting! Even so, it is very important that you understand the answers.

1. Launch Mesquite and open the file ***primate-mtDNA.nex*** by going to "**File → Open File.**"
2. Click on "Show Matrix" on the left-hand side of the Mesquite Window. You will now see a *character matrix* revealed showing DNA sequences for several *orthologs*. Along the left side of this matrix you will see the names of twelve primate species. The numbers along the top represent the 898 positions along this gene. Each cell in the matrix has a color-coded "character state". **Identify the following:**
 - What does each row of the matrix represent?
 - What does each column of the matrix represent?
 - What does each cell in the matrix represent and what are the different letters?
 - How many different *character states* are in the tree? Hint: It's not 4! What do the unexpected state(s) represent?

3. You can see that at some positions (for example, the first three), all the groups share the same state; while at others the states differ between groups. **Do you think the states the following positions will help to determine relationships among these primates? Why or why not? In addition, which of these positions do you think is the most informative?**
 - Position 4
 - Position 5
 - Position 7
 - Position 10
4. Let's make a tree.. the bad way! Click "**Taxa & Trees**" → **New Tree Window** → **With Trees to Edit by Hand**. You should see a default tree with the 12 primate species at the tips of the branches.
5. First, make your life easier by going to "**Display** → **Font Size**" and clicking a much larger font size. Now, you will notice this symmetrical tree was simply arranged in the order in which species are listed in the matrix. This is almost certainly NOT the most parsimonious tree possible.
6. We can find the **tree length** of this tree by clicking "**Analysis:Tree** → **Values For Current Tree...**". In the window that pops up, click "**-Treelength**" (second row). A bright cyan box will appear in the top left and it tells you the Treelength is 1337. **This means a total of 1337 evolutionary changes occurred along branches of this tree.** In other words, if this tree correctly shows relationships among these primates, 1337 mutations had to occur in this gene.
7. We are going to try and turn this tree into a more parsimonious tree by clicking and dragging branches to be on different nodes. After you drag a branch to connect to a different node, the Treelength value will update. Play around with dragging branches and try to get as low a Treelength as you can.
 - During this, you might also wish to formally select an **outgroup**, i.e. the species in the tree most distantly related to everything else. **The outgroup of these species is Ring-Tailed Lemur.** If you wish to set this as the outgroup, click the name of this taxon in tree itself. A gray box will appear around the name Then, go to "**Tree** → **Alter/Transform Tree** → **Root tree with selected taxa as outgroup.**"

8. Once you have gotten tree length as low as you can get it, confer with your lab group and figure out which person has the **lowest Treelength**. That person has the **most parsimonious tree** at the table. **EVERYONE record its tree length and DRAW the tree by hand for future reference!**
9. Given that there are over **315 billion (!)** different ways to draw a tree for 12 species, it is pretty unlikely anyone found the best tree. So, let's ask Mesquite to find the best tree for us, using **treelength** as its optimality criterion. To do this, click: "**Tree → Alter/Transform Tree → Search for Better Tree**". A window will appear. Click the following options:
 - Tree Rearranger: **SPR Rearranger**
 - Statistic to Calculate for Tree: **Treelength**
 - i. This is us specifying optimality criterion!
 - Make sure both boxes are checked in the next pop up screen, and click ok.

Mesquite will now, live!, try to find a better tree that lowers the treelength. You can watch as the treelength goes down until it reaches its lowest value. **What is the treelength of the most parsimonious tree? EVERYONE record its tree length and DRAW the tree by hand for future reference.**

10. Now, by hand, make one rearrangement to the tree: Move "Gorilla" so that gorilla and chimpanzee are **sister taxa**. How did this change the tree length? Given what you have observed, can you say with confidence who human's closest primate relative is? **With your group**, brainstorm different (hypothetical) ways you could answer this question with more confidence.
11. **Do not close the tree window**, but click back for a moment to the "Character Matrix" window. Each of you will be assigned a COLUMN in this matrix. You may wish to write down the values of this column for convenience. Using your two drawn trees (your group's most parsimonious tree and Mesquite's most parsimonious tree), **map the character changes onto each tree for your column.**
12. Mesquite should still have open the most parsimonious tree. In the tree window, go to "**Analysis:Tree → Trace Character History → Parsimony Ancestral States**". A window will appear in the bottom right showing how a given column (it will open with character 1) in the character matrix has evolved, under "Trace

Character." There is a small phrase written in the box ["Steps: 0"], which tells us the treelength for THIS COLUMN only, i.e. how many changes occurred at this column. For column 1, all taxa have the nucleotide "A" so there was no change, hence 0 steps.

13. You can tell Mesquite which column you want to trace by going to the **Trace** menu: "**Trace → Choose Character**". Scroll to find your column, and Mesquite will reveal the most parsimonious explanation for how this column evolved. Compare your results with Mesquite's - did you get it right? Understand how to reproduce Mesquite's results as needed.

Pictures!

Gorilla gorilla (gorilla)



Pan troglodytes (chimpanzee)





orangutan

Gibbon



crab-eating macaque



Japanese macaque



rhesus macaque



barbary macaque



squirrel monkey



tarsier



ring-tailed lemur

