Genetics Education

How Are Humans Related to Other Primates?
A Guided Inquiry Laboratory for Undergraduate Students

Steven T. Kalinowski¹
Mark L. Taper¹
Anneke M. Metz²

¹Department of Ecology,
²Department of Cell Biology and Neuroscience

Montana State University
Running head: Human evolution lab
Key words: Evolution, education, laboratory, primate, human
Word count (total): 3571

Corresponding author:

Steven Kalinowski
Department of Ecology
Montana State University
310 Lewis Hall
Bozeman, MT 59717

skalinowski@montana.edu
Phone (406) 994-3232
FAX (406) 994-3190
ABSTRACT

Understanding that phylogenies depict the evolutionary history of species is a critical concept for undergraduate biology students. We present an inquiry based laboratory exercise exploring this concept in the context of the human phylogeny. This activity reinforces several important biological concepts and skills. Bolstered concepts include that evolution is descent with modification, that evolution is a genetic process, and that humans are closely related to apes. In terms of thinking skills, the lab gives students practice with hypothetical-deductive thinking, quantifying patterns from complex data, and evaluating evidence.

“Light will be thrown on the origin of man and his history.” —Darwin (1859)

“Inquiry... is the central strategy for teaching science.”—National Research Council (1996)

There is an emerging consensus that undergraduate biology coursework should teach thinking skills as well as content—and that student inquiry is an essential tool for reaching both goals (e.g., NATIONAL RESEARCH COUNCIL 1995; 2000; 2003). Despite the recognition that inquiry is an important component of science education, there is a daunting shortage of inquiry-based lessons available for instructors teaching undergraduate biology. The shortage is particularly acute for laboratory exercises, which is unfortunate, because laboratory exercises offer students an ideal opportunity for practicing scientific investigation.

One potential reason for this shortage is that inquiry is a multifaceted activity that is difficult to define, and therefore, difficult to teach. Each of the following activities, for example, fit within most definitions of inquiry: asking questions, reviewing available knowledge,
formulating hypotheses, testing hypotheses, evaluating evidence, relating results to previous knowledge, and communicating results. In addition, activities designed to exercise inquiry skills can vary by how much self-direction is required of students (e.g., NATIONAL RESEARCH COUNCIL 2002). University faculty hope that a doctoral student will be able to identify a meaningful question, propose hypotheses to answer the question, design tests for these hypotheses, interpret results, and then publish their findings, but would not expect such independence from undergraduate students in an introductory biology class.

While designing labs for an introductory biology class for biology majors, we have decided that each investigation should require students to figure out something for themselves. In this paper, we describe a two-part laboratory lesson that helps students answer one of the “biggest” most meaningful questions in biology: how are humans related to other animals? Students answer the question by analyzing skull morphology and DNA sequences among primate species. The lab requires both creativity and critical thinking. Creativity is required to develop methods to infer phylogeny from skull morphology and DNA sequences. Critical thinking is required when students discover that the phylogeny they reconstruct from DNA sequences from ten primate species (including humans) to answer the question. This is the class’s first experience estimating phylogenies, so the lab forces them to answer a series of subsidiary questions: What does it mean for two species to be “related?” How can relatedness be estimated? And, which data is more reliable, morphological or genetic? This last question is encountered when students discover that the phylogeny they estimate from DNA sequences does not agree with the phylogeny they reconstruct from skull morphology. This twist to the lab requires students to think deeply about how evolution works. In our experience, most
undergraduate students are not prepared to tackle this series of questions without preparation.

Consequently Therefore, students are given a series of introductory problems in which they learn the skills needed to analyze skulls and DNA sequences.

This activity lab reinforces several important biological concepts and skills. These concepts include: biological diversity is hierarchal; i. evolution is descent with modification; ii. evolution is a genetic process; iii. biological diversity is hierarchal, and iv. humans are closely related to apes (FIGURE 1). Skills practiced include: hypothetical-deductive thinking; quantifying patterns from complex data; and critically evaluating evidence. Less tangibly, but perhaps more importantly, we hope this lab increases students’ ability and willingness to confront difficult questions.

**LAB DESCRIPTION**

We use this lab in a sophomore level course on ecology and evolution that is part of a three-semester introductory biology sequence. This course is most students’ first introduction to evolution. Students attend lecture three times a week for an hour, and have lab once a week for three hours. In lab, students work in groups of 2-4 under the guidance of graduate student teaching assistant (TA). The lab we present here is broken into two parts, each designed for a single three-hour lab period. Instructor notes, practice problems, and the mtDNA sequences referred to below are posted online as Supplementary Materials.

The first lab session begins with a fifteen-minute presentation introducing the focal question of the lab to the class. The theme of the presentation is that human evolution has aroused fascination and controversy since the *Origin of Species* was published in 1859. In this presentation, the lab instructor briefly discusses the biblical story of Adam and Eve, Darwin’s
reluctance to discuss humans in the *Origin*, the debate between Huxley and Wilberforce, and the contemporary disagreement between biologists and creationists. Next, the presentation shows photographs of chimpanzees, gorillas, and other primates, and suggests that if humans have evolved from other animals, those animals were almost certainly primates. Slides are shown of a diverse array of living primates from Prosimians to the chimpanzee (including all the primates listed in Table 1), along with a brief natural history of each species. The presentation concludes with the central question for the students to answer: “How are humans related to other primates?” A short discussion follows to clarify the question (an important skill itself). The class is asked what it means for species to be “related” and the question is rephrased as “What is the evolutionary history of primates?” This question is written on the board for the remainder of the lab in order to emphasize the purpose of the lab.

Next, students are introduced to the lab materials. Ten primate skulls (Table 1) are arranged on a bench top, and the students are introduced to each skull. We use full sized resin casts produced by BoneClones (www.boneclones.com) in place of real skulls. Each cast costs between $100 and $200, and is realistic enough that students often mistake them for real skulls.

Each lab group is also given 956 base pairs of mitochondrial DNA (NADH dehydrogenase subunit 1mtDNA) sequence for each species. W.\(^1\) choose NADH dehydrogenase it is a

\(^1\) Mitochondrial sequences were chosen because there is no recombination between mtDNA sequences, and because mtDNA has a mutation rate ten times greater than nuclear genes with similar function. The entire mitochondrial genome has been sequenced for nine of the ten species used in this lab (DNA sequence from a capuchin monkey was used in place of the howler monkey), and is available for download in the National Institute of Health database, GENBANK (www.ncbi.nlm.nih.gov). (In order to obtain the complete mtDNA sequence for gorillas, for example, search the nucleotide database for “mitochondrion AND complete genome AND gorilla”. The NADH dehydrogenase sequence discussed below can be obtained by searching for “ND1 AND gorilla.”) Sequences for each species were aligned using the software package MEGA3 using default alignment parameters (Kumar et al. 2004; www.megasoftware.net), resulting in 16,211 base pairs of sequence (including gaps) shared by all ten species. A phylogeny (UPGMA, number of nucleotide differences) constructed from the entire mtDNA sequence was consistent with current understanding of primate evolution (Figure 1) (Jones et al. 1992; Freeman and Herron 2003). From the 16,211 base pairs available for analysis, we chose the first subunit of NADH for students to analyze because this sequence yields a phenogram very similar to the phenogram constructed from the entire mtDNA sequence. In addition, NADH
familiar enzyme to students and because this sequence produces a phenogram consistent with current understanding of primate evolution (Fig. 1) (Jones et al. 1992; Freeman and Herron 2003). Mitochondrial sequences for each of the ten primate species described in this lab are available for download from the National Institute of Health database, GENBANK (www.ncbi.nlm.nih.gov/).

Our experience has been that students are unable to reconstruct phylogenies of ten species without at least some guidance. We do two things to simplify the task. First, students are given a series of training problems to help them to develop basic principles of phylogeny reconstruction. Second, after these training exercises, students are given only three species to analyze for themselves. Each lab group analyzes a different set of species, but all groups have skulls and mtDNA sequence for a human, an ape, and a monkey (e.g., human / gorilla/ rhesus macaque or human / chimpanzee /howler monkey).

The training exercises begin with a question that illustrates the difficulty of phylogeny reconstruction: how many different phylogenies are possible for the ten species represented by the skulls on the bench top? Students break into their lab groups to work on this and subsequent problems. They quickly realize that there are many different possible evolutionary trees, and that dehydrogenase is a familiar enzyme to students because its function in cellular respiration is discussed earlier in the introductory biology sequence.
calculating the exact number is difficult. The instructor then leads a brief discussion of how to simplify the problem. The class is guided to the simpler question of how many trees can be constructed from two, three, and then four taxa species. This turns out to be 1, 3, and 15 rooted phylogenies. We have the students draw each possible phylogeny for these cases in order to practice combinatorial reasoning (see Lawson 1995 for a discussion of the importance of combinatorial thinking). To emphasize the complexity of the problem, the instructor then tells the class that for ten species, there over 34 million ways that ten species could be related.

Next, the class practices estimating phylogenies for hypothetical taxa. We use Chernoff faces (FIGURE 2) for these exercises because they are similar to the skulls students will use, and because it is easy to construct Chernoff faces using readily available statistical graphing software (e.g., Systat 10.0). We give the students three problems: one with three taxa, one with four taxa, and one with six taxa. After working on the problems, the class is asked what they learned from this exercise. Students should identify skills such as determining which features differ among faces and counting numbers of similarities and differences between faces.

Next, students are given 50 base pairs of mtDNA sequence data from three primates and asked to estimate the evolutionary history of the species using the lessons they learned from the Chernoff faces. After some deliberation, they infer that species with similar DNA sequences are most likely most closely related.

Last, students are given 50 base pairs of hypothetical mtDNA sequence (labeled as coming from a mouse, possum, and shark). The mouse and possum sequences are identical except for a point mutation and a ten base pair inversion. Interpretation of the data depends on how the inversion is treated. If all ten base pairs are considered independent characters, the mouse and shark sequences are most similar. On the other hand, if the inversion is treated as a
single mutation (which it was), the mouse and possum sequences are most similar. This illustrates the importance of using independent characters—a lesson that is important when analyzing skull morphology. The first lab session ends at this point.

During the second lab period, students collect data analyze their three skulls and 956 base pairs of mtDNA sequence and present preliminary results to the class. Invariably, students find that their skull and DNA analyses give contradictory results (Figure 3). Data from skulls suggest that monkeys and apes are more closely related, while DNA sequence analysis suggests humans and apes are more closely related. The class then discusses which evidence is stronger. With guidance from the instructor, the following points are developed through Socratic dialogue. i. No single morphological or molecular trait will answer the problem. ii. Analysis of multiple traits is necessary. iii. Independent traits are more informative than correlated traits. iv. DNA differences between primate species are numerous and independent. v. Morphological differences between primate species are numerous but may not be independent.

Students complete the lab by writing a lab report due the following week. The format for this report is similar to the structure for a scientific paper: Introduction, Methods, Results, and Discussion.

INTEGRATING LAB AND LECTURE

We have defined an inquiry-based lab as a lab in which students figure out something for themselves. In the lab described above, students are given the task of reconstructing the evolutionary history of monkeys, apes, and humans. Solving this problem requires three steps: i. developing a method for estimating phylogenies from skulls and DNA sequences, ii. collecting and analyzing morphological and genetic data from monkeys, apes, and humans, and iii.
evaluating which evidence (genetic or morphological) is stronger. This requires a substantial amount of independent problem solving from students, and because of this, there is a risk that some students will not learn the lessons the lab was designed to teach. In order to prevent this, instructors will need to carefully integrate the lab with the rest of the course. This includes using lecture time to prepare students for the lab and reinforcing the main points of the lab in lecture afterwards. This strategy of having students first grapple with a problem before being taught the solution is a particularly effective strategy for teaching deep understanding (see NATIONAL RESEARCH COUNCIL 2000 Box 3.6).

Preparing students for this lab is straightforward, for the lab assumes only that students have a basic understanding of evolution (including mutation). However, because human evolution is a controversial concept for many students, instructors may wish to emphasize evidence for evolution, especially evidence for human evolution. For example, we discuss vestigial features such as the human appendix, developmental homology similarities between vertebrates, homologous bones in mammalian forelimbs structural homology, and the fossil evidence for Neanderthals record (see FREEMAN & HERRON 2003 for a review).

We recommend that instructors follow the lab with a lecture or discussion that reinforces the lessons learned in the lab, and clarifies any residual confusion among students. We begin our discussion of human evolution and phylogeny reconstruction this discussion by closely examining the conflict between morphological and genetic data (FIGURE 3). In our experience, students conclude that the genetic data is more reliable because genetic differences are numerous, independent, and easy to quantify. This interpretation is reasonable, but a more refined explanation is not difficult to construct. We initiate the discussion by telling the class that most of them concluded that humans and apes were most closely related. The instructor then asks
the class if this means that he and a chimpanzee have a relative in common—a great-great-great… grandmother living five million years ago. The answer, of course is yes (e.g. Stauffer et al. 2001), and the instructor presents a family tree of a baboons, chimpanzees, and humans with a photograph of himself representing humans. Next, the instructor asks the class how his mtDNA has come to be different from that of his distant cousin. With a little prompting, the class indicates that: 1. the instructor inherited his mtDNA from his mother, and she from her mother, and so forth for a million generations (more or less);, 2. every once in a while there was a mutation; and, 3. as time passed the mitochondrial sequences in each lineage grew more and more different. This results in distantly related species having DNA sequences that are more different than closely related species—a property that can be used as a “molecular clock” to measure how much time has passed since speciation. The instructor then asks how this idea can be tested, and leads the class to a discussion of molecular clocks and their calibration with fossil evidence. Once the class understands the role of mutation in DNA sequence divergence, the instructor asks how selection would be expected to affect new mutations. Selection would eliminate DNA sequences that interfered with protein function, but would not affect mutations that produced functionally equivalent proteins. The instructor can then point out that most of the DNA differences observed in the mtDNA are “silent” or synonymous mutations—substitutions that do not change the amino acid sequence. For example, there are 90 DNA substitutions between the human and chimpanzee sequences, but only 19 amino acid differences (see Table 2 for an example). Selection will not affect these polymorphisms, so they should be ideal for estimating phylogenies.

Next, the instructor asks why the skulls produced a different phylogeny (Figure 3). To begin this discussion, he asks how many genetic differences are responsible for the skull
morphology. The answer is not known, but developmental biology offers a clue (FIGURE 4). Human and chimpanzee skulls are very similar in the fetal stage of development—each species has a notably rounded skull. Chimpanzee skulls elongate as they grow. In contrast, humans retain the fetal shape. This suggests that evolution of skull morphology from chimp-like to human-like might require only stopping skull elongation at a relatively early stage in development—an evolutionary change that might require few mutations and therefore could evolve quickly. Thus it is reasonable that the uniqueness of the human skull could have evolved quickly and recently (i.e. after the human-chimpanzee divergence).

After proposing that human skull morphology may have evolved recently (i.e. after the human-chimpanzee split), we ask the class, how this could be tested. We use this question to begin an exploration of the fossil record of human ancestors—which show that that the distinctive morphology of human skulls has evolved recently. Once we have concluded a discussion of the data the students collected in lab, we use the remainder of the lecture to present a contemporary understanding of human evolution (FIGURE 2). We present additional genetic data that supports the students’ conclusions and describe major fossil evidence for human evolution.

DISCUSSION

Inquiry based instruction is sometimes criticized for sacrificing content and for being difficult to design. We conclude this paper with a response to these comments.

We believe that the thinking skills / content dichotomy is a false one (See NATIONAL RESEARCH COUNCIL 2000 for a review). There is considerable content in the primate phylogeny lab we present, and students recognize this. For example, eighty-three percent of our class
believed that the lab was a “valuable learning experience” (Table 2). This was especially gratifying to us, because many of our students seemed to favor lectures and labs that presented material to memorize. In addition to teaching content, we believe inquiry oriented labs such as ours increase students’ curiosity and thereby improves their receptiveness to subsequent lectures and homework. Course evaluation supports this hope (Table 2). Eighty percent of the class classified the lab as being “interesting,” which is quite favorable for students that expressed preference for investigations using “modern” (i.e. molecular) laboratory techniques.

Our experience designing this lab confirms that inquiry labs are difficult to construct. We found two challenges. First, we had to decide what the goal of the lab was, and second, we had to construct a lab in which students could achieve this goal. The second task was more difficult, for students are typically not able to answer interesting questions on their own in one or two lab sessions. Our response to this problem was to break the problem into solvable steps and to attempt to keep the guidance of the instructor was inconspicuous as possible. This required extensive planning and revision, but watching students successfully answer an authentic scientific problem was worth the effort.

We thank the National Science Foundation and the Howard Hughes Medical Institute for funding (NSF grant DEB-0415932 to MLT; HHMI Undergraduate Science Education Program grant to Montana State University).

LITERATURE CITED


Table 1. Classification of the 10 primate species used in the laboratory exercise described in the text.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Description</th>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>—</td>
<td>Hominidae</td>
<td>Homo</td>
<td>sapiens</td>
</tr>
<tr>
<td>Chimpanzee</td>
<td>Great ape</td>
<td>Pongidae</td>
<td>Pan</td>
<td>troglodytes</td>
</tr>
<tr>
<td>Bonobo</td>
<td>Great ape</td>
<td>Pongidae</td>
<td>Pan</td>
<td>paniscus</td>
</tr>
<tr>
<td>Gorilla</td>
<td>Great ape</td>
<td>Pongidae</td>
<td>Gorilla</td>
<td>gorilla</td>
</tr>
<tr>
<td>Orangutan</td>
<td>Great ape</td>
<td>Pongidae</td>
<td>Pongo</td>
<td>pygmaeus</td>
</tr>
<tr>
<td>Gibbon</td>
<td>Ape</td>
<td>Hylobates</td>
<td>Hylobates</td>
<td>lar</td>
</tr>
<tr>
<td>Vervet monkey</td>
<td>Old world monkey</td>
<td>Cercopithecida</td>
<td>Cercopithecus</td>
<td>aethiops</td>
</tr>
<tr>
<td>Rhesus macaque</td>
<td>Old world monkey</td>
<td>Cercopithecida</td>
<td>Macaca</td>
<td>mulatta</td>
</tr>
<tr>
<td>Baboon</td>
<td>Old world monkey</td>
<td>Cercopithecida</td>
<td>Papio</td>
<td>hamadryas</td>
</tr>
<tr>
<td>Howler monkey</td>
<td>New world monkey</td>
<td>Cebidae</td>
<td>Alouatta</td>
<td>pigra</td>
</tr>
</tbody>
</table>
Table 2. Partial mtDNA sequence for humans and chimpanzees for NADH subunit 1 (base pairs 756-770 of the sequences provided). Note that none of the four substitutions change the amino acid sequence.

<table>
<thead>
<tr>
<th>Human mtDNA sequence</th>
<th>TTC – AAC – ATC – GAA – TAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chimpanzee mtDNA sequence</td>
<td>TT&lt;T – AA&lt;T – ATC – GAG – TAT</td>
</tr>
<tr>
<td>Amino Acid acid sequence for both species</td>
<td>Phe – Asn – Leu – Glu – Tyr</td>
</tr>
</tbody>
</table>
**Table 3.** Student evaluation of the human evolution lab. Student responses were anonymous.

Evaluation was conducted immediately following the lab. Student responses were anonymous.

The total sample size was 41.

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Agree</th>
<th>Disagree</th>
</tr>
</thead>
<tbody>
<tr>
<td>The lab was interesting</td>
<td>80%</td>
<td>20%</td>
</tr>
<tr>
<td>The lab was clearly organized and presented</td>
<td>85%</td>
<td>15%</td>
</tr>
<tr>
<td>The lab was a valuable learning experience</td>
<td>83%</td>
<td>17%</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

Fig. 1. Phenogram of ten primate mtDNA sequences constructed from the entire mitochondrial genome of each species. The phenogram was constructed using the UPGMA method and percent sequence difference as a genetic distance. Its topology is consistent with current understanding of primate evolution.

Fig. 2. Phenogram of ten primate mtDNA sequences constructed from the entire mitochondrial genome of each species. The phenogram was constructed using the UPGMA method and percent sequence difference as a genetic distance. Its topology is consistent with current understanding of primate evolution.

Fig. 3. Evolutionary relationships between humans, apes, and monkeys suggested by skull morphology (a) and mtDNA sequence variation (b).

Fig. 4. Skull morphology of fetal and adult humans and chimpanzees. From Campbell and Reece (2005). Copyright Pearson Education. Reprinted by permission of Pearson Education, Inc.
FIGURE 1.

human
chimpanzee
bonobo
gorilla
orangutan
gibbon
baboon
rhesus
vervet
howler
**Figure 3.**

- a) Skull morphology
- b) mtDNA
SUPPLEMENTARY MATERIAL

The following resources will be posted as supplementary material on the *Genetics* webpage. For now, they are available at

http://www.montana.edu/kalinowski/PrimateEvolutionLab/PrimateLab.htm

Instructor/TA Notes
Chernoff Face Problem #1
Chernoff Face Problem #2
Chernoff Face Problem #3
DNA Sequence Problem #1
DNA Sequence Problem #2
Data file for MEGA software
DNA Sequences for 10 primate species (continuous)
DNA Sequences for 10 primate species (interleaved)