## Using the Sequence Server at the CSHL DNA Learning Center

Objectives:
You should be able to view your class data in the Cold Spring Harbor Laboratory Sequence Server database.
You should be able to perform pair-wise sequence alignments between diverse modern humans.
You should be able to perform pair-wise sequence alignments between diverse modern humans and Neandertals.
You should be able to set the "molecular clock" based on the number of sequence differences between modern humans.
You should be able to use a "molecular clock" to estimate when Neanderthals and modern humans diverged.

The DNA Learning Center at Cold Spring Harbor Laboratory has developed a number of **bioinformatics** tools for student use. Bioinformatics tools are computer programs used to help scientists make sense of biological data and solve biological problems. You will be using the Sequence Server for three different activities to help you learn more about the origins of our species.

In the following exercise, you will compare DNA sequence between individuals from several different population groups. You will first compare sequences between modern humans. This information will be used to set a "molecular clock" (described on page 19). You will then compare modern humans to Neandertals to see if Neandertals might have contributed to our gene pool. The molecular clock you derive will be used to determine when modern humans and Neandertals diverged. In your final comparison, you will align modern human sequences to that of a chimpanzee to derive a new molecular clock. The molecular clocks will be used to estimate when modern humans first appeared.

### Using the Sequence Server to Align Mitochondrial DNA Sequences

Your class data has been entered into the Sequence Server database at the Dolan DNA Learning Center at the Cold Spring Harbor Laboratory. Use the following steps to access and utilize that data.

| **Mitochondrial DNA Sequence Comparisons** | |
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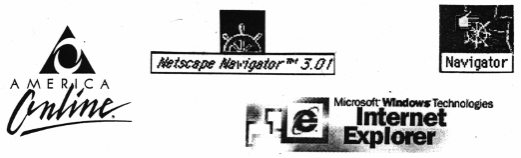
| 1. Click on the icon for your Internet Service Provider to gain access to the internet. (This might be America Online, Netscape Navigator, Microsoft Internet Explorer, etc.) |   |
| 2. In the internet address box, type in the following URL: http://vector.cshl.org and press the Enter (or Return) key on the keyboard. The DNA Learning Center main page will be brought up. |   |

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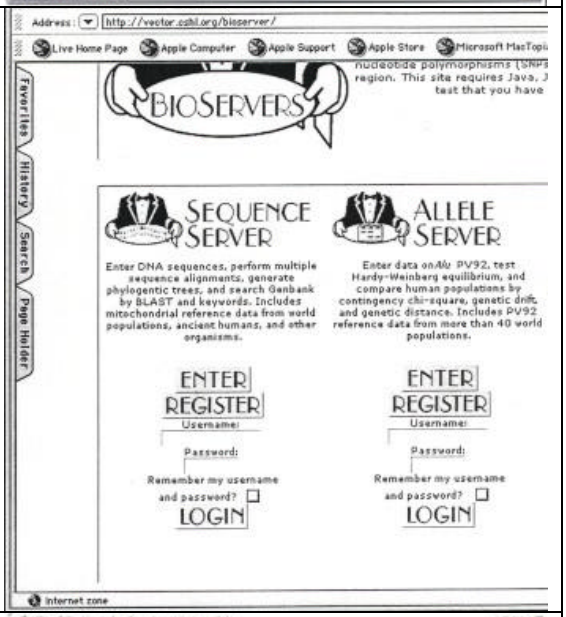
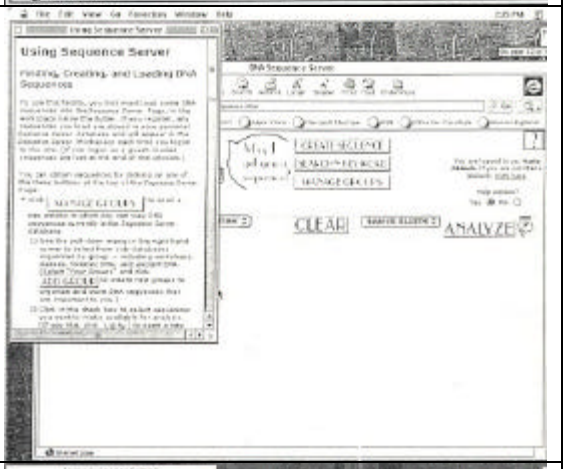
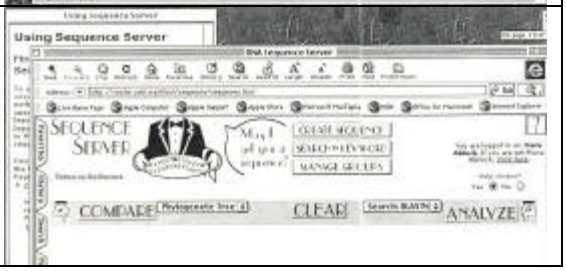
| 3. In the DNA Learning Center main page, click on the "Bioservers" image towards the bottom right of the page. |  |
| 4. Click on the "REGISTER" button if you have not previously registered with Bioservers. Fill out the required information and then hit "SUBMIT." If you are already registered, enter your username and password, then press "LOGIN." |  |
| 5. The "Using Sequence Server" instruction window will appear on top of the Sequence Server Workspace. The instructions contained in this box can be used if you need more information about using this site. Click on the Sequence Server Workspace to bring it forward on the desktop. |  |
| 6. Click on the "MANAGE GROUPS" box. This is where you can identify groups of data to add to your workspace. |  |

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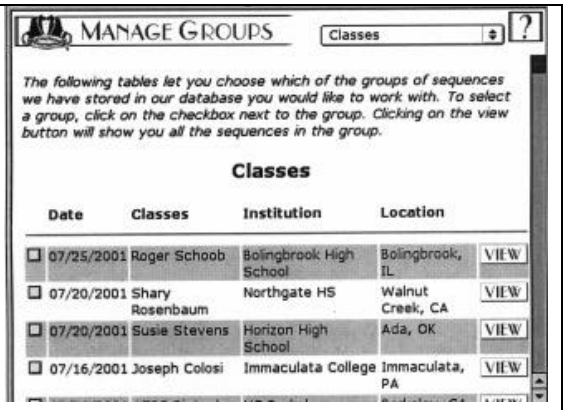
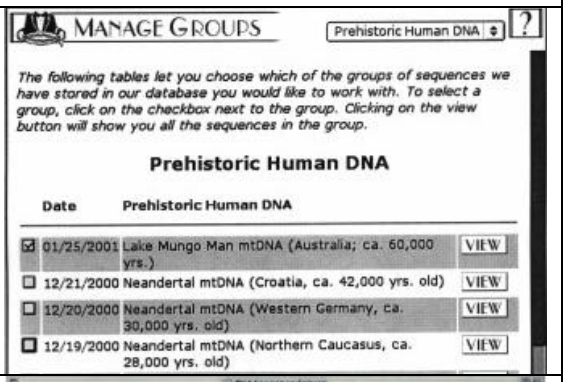
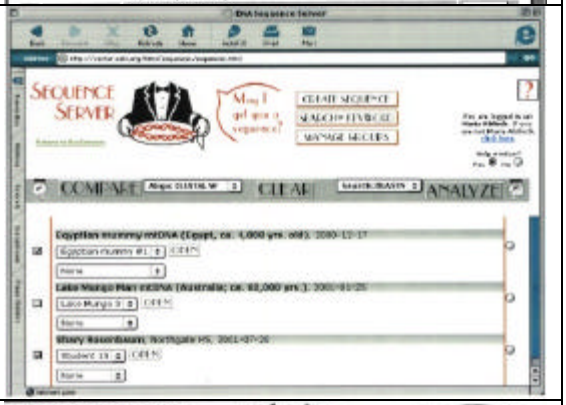
| 7. In the upper right hand corner of the Manage Groups window is a scroll menu. If it isn't already showing, select "Classes." This will bring up a list of classes from across the country that have sequence data stored in the Sequence Server database. Use the scroll bars on the right side of the window, if needed, to locate your class. When you have located your class, click the box to its left to select it.<br><br>**Note:** The entire window must load before attempting to change the selection. |  |
| 8. Select "Prehistoric Human DNA" from the Manage Groups window. Click the box to the left of one or more of the following: "Lake Mungo Man," "Otzi, the Iceman," "Egyptian Mummy," "Yixi mtDNA," or "Cahokian mtDNA." Press "OK" when finished. |  |
| 9. Your class data and several prehistoric humans' data should now be added to your workspace. Select your sequence by using the scroll menu below your class name. Deselect all the check boxes on the left except for your sample and one prehistoric human of your choice. |  |
| 10. Next to the "COMPARE" button below the Sequence Server icon, use the arrows to scroll to "Align:CLUSTAL W" then click the "COMPARE" button. |  |

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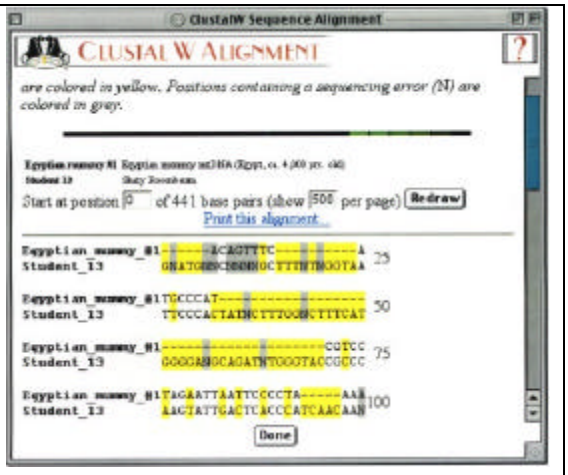
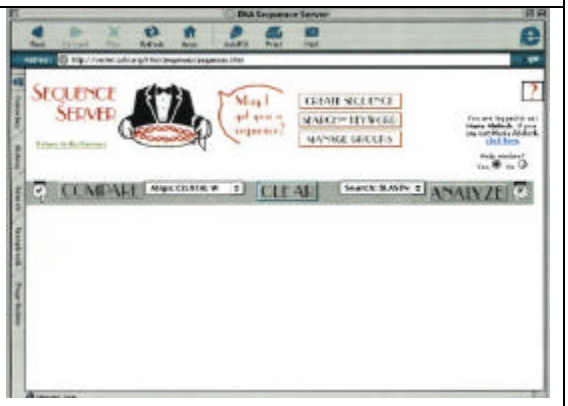
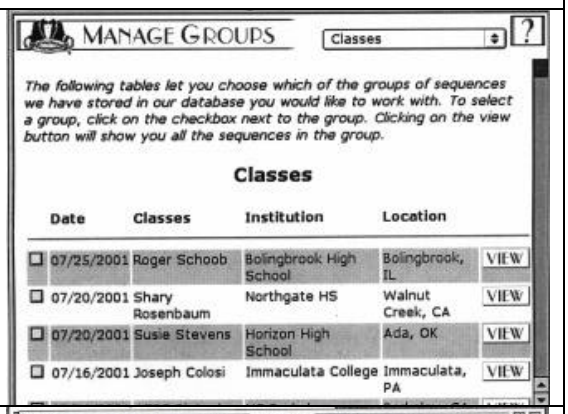
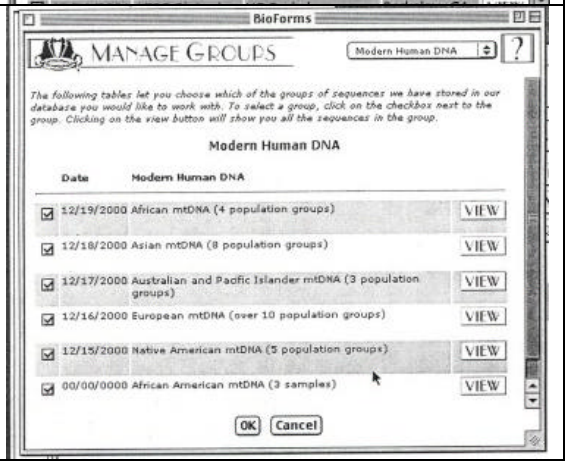
| 11. Your sequence and the prehistoric human sequence you chose should align where bases are complementary. You may notice some yellow highlight regions, dashes and gray highlight regions with "N's." What do you think these indicate?<br><br>***Note to teachers:***<br>*yellow = mismatch*<br>*gray = "N" or unknown base*<br>*dashes = gaps due to insertions, deletions or sequencing errors* |  |
| 12. When you have examined the alignment to your satisfaction, press the "CLEAR" button to clear your workspace. Next, you will work with various diverse modern humans, Neandertals, chimpanzee, and your classmates' data to investigate your genetic origins. The following steps will guide you through the procedure to add members of these groups to your workspace. |  |
| 13. In the Manage Groups window, use the scroll menu to locate your class. Select it by checking its box. |  |
| 14. Using the scroll menu again in the upper right corner of the Manage Groups window, locate and select "Modern Human DNA." Check all boxes in this Modern Human DNA window. |  |

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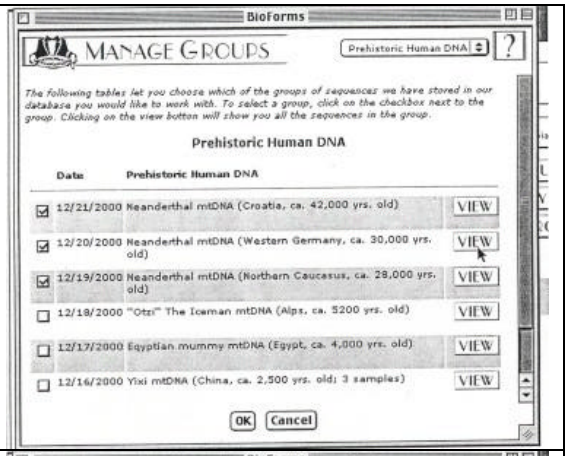
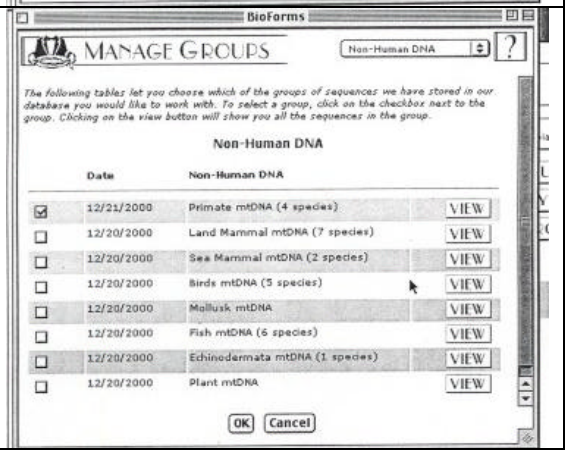
| 15. Locate and select the “Prehistoric Human DNA” category from the Manage Groups window. Place check marks in the boxes to the left of the Neanderthal mtDNA sequences. |  |
| 16. Select the “Non-Human DNA” category from the Manage Groups window. Click the box to the left of “Primate mtDNA (4 species).” Click on the “OK” button at the bottom of the window. This will place all selected DNA sequences onto the Sequence Server Workspace. |  |

**Sequence Comparisons**

You will perform a series of sequence alignments that will allow you to estimate a mutation rate and to calculate the timing of crucial events in human evolution.
Use the following guidelines for each comparison.

- Identify a region spanning 200 bases where there is good alignment between the two sequences you are comparing. This region should contain few, if any, "N's."
- Excluding N's (in gray) and dashes that may occur at the beginning or end of the alignment, count how many yellow-highlighted base positions are found in the alignment. If you find a run of three or more dashes in a row, count such a run as a single nucleotide difference.

***Note to teachers:*** *This is based on the assumption that it is more likely that a single event, rather than multiple, independent events, will lead to the insertion/deletion of 3 or more bases at a particular site.*

- If you find a sequence that does not align for 200 bases, use a different sequence.

Follow the steps below to fill in the spaces in the chart that follows.

1. **Modern Human vs. Modern Human**
   a. Select any two modern humans from the groups on your workspace. Fill in the identifying information in the table.
   b. Compare these two individuals by ClustalW alignment. Count the number of mismatches, or SNPs, and record this number in the table.
   c. Repeat steps "a" and "b" using different modern humans.
   d. Now compare two students in the class and fill in all the appropriate information in the table.
   e. Calculate the average number of SNPs for this group and record in the table.
2. **Modern Human vs. Neandertal**
   a. Select any African modern human and any Neandertal to compare by ClustalW alignment. Fill in the identifying information in the table.
   b. Compare these two individuals and record the number of SNPs in the table.
   c. Repeat steps "a" and "b" with any Asian modern human and any Neandertal.
   d. Repeat steps "a" and "b" again using any European modern human and any Neandertal.
   e. Now compare your (or another student's) sequence with any Neandertal and record all appropriate information in the table.
   f. Calculate the average number of SNPs for this group and record in the table.
3. **Neandertal vs. Neandertal**
   a. Select Neandertal #1 and Neandertal #2 to compare by ClustalW alignment. Record the number of SNPs in the table.
   b. Do the same with the other two combinations of Neandertals.
   c. Calculate the average number of SNPs for this group and record in the table.
4. **Modern Human vs. Chimpanzee**
   a. Select any modern human to compare with Chimp #2. Fill in the identifying information in the table.
   b. Compare these two sequences by ClustalW alignment. Count the number of SNPs and record this number in the table.
   c. Repeat steps "a" and "b" using different modern humans.
   d. Now compare your (or another student's) sequence with Chimp #2 and record the number of SNPs along with the appropriate identifying information.
   e. Calculate the average number of SNPs for this group.

## Student Data

| Sequence Server Clustal W Alignments: SNPs | | | |
|---|---|---|---|
| **Modern Human vs. Modern Human** | **Number of SNPs** | **Your Average** | **Class Average** |
| _____ vs. _____ | | | *~6* |
| _____ vs. _____ | | | |
| _____ vs. _____ | | | |
| Student _____ vs. Student _____ | | | |
| **Modern Human vs. Neandertal** | | | |
| African _____ vs. Neandertal # _____ | *~18* | | *~18* |
| Asian _____ vs. Neandertal # _____ | *~18* | | |
| European _____ vs. Neandertal # _____ | *~19* | | |
| Student _____ vs. Neandertal #____ | | | |
| **Neandertal vs. Neandertal** | | | |
| Neandertal #1 vs. Neandertal #2 | *~7* | | *~5* |
| Neandertal #1 vs. Neandertal #3 | *~4* | | |
| Neandertal #2 vs. Neandertal #3 | *~4* | | |
| **Modern Human vs. Chimpanzee** | | | |
| _____vs. Chimp #2 | | | *~42* |
| _____vs. Chimp #2 | | | |
| _____vs. Chimp #2 | | | |
| Student _____ vs. Chimp #2 | | | |

***Note to teachers:*** *Since there are so many possible combinations to choose from when making these comparisons, the values you obtain in your class may be different than those given in this table. This can bring up a good discussion topic: How might these numbers vary depending on the samples (and groups) used for comparison? You may notice how small changes in the numbers can make a huge difference in the values calculated for the evolution divergence points.*

***Note to teachers:*** *After students complete the ClustalW alignments and calculate their averages, they will need to calculate the class averages before proceeding with the following questions.*

**1. Calculating a Molecular Clock**

Archaeologists use a number of different techniques to estimate the age of fossils. These include radiocarbon dating, measuring changes in carbonates and tooth enamel brought about by exposure to radiation over time, and determining the age of the geological strata in which the fossil was found. By dating human fossils discovered in Africa, scientists estimate that modern humans first appeared approximately 150,000 years ago. Using this value and the class average number of differences for "Modern Humans vs. Modern Humans," derive a **molecular clock**, or mutation rate, in years/mutation. Use the formula below.

$$\frac{150{,}000 \ \ years}{6 \ \ mutations} = \underline{25{,}000} \ \ years / mutation$$

**2. Did Modern Humans Evolve from Neanderthals?**

Neandertal fossils have been discovered in Europe and the Middle East. Dating the fossils by radiocarbon decay suggests that Neandertals inhabited the European continent as recently as 28,000 years ago. Estimates of when Neandertal first appeared in Europe are far less precise but many scientists believe it may have been as long as 300,000 years ago. Although they are frequently depicted as stocky and brutish individuals, Neandertals cared for their sick and injured, fashioned stone tools, used fire, lived and hunted in social units, and ritually buried their dead.

As far as we know, Neandertals did not inhabit regions far outside the European continent. If modern Europeans descended from Neandertals, you would expect that Neandertals would be more closely related to modern European populations than to any other modern human population in the world. Based on your "Modern Human vs. Neandertal" data, does it appear as though Europeans or any other modern world population descended from the Neandertals? Explain.

*No. The number of differences between Neandertals and Modern Europeans is not significantly less than the number between Neandertals and any other modern human population.*
***Note to teachers:*** *Students may want to discuss what would be a significant difference.*

**3. Human - Neandertal Divergence**
How many years ago did the common ancestor of modern humans and Neandertals live? In the equation below, use the average number of differences (mutations) you found between modern humans and Neandertals and your calculated mutation rate (page 20) to estimate this number.

$$18 \ \textit{mutations} \times \frac{25{,}000 \ \textit{years}}{\textit{mutation}} = 450{,}000 \ \textit{years}$$

**4. Did Neandertals Contribute to the Modern Human mtDNA Gene Pool?**
A **gene pool** is the collection of all genes in a population. Members of a single gene pool would be expected to have fewer differences between them than would be expected between members of different gene pools. Did Neandertals have a separate gene pool from that of modern humans? Could Neandertals have contributed their mitochondrial DNA to the gene pool of modern humans? Use the comparisons below (4a through 4e) to answer this question.

**a.** Average difference between Neandertals = *~5*

**b.** Average difference between modern humans and Neandertal = *~18*

**c.** Average difference between modern humans = *~6*

**d.** The *closest* modern human/Neandertal alignment discovered by your class showed *~14* differences.

**e.** The two most divergent modern humans discovered by your class showed ___*~11*____ differences.

**f.** Do you think the Neandertals used in this study are members of a single gene pool (assume that modern humans are of a single gene pool)? Explain.

*If Neandertals passed on their mitochondrial DNA to modern humans, you might expect that the average number of SNPs between "Modern Human vs. Modern Human" and "Modern Human vs. Neandertal" would be similar. If Neandertals had their own gene pool, you would expect that the average number of SNPs between "Neandertal vs. Neandertal" and "Modern Humans vs. Neandertals" would be different. Neandertals, therefore, are probably of a single gene pool since the average difference between Neandertals is similar to the average difference between modern humans and modern humans are considered to be of a single gene pool. However, we are only looking at a very small sample size so it is dangerous to draw a strong conclusion.*

***Note to teachers:*** *Your students may argue the definition of a gene pool. If there are barriers (lakes, mountains, etc.) between two populations, would they be in the same gene pool?*

**g.** Do you think Neandertals contributed their mitochondrial DNA to the modern human mtDNA gene pool? What other data would you want to answer this question?

*It was shown in Problem 2 that modern humans most likely did not evolve from Neandertals. Furthermore, the difference between any two modern humans is not as great as that between Neandertal and any modern human. To appropriately address this question, however, it would be useful to look at the particular polymorphisms. If modern humans share some of the same mutations with Neandertals, then perhaps Neandertals did contribute their mtDNA to the modern human gene pool? Even if Neandertals did not contribute their mitochondrial DNA to modern humans, it does not mean that they did not contribute their nuclear genomic DNA (i.e., the result of a mating between a male Neandertal and a modern human female).*

**5. A Molecular Clock Based on Chimpanzee/Hominid Divergence**

Based on the fossil record, scientists believe that chimpanzees and modern humans may have diverged 5,000,000 years ago.

**a.** Would the molecular clock be different if you used the time since chimpanzees and modern humans evolved to determine the mutation rate? Calculate a new mutation rate using the formula below and the 5 million year divergence estimate.

$$\frac{5{,}000{,}000 \ \ years}{\underline{42} \ \ mutations} = \underline{119{,}000} \ \ years/mutation$$

**b.** Is this value different than the one you calculated based on "Modern Human vs. Modern Human" differences? Explain.

*Yes. 25,000 years/mutation vs. 119,000 years/mutation*

*The number of SNPs for the "Modern Human vs. Chimpanzee" comparison might be an underestimate; there may be more differences in actuality for the following reasons:*

- *Back mutations would not be noticed.*
- *Multiple mutations at one base position would be counted as only one change.*

*In addition, one of the estimates of species divergence is incorrect.*

**c.** Using the mutation rate you calculated in 5a, when did "Mitochondrial Eve," the mitochondrial ancestor of all modern humans, live? Use the formula below for this calculation.

$$\underline{6}\ \textit{mutations} \times \frac{\underline{119{,}000}\ \textit{years}}{\textit{mutation}} = \underline{714{,}000}\ \textit{years}$$

How does this estimate compare with the value you used to calculate a molecular clock in Problem 1 (pg 20)?

*In Problem 1, it was stated that modern humans arose 150,000 years ago. The value calculated here may range from 3 to 7 times greater than 150,000 (depending on your particular class' data).*

**d.** Using the same molecular clock (calculated in 5a, pg 22), when did Neandertals and modern humans diverge and how does this estimate compare with the value you calculated in Problem 3?

$$\underline{18}\ \textit{mutations} \times \frac{\underline{119{,}000}\ \textit{years}}{\textit{mutation}} = \underline{2{,}142{,}000}\ \textit{years}$$

*In Problem 3, a value 3 to 5 times less than this would be calculated (depending on your particular class' data).*

**e.** How many mutations would you need between chimpanzee and modern humans to give the mutation rate you calculated in Problem 1 (pg 20)? Use the equation below for your calculation.

$$\frac{5{,}000{,}000 \ \ years}{x \ \ mutations} = \underline{25{,}000} \ \ years / mutation$$

$$x = 200 \ \ mutations$$

How does this number compare with the average number of SNPs your class found for the "Modern Human vs. Chimpanzee" comparisons and how can you account for any discrepancy?

*Your class probably found close to 50 SNPs when making these comparisons. The number of SNPs could be underestimated for reasons outlined in 5b.*

**f.** Which mutation rate might be more accurate, that derived from the modern human/modern human comparisons or that derived from the chimpanzee/modern human comparisons? Explain.

*The mutation rate derived from modern human/modern human comparisons is probably closer to the actual rate. There are other lines of evidence (archeological, geological, etc.) that support the 150,000 year ago divergence. The longer ago an event occurred, the more difficult it is to estimate that point in time when it actually occurred. The small chimpanzee sample size is also a problem when trying to make an estimate of mutation rate.*

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