

Human Race(s)

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What exactly is “race”? Is race best defined as a social, a geographical, or a biological concept? Is race strictly a cultural characteristic? How should we define race or distinguish one racial group from another? More importantly, *should we* distinguish people by race? Students begin this experiment by calculating the genetic diversity within and between three subspecies of chimpanzees to demonstrate how DNA sequences can be used to identify the relationships between organisms. The hypothesis that human races are biologically different from each other is evaluated using mitochondrial DNA to determine the genetic relatedness between people of different geographic origins. Phylogenetic trees are used to visualize the relationships first among subspecies of chimpanzees and then among humans from different regions of the World.

Keywords: bioinformatics, human race, phylogeny

Introduction

This lab exercise was developed to fill a curricular need for an experiment on biodiversity. It was originally designed for Human Biology, a non-majors introductory biology course that focuses on the interactions between humans and society. While there were biodiversity lab exercises available utilizing any number of different organisms, it seemed most appropriate in this course to consider diversity among humans.

The lab exercise begins with mitochondrial DNA comparisons of three different chimpanzee subspecies. This provides students with instruction on how to use the online sequence comparison software (www.bioservers.org) to compare mtDNA sequences. More importantly, the chimpanzee comparisons demonstrate the degree of mtDNA sequence similarity present in individuals known to be of different subspecies. The principal question when determining the genetic relatedness between humans of different geographic origin is whether different races of humans constitute biological subspecies. Therefore, the analysis of chimpanzee subspecies provides an important contrast.

This experiment is derived from classroom activities developed by the Dolan DNA Learning Center at Cold Spring Harbor Laboratory (<https://www.dnalc.org/>) and utilizes their bioinformatics tools (www.bioservers.org) for the mtDNA sequence comparisons and the construction of a phylogenetic tree. The online resources available at the Dolan DNA Learning Center are extensive and this lab exercise could easily be

modified or expanded to include analyses of other modern humans, of ancient humans, and even of *H. neanderthalensis*.

In addition to the Human Biology non-majors course, I have also used this lab experiment in a first-year introductory biology course that focuses on evolution, ecology, and natural history. In this course it also serves to cover the topic of biodiversity, introduces students to the use of bioinformatics, and reinforces the use of phylogenetic analysis to evaluate genetic relatedness. In both courses the student response to this experiment has been positive. The proportion of students rating this lab exercise as “excellent” or “very good” ranges from 49 – 74%.

Student Outline

Objectives

- Use bioinformatics tools
- Evaluate DNA sequence differences among subspecies and among individuals
- Visualize relationships using phylogenetic trees

Introduction

Race is a significant topic in the news lately. Many cities throughout the United States are struggling with issues arising from different racial groups living in their communities. But what exactly is “race”? Is race best defined as a social, a geographical, or a biological concept? Is race strictly a cultural characteristic? How should we define race or distinguish one racial group from another? More importantly, *should we* distinguish people by race? The idea that human races are biologically different from each other can be investigated by examining the genetic relatedness between people. The first step is to understand how DNA can be used to see the relationships between organisms.

Data Analysis to Determine Relatedness

As you already know, the DNA sequence contained in the genes of an organism determines the identity of that organism. Not only is the DNA sequence of a dog different from that of a mosquito or a geranium, but DNA sequences are so specific to the individual that we use the sequences to distinguish one individual from another. This is the basis of such DNA analyses as paternity testing and crime scene investigation techniques to determine who committed the crime. When studying evolutionary relationship, biologists compare DNA sequences between two species to determine how closely related they are. The more DNA sequence similarity between the species, the more closely related they are. There are some DNA sequences that are especially useful for determining the relatedness between species and individuals. One example is mitochondrial DNA.

Within all eukaryotic cells are structures called **mitochondria**. Mitochondria are the organelles within the cell in which sugar is broken down to release energy for the cell. Mitochondria contain a small amount of DNA referred to as **mitochondrial DNA** or **mtDNA**. There are a few reasons why mtDNA is especially good for determining the relationships between species or individuals. First, unlike chromosomal DNA that is inherited from both parents, mtDNA is inherited only from the mother. A human egg cell contains over 10,000 mitochondria (Edvotek, 2013) while a human sperm cell contains very few. During fertilization, the few mitochondria from the sperm cell are destroyed, leaving all of the new zygote’s mtDNA to be inherited from the mother. This allows researchers to trace these mtDNA sequences through the maternal inheritance of the individual.

Another feature of mtDNA that facilitates the comparison of different sequences is that mtDNA has a higher mutation rate than chromosomal DNA. A **mutation** is a change in the DNA sequence. The **mutation rate** indicates how frequently mutations are occurring in the DNA. A higher mutation rate in mtDNA means there are a sufficient number of differences in in mtDNA that can be compared between individuals. Over time, mutations accumulate as mtDNA is passed from one generation to the next, leaving a genetic "footprint" of one's mother's mother's mother's mother, etc. These accumulated mutations can be seen as mtDNA sequence differences in people around the world.

In order for populations to evolve into distinct **subspecies** (the taxonomic category that corresponds to races), they must be isolated from one another long enough for sufficient DNA mutations to accumulate in one population to differentiate them from other populations. One way to find out is to examine sequence differences that have accumulated in our mitochondrial DNA (mtDNA). Scientists approximate the evolutionary time back to the "common ancestor" of two different mtDNA sequences by assuming an average rate of mutation over the millennia. In this way, some scientists have calculated that all extant mtDNA sequences can trace their ancestry to a single woman in East Africa approximately 180,000 years ago. She is referred to as the Most Recent Common Maternal Ancestor - or simply, Mitochondrial Eve. (That's not to say that all humans descended from this Mitochondrial Eve; just that hers is the only mitochondrial line to survive.)

Relatedness of Chimpanzee Subspecies

In this first part of the lab exercise you will analyze mtDNA sequences from three subspecies of chimpanzees. The goal is to gain a better understanding of what genetic "race" actually is by measuring the genetic diversity within and between known subspecies. Comparisons of mtDNA sequences will be used as evidence for or against the idea that different chimpanzee

racess (subspecies) are genetically distinct. After analyzing chimpanzee genetic diversity, you will use this information to explore race as a genetic concept within human populations.

The three chimpanzee subspecies to be analyzed are *Pan troglodytes troglodytes* (Central Common Chimpanzee), *Pan troglodytes verus* (Western Common Chimpanzee) and *Pan troglodytes schweinfurthii* (Eastern Common Chimpanzee). The scientific names of these chimps include the name of their genus (*Pan*), their species (*troglodytes*), and a designation of their subspecies (either *verus*, *schweinfurthii*, or *troglodytes*).

Subspecies arise when different populations of a species are isolated from one another for a long period of evolutionary time. Over generations, populations naturally accumulate their own set of DNA mutations. When no interbreeding occurs between two animal populations, they can eventually become so genetically different from one another that they evolve into distinct subspecies or races. Further isolation and differentiation can lead to a new species altogether. If chimpanzee populations have evolved into distinct subspecies, there would be more genetic diversity **between** two subspecies of chimpanzees than **within** a single subspecies of chimpanzees. In other words, one would expect to find more mtDNA sequence differences between Western Chimpanzees and Eastern Chimpanzees than between two Eastern Chimpanzees or between two Western Chimpanzees. On the other hand, if chimpanzee populations have not been isolated long enough to evolve into distinct subspecies, there would be as much, if not more, genetic diversity within a so-called subspecies as between two separate subspecies. In other words, one would expect to find just as many mtDNA sequence differences between two random Western Chimpanzees as between a Western Chimpanzee and an Eastern Chimpanzee.

To determine the relatedness among chimpanzee subspecies you will make comparisons between chimpanzees within the same subspecies and between chimpanzees of different subspecies.

Method

Open a computer and follow the instructions to determine the percent of sequence differences in the mtDNA of three different pairs of individuals from the chimpanzee subspecies.

1. Navigate to the Sequence Server Web site located at <http://www.bioservers.org>
2. Under the section called Sequence Servers, click on the ENTER button to login as a guest. This will give you access to the server. In addition, a small window containing instructions for using the Sequence Server will appear. You can close, or at least minimize, this window.
3. Click "Manage Groups" near the top of the screen. This will open a new window with folders of mtDNA sequences from high school classes around the country. You may want to enlarge this window to view the list of DNA sequence groups better.
4. Click on the drop-down menu labeled "Sequence Sources." You should see other types of DNA sequence groups in the pull-down menu.
5. Choose "Public" on the list. This will pull up a list of groups.
6. Scroll down until you find "Race - chimps". Keep scrolling – it's a long way down the list. To select this group of mtDNA sequences, check the box to the left of the group, and click the "OK" button at the bottom of the window. This will open a new window and will bring sequences from this group into the new window.
7. There are a total of 14 mtDNA sequence files from different chimpanzees. In the drop-down menu is the first mtDNA sequence file called "chimp 10s." (The letter "s" refers to *schweinfurthii*, the Eastern Common Chimpanzee. Chimpanzee samples ending in "v" refer to the "verus" subspecies of Chimpanzee, and sequences ending in "t" refer to the "troglodytes" subspecies of Chimpanzee.) Notice that the program automatically checks the box to the left of the sequence file. The box must be checked to select this sequence for comparison.
8. After you check the box for the DNA sequence file chimp10s, a second drop-down menu will appear underneath. Scroll down and choose a different chimp of the *schweinfurthii* subspecies. Notice that the computer automatically checks the box to the left. This box must be checked to select this sequence for comparison.
9. Compare the two sequences by clicking the "Compare" button in the upper left corner of the page. The total number of DNA nucleotides used in the comparison is shown above the aligned sequences ("Showing #). Differences between the sequences will appear highlighted in yellow. **Calculate the Percent Difference (%) by dividing the number of differences highlighted in yellow by the total number of DNA nucleotides used in the comparison.** Complete 3 trials for each comparison listed in Table 1, making sure to use different individuals in each comparison.

Results

Record your data in Table 1 and **calculate the Average Percent Difference using the data from the 3 trials.**

Table 1. Comparison of mitochondrial DNA sequences of chimpanzee subspecies.

Comparison Group	Percent Differences (%)			
	Trial 1	Trial 2	Trial 3	Average
schweinfurthii mtDNA & schweinfurthii mtDNA				
verus mtDNA & verus mtDNA				
troglydytes mtDNA & troglydytes mtDNA				
schweinfurthii mtDNA & verus mtDNA				
schweinfurthii mtDNA & troglydytes mtDNA				
verus mtDNA & troglydytes mtDNA				

Consider all of the mtDNA sequence comparisons of these groups of chimpanzees as you answer the following questions.

1. Are there more genetic differences within the subspecies or between the subspecies of chimpanzees? Use examples from the data in Table 1 to explain your answer.
2. Do the results from the mtDNA comparisons support the idea that different subspecies (or different races) are genetically distinct? Use the data from Table 1 to explain your answer.

One way to visualize the evolutionary relatedness between different organisms is with the use of a **phylogenetic tree**. This analysis arranges the sequences so you can view the genetic relationships. The closer two sequences are to each other, the closer they will appear on the tree. The Sequence Server website can compare multiple mtDNA sequences at one time to explore the genetic relationship of populations in the form of a phylogenetic tree. Instead of performing CLUSTALW sequence alignments, pull the tool bar down next to the word CLUSTALW. You should see an option for 'phylogenetic tree.'

1. After selecting a group of sequences to work with (see instructions above for bringing the "Race-chimps" group into your workspace), set the sequence comparison tool to the phylogenetic tree function. Select any 3 schweinfurthii mtDNA sequences, any 3 verus mtDNA sequences, and the 3 troglydytes mtDNA sequences.
2. With all nine chosen, click the "Compare" button. It will take a few seconds to perform the multiple sequence analysis. Do not use more than nine sequences.

Use the space below to draw the cladogram showing the relationships between these groups of chimpanzees.

Figure 1. Cladogram of three chimpanzee subspecies.

Comparison of Human Races

We can explore our species' shared ancestry - and what it tells us about the concept of "race" - by deciphering the DNA sequences of people from around the world. Are the different "races" of humans genetically distinct? Or is there just as much genetic diversity within so-called races as there is between them? To investigate this, let's begin with the following hypothesis:

Hypothesis: Within the species *Homo sapiens* there are multiple races, or subspecies, that are genetically distinct from one another.

To test this hypothesis you will compare mtDNA sequences from multiple people of different origins: European, Asian, and African. You will calculate the percent of sequence difference both within each group and between each group of people. Before beginning this comparison, assume that the hypothesis is correct, and predict the results of the data analysis.

Predicted Results:

Method

1. Navigate to the Sequence Server Web site located at <http://www.bioservers.org>
2. Under the sections called Sequence Servers, click on the ENTER button to login as a guest. This will give you access to the server. In addition, a small window containing instructions for using the Sequence Server will appear. You can close or at least minimize this window.
3. Click "Manage Groups" near the top of the screen. This will open a new window with folders of mtDNA sequences from high school classes around the country. You may want to enlarge this window to view the list of DNA sequence groups better.
4. Click on the drop-down menu labeled "Sequence Sources." You should see other types of DNA sequence groups in the pull-down menu.
5. Choose "Public" on the list. This will pull up a list of groups.
6. Scroll down until you find "Race - lesson 2". Keep scrolling – it's a long way down the list. To select this group of mtDNA base-pair sequences, check the box to the left of the group, and click the "OK" button at the bottom of the window. This will open a new window and will bring sequences from this group into your workspace in the main window.
7. By clicking on the tool bar, you should see 9 mtDNA sequence files from different parts of the world. At the top of the list is the mtDNA sequence file called "Africa #1." Notice that the computer automatically checks the box to the left of the sequence file. The box must be checked to select this sequence for comparison.
8. After choosing your first sequence, a second pull-down menu will appear underneath. Scroll down until you see a sequence file called "Africa #2." Click on it to choose it. Notice that the computer automatically checks the box to the left. This box must be checked to select this sequence for comparison.
9. Compare the two sequences by clicking the "Compare" button in the upper left corner of the page. The total number of DNA nucleotides used in the comparison is shown above the aligned sequences ("Showing #). Differences between the sequences will appear highlighted in yellow. **Calculate the Percent Difference (%) by dividing the number of differences highlighted in yellow by the total number of DNA nucleotides used in the comparison.** Complete 3 trials for each comparison listed in Table 2, making sure to use different individuals in each comparison.

Results

Record your data in Table 2 and calculate the Average Percent Difference using the data from the 3 trials.

Table 2. Comparison of mitochondrial DNA sequences from humans of different origin.

Comparison Group	Percent Differences (%)			
	Trial 1	Trial 2	Trial 3	Average
African mtDNA & African mtDNA				
European mtDNA & European mtDNA				
Asian mtDNA & Asian mtDNA				
African mtDNA & European mtDNA				
African mtDNA & Asian mtDNA				
European mtDNA & Asian mtDNA				

Use the Sequence Server program to construct a phylogenetic tree representing the sequence comparisons of all nine mtDNA sequences of *Homo sapiens*. Draw the cladogram in the space below.

Figure 2. Cladogram of *Homo sapiens* mtDNA comparisons

Use the mtDNA sequence comparisons (Table 2) and the phylogenetic tree (Figure 2) of the different groups of *Homo sapiens* to answer the following questions:

3. Consider the cladogram you drew in Figure 2 to answer this question. *Then provide a written explanation for your answer.* The person called “Europe_3” is

- most closely related to “Europe_2”
- more closely related to “Africa_3” than it is to “Europe_2”
- equally related to “Asia_2” and “Europe_1”
- equally related to “Asia_1” and “Asia_3”

4. Of the 9 individuals used in this analysis, which 2 are most closely related to each other? What is the next most closely related pair of individuals? What evidence do you have from both Table 2 and Figure 2 to explain your answer?

5. Do the comparisons of mtDNA sequences and the phylogenetic tree of these individuals provide support for the hypothesis of this experiment? What evidence do you have from both Table 2 and Figure 2 to explain your answer?

6. Implications. If the division of humans into different races cannot be based on a genetic distinction between different populations, what is the basis of “race”? Please explain your answer.

7. Your opinion. With respect to humans, does the concept of “race” have a useful function in society today, or should it be eliminated as a method of categorizing people?

Cited References

Edvotek, Inc. 2013. Mitochondrial DNA Analysis Using PCR. Retrieved September 17, 2015 from <http://www.edvotek.com/site/pdf/332.pdf>

Materials

A computer with Internet access is required for each pair of students. A LCD projector and computer are required for showing video clips and screen shots of the Bioservers website.

Notes for the Instructor

I usually begin the lab period with a short video (<https://www.youtube.com/watch?v=V9YMCKp5myI>) to introduce the idea of biological “race”. This 5 minute video provides an explanation of race and asserts that there is no biological basis of race within humans. The video also follows a group of students as Scott Bronson, from the Dolan DNA Learning Center, leads them through a comparison of their own mtDNA sequences. The video is the first episode of a larger documentary, “Race – The Power of an Illusion” produced by California Newsreel (California Newsreel, nd). This documentary has a companion website hosted by PBS (http://www.pbs.org/race/000_General/000_00-Home.htm) containing extensive resources. After my students complete the chimpanzee mtDNA sequences analysis and the human mtDNA sequences comparisons, another video clip from the documentary (<https://www.youtube.com/watch?v=GyuKJAG11Cw>) provides a good summary of genetic similarity among humans.

The lab report that student write following this lab exercise focuses on an evaluation of the hypothesis provided in the lab exercise. Their lab reports include the completed tables 1 and 2 and a written discussion. Within the discussion, students must use the percent differences between the mtDNA samples to argue that the hypothesis either is, or is not, supported by the data. They are asked to address the implications of their conclusion by answering questions 6 and 7 from the lab exercise (contained in the Student Outline above).

Cited References

California Newsreel, nd. Race – the power of an illusion, <http://newsreel.org/video/RACE-THE-POWER-OF-AN-ILLUSION>

Acknowledgments

Portions of this lab exercise were adapted from:

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About the Author

Amy Marion has been the Laboratory Coordinator in the Biology Department at New Mexico State University since 2001. She focuses on introductory laboratory courses for biology majors and non-majors as well as biology education.

Appendix A

The following tables provide the percent differences for the chimpanzee mtDNA comparisons and the human mtDNA sequence comparisons.

Table 1. Comparison of mitochondrial DNA sequences from different chimpanzee subspecies.

Comparison Group	Percent Differences (%)			
	Trial 1	Trial 2	Trial 3	Average
schweinfurthii mtDNA & schweinfurthii mtDNA	Range from 0.6-1.8%			~ 1.5%
verus mtDNA & verus mtDNA	Range from 2.6 – 9.7%			~ 6.3%
trogloodytes mtDNA & trogloodytes mtDNA	Range from 1.2 – 1.5%			~ 1.4%
schweinfurthii mtDNA & verus mtDNA	Range from 7.1 – 12.6%			~ 9.5%
schweinfurthii mtDNA & trogloodytes mtDNA	Range from 4.1 – 5.9%			~ 5.0%
verus mtDNA & trogloodytes mtDNA	Range from 9.1 – 14.1%			~ 11.5%

Table 2. Comparison of mitochondrial DNA sequences from humans of different origin.

Comparison Group	Percent Differences (%)			
	Individuals 1 vs 2	Individuals 1 vs 3	Individuals 2 vs 3	Average
African mtDNA & African mtDNA	3.4	0.8	4.2	2.8%
European mtDNA & European mtDNA	1.9	2.3	3.4	2.5%
Asian mtDNA & Asian mtDNA	2.6	2.3	3.4	2.8%
African mtDNA & European mtDNA	Range from 1.1 – 4.5%			2.4%
African mtDNA & Asian mtDNA	Range from 0.8 – 4.9%			2.5%
European mtDNA & Asian mtDNA	Range from 1.9 – 3.8%			2.7%

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