Article

Teaching the Process of Molecular Phylogeny and Systematics: A Multi-Part Inquiry-Based Exercise

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Three approaches to molecular phylogenetics are demonstrated to biology students as they explore molecular data from *Homo sapiens* and four related primates. By analyzing DNA sequences, protein sequences, and chromosomal maps, students are repeatedly challenged to develop hypotheses regarding the ancestry of the five species. Although these exercises were designed to supplement and enhance classroom instruction on phylogeny, cladistics, and systematics in the context of a postsecondary majors-level introductory biology course, the activities themselves require very little prior student exposure to these topics. Thus, they are well suited for students in a wide range of educational levels, including a biology class at the secondary level. In implementing this exercise, we have observed measurable gains, both in student comprehension of molecular phylogeny and in their acceptance of modern evolutionary theory. By engaging students in modern phylogenetic activities, these students better understood how biologists are currently using molecular data to develop a more complete picture of the shared ancestry of all living things.

INTRODUCTION

Teaching fundamental mechanisms of evolution by natural selection is more important than ever, both to biology students and the general student population, and fresh pedagogical approaches to accomplish this are needed at all levels (Dagher and BouJaoude, 1997; Robbins and Roy, 2007; Labov and Kline Pope, 2008). Perhaps more than any other subdiscipline of biological sciences, the study of systematics tangibly and powerfully invokes the biological results of evolution and selection. Thus, its inclusion throughout all levels of the biology curriculum has long been strongly recommended. However, as powerfully argued by Rudolph and Stewart (1998), misunderstandings about evolution are largely philosophical, rooted in a poor understanding of the scientific method and how it applies to the study of natural history. Indeed, even advanced biology students can harbor striking misconceptions regarding the fundamental basis of evolutionary history (O'Hara, 1997; Robbins and Roy, 2007).

This lack of fundamental understanding by students is compounded by the seemingly subtle differences among different systematic approaches. Phenetics seeks to classify organisms based on observable differences regardless of evolutionary history, while cladistics, which also examines observable characteristics, has the explicit goal of inferring shared ancestry and constructing a hierarchical classification. Molecular phylogenetics is similar to cladistics, but instead of relying on primitive and derived morphological characteristics, it uses molecular sequence data and other quantifiable measures to establish data matrices to infer likely evolutionary relationships. Further, there exists a variety of possible forms for expressing the results of systematic analyses: phylogenetic trees, cladograms, phylograms, dendograms, ultrametric trees, etc. Thus, it is not surprising that students sometimes fail to grasp the larger conceptual framework amid this disciplinary complexity.

Explicitly teaching the process and nature of scientific research results in considerable learning gains, among science majors and nonmajors alike (Lederman, 1992, 1999; Lombrozo *et al.*, 2008). The National Academy of Science (NAS) and the National Science Foundation (NSF) have explicitly called for the teaching of the practice of science within the existing science curricula, especially in relation to evolutionary theory (Alberts and Labov, 2004; Miller *et al.*, 2006; Ayala, 2008). However, this pedagogical approach is not trivial to implement. First, there are at least two distinct conceptual frameworks to consider: the philosophical *nature* of the scientific pursuit, and the true-to-life realities of the modern scientific practice, which are markedly different among disciplines (Matthews, 1994; Rudolph and Stewart, 1998; Staver, 1998; Schwartz and Lederman, 2002). Second, using real datasets that challenge students to apply scientific concepts and analysis is key to learning scientific thinking (Wise and Okey, 1983; Soloway *et al.*, 1999). These active-learning methods are often met with student confusion and resistance, especially if they have not learned this way before (Gosser, 2003; Shetlar, 2005). Considerably more effort and thought is required of students, compared with traditional passive learning approaches involving didactic lectures and protocol-driven laboratory exercises in which students simply follow clear experimental procedures and interpret data as instructed (Hofstein and Lunetta, 2004; Hanauer *et al.*, 2006).

Several new educational resources have emerged that specifically give attention to the methods and practice of the modern field of systematics (Clough, 1994; Alles, 2001; Perry *et al.*, 2008). Because the majority of biology students don't properly grasp discrete information conveyed by a simple phylogenetic tree, groups including the "tree-thinking group" (http://tree-thinking.org) have resolved to develop resources and support for biology teachers at all levels (O'Hara, 1997; Baum *et al.*, 2005). Other tools include the Understanding Evolution resource (http://evolution.berkeley.edu) from the University of California Museum of Paleontology in Berkeley, CA, resources from the NAS (www.nationalacademies.org/evolution/index.html), the Public Broadcasting Station (www.pbs.org/wgbh/evolution), and Visionlearning (visionlearning.com), which is funded by the NSF and the U.S. Department of Education.

In designing the educational method described herein, we aimed to develop another teaching tool for demonstrating the modern practice of molecular phylogenetics by using actual datasets and challenging students to interpret those data using their own skills in deductive reasoning. We do this by providing DNA sequences, protein sequences, and chromosomal electron density maps of five closely related species, and then asking students to make simple hypotheses regarding the phylogeny of these species. There are several unique features of this approach. First, by having students participate in the scientific process of hypothesis-making, they gain familiarity with "what scientists do" with experimental data. Second, by engaging several types of data addressing the same underlying question, we demonstrate to students how scientists use multiple lines of evidence to support or refute hypotheses. Third, by exposing students to raw data that can be used to elucidate the common evolutionary origins of related species, we may break through resistance that some students have to evolution in general (Clough, 1994; Lombrozo *et al.*, 2008; Perry *et al.*, 2008). In our chosen method of implementation, unbeknownst to the students, the raw data they will be handling are taken from *Homo sapiens* and four closely related primates, thus shedding light on the biological origin of humanity. Fourth, the method that students will use, comparative genomics, is currently used by evolutionary biologists in exactly this context (Zhu *et al.*, 2007), thus accurately "mimicking" a relevant and cutting-edge scientific practice.

EXPERIMENTAL METHODS

Student Assessment Groups

Assessment took place in spring of 2009 in the course Biology 104 (Bio104), Principles of Modern Biology II, the second semester of the majors-track introductory biology course at John Jay College, a large, urban, minority-serving institution, and part of the City University of New York (CUNY) university system. All three activities were conducted in one 2.5-h laboratory session, with students working in their normal laboratory group (pairs). The laboratory took place after the second week of the course, immediately after the course lecture on phylogeny and systematics, which follows lectures on natural selection, micro- and macroevolution, speciation, and Hardy–Weinberg equilibrium. Two laboratory sections (28 students each) meet jointly for course lectures. For the assessment, both lab sections met with the same course instructor at the same time, thus providing for a case-controlled experimental design. One lab section completed the traditional laboratory exercise [chapters 20 and 21 of the Helms biology laboratory manual (Helms *et al.*, 1998)] and is referred to as the control section, while the experimental section completed the exercises described herein.

Performance on Exam Questions

The three phylogeny-related exam questions referred to in the *Assessment of the Activity* section were as follows:

Q1. If two modern organisms are distantly related in an evolutionary sense, then one should expect that…

A1. they should share fewer homologous structures than two more closely related organisms.

Q2. In evolutionary terms, the more closely related two different organisms are, the…

A2. more recently they shared a common ancestor.

Q3. The theory of evolution is most accurately described as…

A3. an overarching explanation, supported by much evidence, for how populations change over time.

These questions were given in multiple-choice format (other answer choices are available upon request), and the results shown in the *Assessment of the Activity* section represent the percentage in each group that selected the correct answer.

Surveys Regarding Perceptions of Evolution

The surveys used in this study (results shown *Assessment of the Activity* section) were devised and twice validated by administration to similar student sections in previous semesters and were deemed exempt from full panel review by the John Jay College Institutional Review Board (IRB). Several "control statements" were included regarding the acceptance of the scientific validity of current understandings of geologic time, which had shown in previous validations of this survey to be relatively stable in group responses before and after learning about evolution in detail. Next, we included a series of overlapping statements about 1) evolution, 2) natural selection, and 3) how those processes contributed to the emergence of *Homo sapiens*, which, in previous validations, had generated responses that were subject to change as students studied the mechanisms of evolution.

For this survey, students were asked to report their acceptance of the statements on a five-point scale: strongly agree, agree, neither agree nor disagree, disagree, strongly disagree. Importantly, similar concepts were repeated in several variations, because studies have shown that students may key in to certain "trigger words" including theory, Darwin, evolution, scientists, and descent; and different wordings can lead to different survey results, even with the same students (Evans, 2001; Scott and Branch, 2009). For most statements, a response of "strongly agree" was scored as a "1" and indicated the strongest acceptance of current scientific theory, while a response of "strongly disagree" was scored as a "5" and indicated the strongest opposition to current scientific theory. However, three statements were expressed as "inverts," such that agreement would indicate a rejection of currently accepted scientific theory. The numerical scoring of these questions was inverted to maintain the pattern that the lowest score indicates the strongest acceptance of scientific theory. The survey questions were as follows:

Control Questions

C1. I agree with the scientific evidence that dates the earth to more than 4 billion years of age.

**C2. Although some scientists claim otherwise, the earth is not more than 10,000 years old.

C3. I agree with the theory that, over the course of time, the positions of the great land masses (continents) have undergone many dramatic changes.

Probative Questions

**Q1. I believe that, with only a few exceptions, the life forms that exist on the planet today are, more or less, the same that have always been here since life first began on earth.

Q2. I believe that, over many generations, natural selection has contributed to the gradual evolution of animals and plants into their present forms.

**Q3. I believe that evolution by natural selection is just one theory about how life on earth came to its present form and I personally don't support it.

Q4. I feel that a large body of evidence supports the Darwinian theory of evolution by natural selection.

Q5. I support the theory that the biological species, Homo sapiens (Human beings) evolved from an earlier species of primates.

Q6. I agree with Charles Darwin, who first suggested that the current form of human beings was influenced through the process of evolution by natural selection.

Q7. Because human beings are mammals, I believe that they have a shared ancestry with all other mammals.

Q8. I believe that human beings descended to their present form through natural processes, including natural selection.

** = inverted statements; scoring is reversed.

Survey responses were tabulated, scores for invert statements were reversed, and group patterns were analyzed. First, responses to the control questions were analyzed to ensure that the two groups were comparable. To assess changes in perception, we scrutinized pre- and posttreatment responses to identical questions and performed the following calculation on the "average group scores" (arithmetic mean) to individual questions: $100\% \times (\text{pre} - \text{post})/\text{pre}$. By placing the pretest values in the denominator, this formula normalizes for beginning differences in the two student groups and expresses change relative to the initial condition. Error bars were added to indicate relative variance in survey responses, as calculated by the following formula: (SD)/(average response) multiplied by the "percent change" score for that question for proper scaling.

DESCRIPTION OF THE ACTIVITIES

This activity, suitable for laboratory, discussion, or any other group work setting, is broken into three parts. Although common connections are drawn at the conclusion, each individual part could be done at different times or stand entirely on its own. Further, each part could be simplified, further extended to include a quantitative parsimony analysis, or otherwise modified, as explained within each description. Thus, these exercises are flexible and can accommodate many teaching environments. The driving theme is to provide actual scientific data to students and challenge them to draw conclusions about the data in ways that lead them to propose a hypothetical phylogram describing the evolutionary relatedness of the species involved. Although it may be best if these activities follow a lecture on systematics that covers the differences between cladistics and phylogenetics, as we have done, this may not be strictly essential and a short primer on systematics (see www.ncbi.nlm.nih.gov/About/primer/phylo.html) might suffice, depending on the academic background of the students. The complete student handout for this exercise is provided as Supplemental Material 1, while the complete instructor guide is provided as Supplemental Material 2.
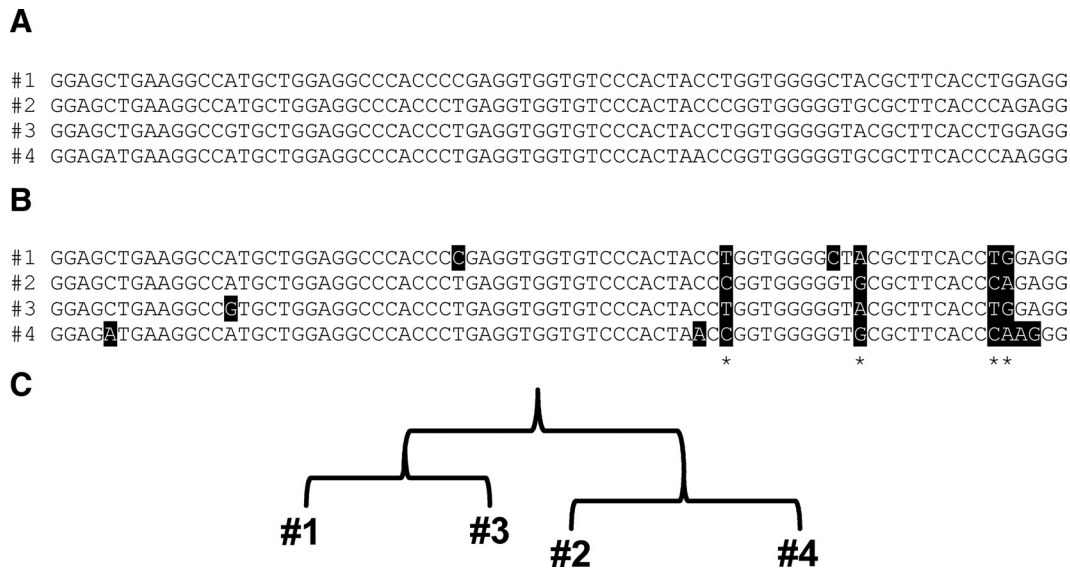
A

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#1 GGAGCTGAAGGCCATGCTGGAGGCCCACCCCGAGGTGGTGTCCCACTACCTGGTGGGGCTACGCTTCACCTGGAGG
#2 GGAGCTGAAGGCCATGCTGGAGGCCCACCCTGAGGTGGTGTCCCACTACCCGGTGGGGGTGCGCTTCACCCAGAGG
#3 GGAGCTGAAGGCCGTGCTGGAGGCCCACCCTGAGGTGGTGTCCCACTACCTGGTGGGGGTACGCTTCACCTGGAGG
#4 GGAGATGAAGGCCATGCTGGAGGCCCACCCTGAGGTGGTGTCCCACTAACCGGTGGGGGTGCGCTTCACCCAAGGG
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B

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#1 GGAGCTGAAGGCCATGCTGGAGGCCCACCCCGAGGTGGTGTCCCACTACCTGGTGGGGCTACGCTTCACCTGGAGG
#2 GGAGCTGAAGGCCATGCTGGAGGCCCACCCTGAGGTGGTGTCCCACTACCCGGTGGGGGTGCGCTTCACCCAGAGG
#3 GGAGCTGAAGGCCGTGCTGGAGGCCCACCCTGAGGTGGTGTCCCACTACCTGGTGGGGGTACGCTTCACCTGGAGG
#4 GGAGATGAAGGCCATGCTGGAGGCCCACCCTGAGGTGGTGTCCCACTAACCGGTGGGGGTGCGCTTCACCCAAGGG
                                                  *           *            **
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C

Figure 1. (A) Aligned genomic DNA sequences from the *GULO* pseudogene taken from the short arm of chromosome #8 (8p21 in humans) of the following species: #1 = *Pan troglodytes*, #2 = *Pongo pygmaeus*, #3 = *Homo sapiens*, and #4 = *Macaca mulatta*. (B) The discrete nucleotide differences among the four DNA sequences have been highlighted. The asterisks indicate key positions that help reveal ancestry. (C) The most likely phylogram indicating the ancestry and divergence of the species based on these DNA sequences.

Activity One: Molecular Phylogenetics Using a Pseudogene

In the first activity, students are given four short DNA sequences (Ohta and Nishikimi, 1999), shown in Figure 1A, with a brief description.

- Below are four gene sequences. These are taken from four animals that are believed to have "recent shared ancestry" (are closely related).
- The gene sequences are from a so-called "broken gene" or pseudogene, the evolutionary remnant of a gene, which is now nonfunctional, in a given species or group of related species. In this case, the gene is called GULO (L-gulonolactone oxidase), which codes for the enzyme which catalyzes a key step in the synthesis of ascorbic acid (vitamin C). Along the way, some animals have lost the function of this gene (by random mutation) and must consume vitamin C in their diet.

Procedure

1. Examine the four gene sequences below and mark any differences among the sequences that you can find.
2. Discuss the following questions with your lab partner: Do you notice any specific pattern? What could this pattern mean regarding the ancestry/relatedness of the four species?
3. Together with your lab partner, make a hypothesis about the ancestry of these four species in the form of a phylogenetic tree. Draw this tree on a separate sheet of paper and make a few notes explaining why you drew it this way.

In an effort to reduce intellectual resistance to the topic, we elect not to reveal the identity of the species until all activities are complete (Lombrozo *et al.*, 2008). Studies have

shown that many self-identified Christians in the United States have brokered a psychological compromise between science and faith by accepting the validity of geologic time and evolutionary change but maintaining that these processes had little to do with the divinely instituted emergence of *Homo sapiens* (Smith, 1994; Meadows *et al.*, 2000; Miller *et al.*, 2006). The DNA sequences are derived from a pseudogene, which opens up an interesting discussion in itself (Nishikimi and Yagi, 1991; Eyre-Walker and Keightley, 1999). As students begin to examine the DNA sequences, they have little trouble identifying the differences between the species, highlighted in Figure 1B. However, if students are then unsure what to do next, we let them wander through the initial confusion and discuss how to approach the problem with their lab partner and other classmates, reinforcing the collaborative nature of scientific research. Eventually, students focus on the differences marked with asterisk in Figure 1B, and nearly all student pairs draw a phylogram similar to that shown in Figure 1C. A quantitative analysis of parsimony might enrich this activity significantly for more advanced students. Based upon such a quantitative parsimony analysis, the phylogram shown in Figure 1C is indeed the most parsimonious relationship based on these DNA sequences (data not shown).

Activity Two: Amino Acid Sequences of Functional Homologous Proteins

In this activity, we present students with sequences from related species and challenge them to deduce a phylogram. However, this exercise is more complicated because there are sequences provided from five species, and students are provided with the amino acid sequences of a functional protein, chromosome-encoded SCML1 protein that func-

tions in male embryonic development and male fertility (van de Vosse *et al.*, 1998; Wu and Su, 2008). Because mutation and evolutionary change are more "constrained" in a protein sequence (Nachman and Crowell, 2000), these sequences utilize the "…/…" symbol to denote long stretches of protein sequence with no differences in amino acids. This opens a discussion of different silent mutations that might be present in these species, both of the wobble and intronic variety. The five sequences provided to students are shown in Figure 2A (Wu and Su, 2008).

The differences between the homologous sequences, highlighted in black in Figure 2B, are more numerous in this activity, but because of the practice they had in activity one, students are more prepared to "see through the noise" and ignore instances in which one species has a unique amino acid at a certain position. Another new challenge faced by students in this activity is the inclusion of data from five species, instead of just four, which will require a more complicated phylogram. Although most of the student groups will notice the early divergence of the ancestor of #1 and #2, from the ancestor of #4 and #5, these same groups

are often split evenly regarding which side of the branch point includes the most recent unique ancestor of species #3. Thus, most students begin by constructing their phylograms according to one of the options shown in Figure 2C, evenly split between the two possibilities.

The fact that two hypothetical phylograms are nearly equally likely provides a good teaching moment as this introduces the nature of scientific controversy and debate. We encourage students to present data for their position, and we have observed that some lab groups argue strongly that, using the positions marked with an asterisk in Figure 2B, there are three examples of species #3 being similar to #1 and #2, and only two examples when #3 is similar to #4 and #5. Because three is more than two, this does argue, albeit weakly, that the convergence of species #3 from #1 and #2 was more recent than its divergence from #4 and #5. This opens a discussion of "weight of evidence" and the need for much larger sets of sequence data, from many genes, to build stronger hypotheses. Further still, this provides a nice segue to the next activity, which is a wholly different method of analysis, and how scientific research relies on
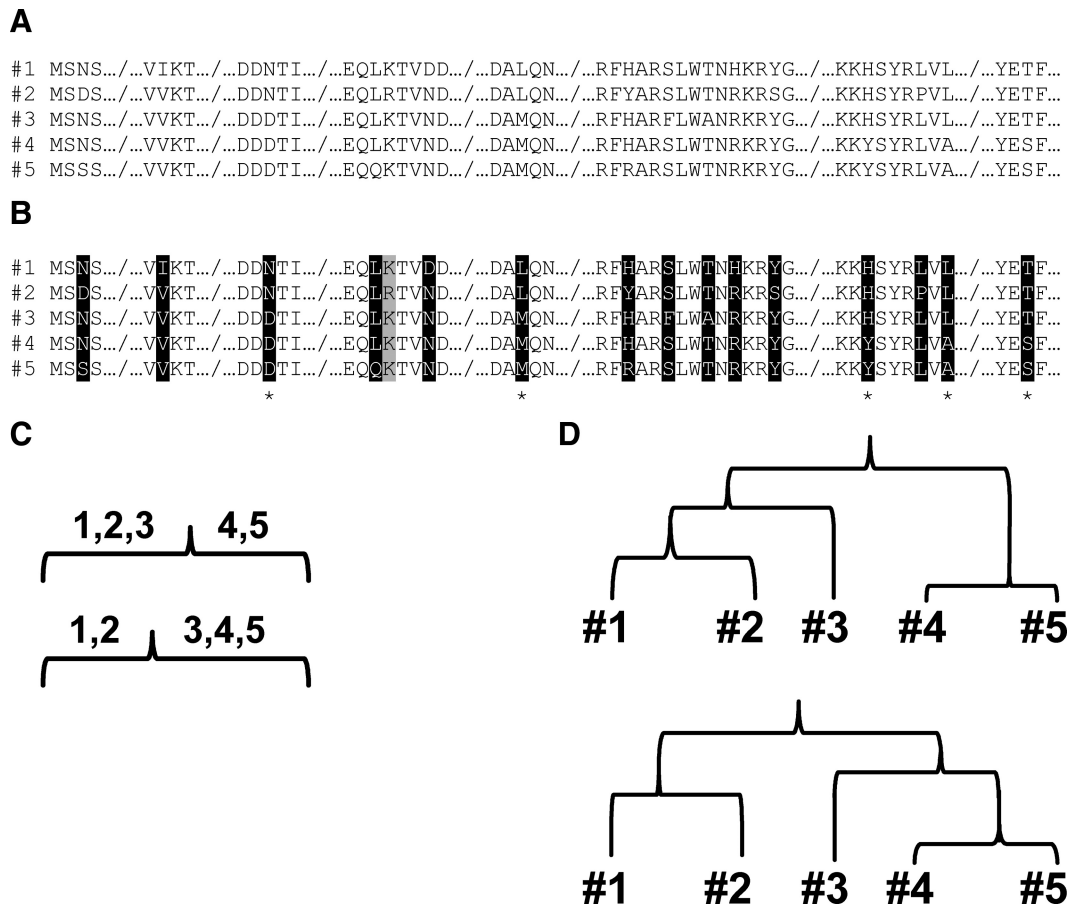

Figure 2. (A) Aligned amino acid sequences from the *SCML1* gene product of the following species: #1 = *Homo sapiens*, #2 = *Pan troglodytes*, #3 = *Gorilla gorilla*, #4 = *Pongo pygmaeus*, and #5 = *Macaca mulatta*. The symbol …/… indicates a long stretch of amino acids with no differences among the species. (B) The discrete amino acid differences among the five protein sequences have been highlighted. The asterisks indicate key positions that help reveal ancestry. (C) The two most likely phylograms indicating the first (most distant) divergence of the species based on these protein sequences. (D) The two most likely complete phylograms indicating the ancestry of the species based on these protein sequences.

multiple lines of evidence from different methodologies, resulting in an inherently self-correcting march toward a more detailed understanding of the natural world.

Activity Three: Electron Density Maps of Chromosomes

In the final activity, students are given chromosomal maps (cytogenetic ideograms) of a few of the larger chromosomes from four different species (Murphy *et al.*, 2005). This opens up a short discussion about euchromatin versus heterochromatin, and how and why some DNA is kept "silent" (Yunis and Prakash, 1982). The maps are shown in Figure 3A and are provided to students with the chromosomes clearly arranged by species. Students are instructed to cut each chromosome out and compare them to each other in a search for homologous chromosomes shared by all four species. After

some time, most student groups identify the three sets of homologues shared by all species (Figure 3B).

Concentrating only on the three sets of homologues, students are challenged to make qualitative comparisons about the similarities and shared features of the homologues, and in so doing, infer the relatedness of the four species. Through a process of hypothesis testing, the students work through the three sets of four homologous chromosomes and most come to recognize that species #1 and #4 are markedly more similar to each other than to the others, and the same is true for species #2 and #3. Thus, most students begin their phylogram as shown in Figure 3C. However, before the students simply further branch the two sides into symmetrical final branches, a new challenge is given. Students are asked to make a hypothesis regarding which divergence occurred more recently. In other words, students were asked to return to the sets of homologues and make qualitative judgments regarding which pair shows more
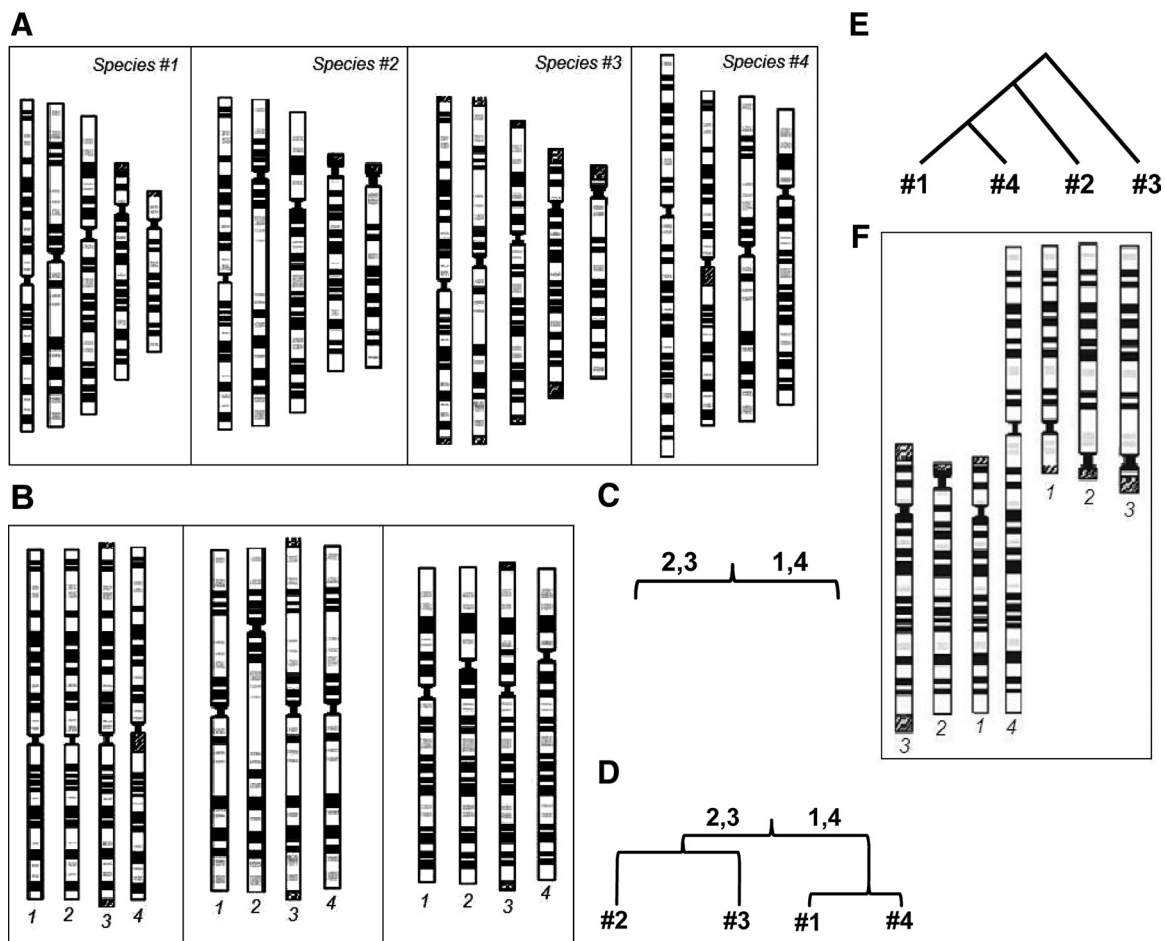

Figure 3. (A) Chromosomal maps (cytogenetic ideograms) for assorted chromosomes from the following species: #1 = *Pan troglodytes*, #2 = *Pongo pygmaeus*, #3 = *Gorilla gorilla*, and #4 = *Homo sapiens*. (B) The same chromosomes, but arranged by homologues that are shared by all four species. (C) The two most likely phylograms indicating the first (most distant) divergence of the species based on the degree of similarity among the chromosomal maps of the homologues. (D) The two most likely complete phylograms indicating the ancestry of the species based on the chromosomal maps. (E) A cladogram showing the ancestry expressed in 3D. (F) The arrangement of chromosomes showing how species #4 has one unique chromosome with two long arms, each of which shares substantial similarity with other chromosomes from the other species. This is evidence that this long chromosome in species #4 is actually the result of a chromosomal fusion of two smaller ancestral chromosomes.

similarity in their chromosome heat maps: #1 with #4 or #2 with #3. Such patterns of similarity can provide one line of evidence regarding the relative relatedness of the species in terms of evolutionary time (Nachman and Crowell, 2000; Murphy *et al*., 2005).

Importantly, each student group will attack this problem with a slightly different approach and this diversity of methodology is encouraged—there is no "right way" to solve the problem and no "answer key" that will verify the correct answer. This reflects how science really works: We speak in "weight of the evidence" and theories that are supported by "multiple lines of reasoning," not in the absolutes of "correct answers" and foregone conclusions. Additionally, the challenge of deducing relative age of the branch points (Figure 3D) in this exercise provides a nice opportunity to connect with another common way of representing evolutionary relationships: the cladogram. Although cladograms are usually constructed based on shared and derived characteristics, they share with phylograms the fundamental basis of evolution and shared ancestry. Thus, students gain important understanding by learning how to interpret both. Figure 3E shows a cladogram that expresses the conclusion that the divergence between species #2 and #3 occurred earlier than the divergence between species #1 and #4.

Following the construction of the phylograms, but before we move to the final discussion, we "resurrect" the outlier chromosomes previously set aside because they did not form part of a homologue set shared by all species. It is obvious that the real outlier is the very long chromosome from species #4. We ask students to set this chromosome in front of them and compare to the other outlier chromosomes, especially those from the species that is most related, which they now know is species #1. The realization being sought is that the lone outlier chromosome from species #4, which has no homologue in the other species, has regions of very substantial similarity with *two* of the other outlier chromosomes from the other species, as shown in Figure 3F. Ayala and Coluzzi (2005) inferred that an ancestor of species #4 possibly suffered a mitotic catastrophe that was repaired erroneously through the fusion of two different chromosomes together. This opens a discussion of chromosomal breakage and repair phenomena such as fusions, translocations, etc.

Because the activities are now finished and the session is about to proceed to the postlab discussion, this is a perfect opportunity to "break the code" and tell the students that "species #4" in activity three is actually *Homo sapiens*. Humans indeed have one fewer pair of chromosomes (23) than all other living primates (Zhu *et al*., 2007). From these analyses, scientists have concluded that the second longest human chromosome is actually the result of a fusion between two smaller chromosomes (#12 and #13 in chimps and great apes), which occurred in a primate ancestor of humans within the last 3 million years (Ijdo *et al*., 1991). This conclusion is strongly supported by extensive DNA evidence, such as the presence of two telomere-like stretches arranged end-to-end within chromosome #2 and the remnants of an additional centromere (Wienberg *et al*., 1994; Navarro and Barton, 2003; Zhang *et al*., 2004). All of these concepts, especially if they have previously been covered in lecture, provide an

excellent discussion with the students to connect this exercise to other material covered in introductory biology.

The Postactivity Discussion

The discussion at the end of the activity is crucial for "driving home" the main points of this pedagogical method. Several points should be explicitly stressed during this discussion (see Supplemental Material 2). First, the sequences shown in Figure 1, A and B were selected for this exercise essentially at random. There is no reason to think that these genes are somehow exceptional and that selecting other genes would paint a significantly different picture. In fact, if an Internet connection is available, these sequences can actually be used to break the code of which species is which, using the BLAST bioinformatics search tool at the National Library of Medicine's website (http://blast.ncbi.nlm.nih.gov/Blast.cgi). This means that the hypothetical phylograms built in activity one can now be redrawn with the species names shown in Figure 4A.

The second activity involves five species, and once again the protein sequences shown in Figure 2 are real, and the identity of these can be revealed through a protein BLAST search. At this point in the discussion, we point out that the SCML1 protein sequences and the GULO pseudogene sequences both led students to conclude that humans and chimpanzees are more related to each other than to macaque and orangutan and vice versa. This reinforces the concept of "multiple lines of evidence." However, because gorilla was not included, the activity one phylograms cannot help re-
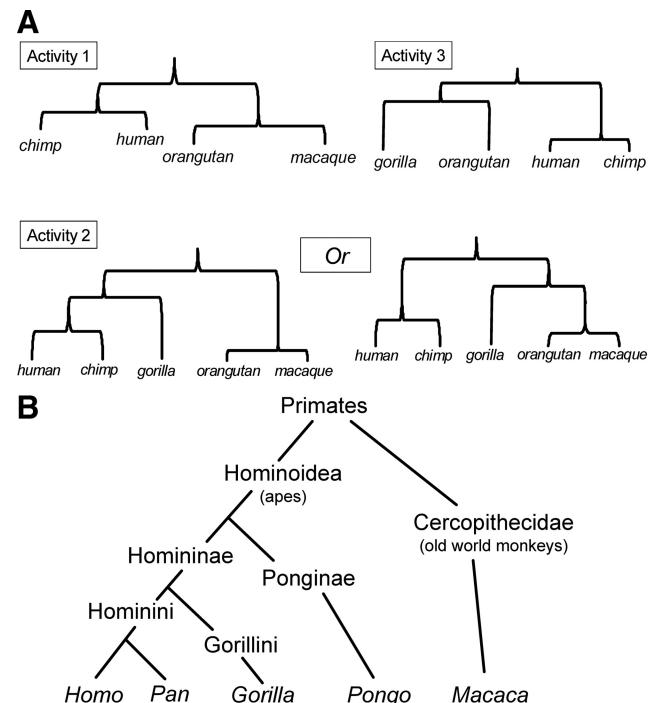

Figure 4. (A) The phylograms derived from the three exercises with the species identities revealed. (B) A representative cladogram expressing the current scientific consensus regarding the shared ancestry of the five genera examined in this exercise.

solve the unanswered question of how gorilla best fits into the evolutionary scheme. For evidence on this question, we move on to activity three.

For activity three, one cannot easily do a bioinformatics search with the chromosome maps. However, an Internet search with the terms "chromosome map [species name]" will show similar examples of these maps so that students can see that these are indeed real maps from these four species. Further, with the addition of the third phylogram, students can now address the question left unresolved from activity two—where gorillas fit into the evolutionary scheme of the apes. The annotated phylogram shown in Figure 4A argues that gorilla and orangutan share a more recent common ancestor than gorilla does with humans and chimps. Thus, the students can return to the sequence data from activity two and observe that, although there were three incidents of gorilla sequence matching humans and chimps and only two where the gorilla sequenced matched with the orangutan and macaque sequence, the chromosome density maps argue that the gorilla is more closely related to orangutans than to humans or chimps. This demonstrates the need for more and longer sequences for comparisons and how evolutionary relationships are explored through many overlapping methods in order to reach a more solidly founded conclusion.

At this point in the discussion, it is often powerful to demonstrate how the phylograms constructed by the students compare with phylograms drawn by experts in the evolutionary biology of apes and humans (Zhu *et al.*, 2007). If an Internet connection is present, simple Internet searches for "phylogram [species names]" will produce hits that link to different phylograms. Importantly, many different phylograms will be found, with different groupings based on which species and taxonomic groups are included. This helps to underscore the concept that phylograms are drawn to express relationships between species of interest: they are not meant to be all-inclusive. Figure 4B shows the current scientific consensus regarding the evolutionary history of the five genera involved in this activity.

ASSESSMENT OF THE ACTIVITY

As this activity was designed, implemented, and refined, we took efforts to assess the degree to which it accomplishes the original goal of gains in student learning through explicitly engaging the scientific process. Toward that end, we monitored several aspects of the student experience. First, each term we collected student work and assessed how successful they were at completing each activity as expected. This resulted in substantial revisions of the activity worksheets and refinement of the activity itself. These revisions to the exercise improved the students' ability to understand and complete the challenges such that, in the present form, the success rate is >80%, >70%, and >60%, respectively, for the three activities (data not shown). The lower rate of success indicates progressively challenging activities, but we observe that even students who are unable to reach the expected conclusions on their own are able to comprehend the methodologies during the postactivity discussions. We have wondered whether guided inquiry or a problem-based research approach assist the students with these challenges.

A second form of assessment that we analyzed was the performance on lecture exam questions related to this topic. For this comparison, we arranged a case-control experimental design and two different sections of Bio104 were selected. Both groups had 28 students and the same instructor for the lecture part of the course, in which all course topics are taught. The control group completed a traditional laboratory exercise on evolution, phylogeny, and classification: chapters 20 and 21 of *Biology in the Laboratory* (Helms *et al.*, 1998), while the experimental group completed the exercise described here. Then, we compared performance on the course exam, which is common among all sections and is relatively unchanged year to year.

As Figure 5A shows, the two groups' general exam scores indicate that the control group was composed of measurably higher-performing students than the experimental group. However, because this difference is <10% and within the 95% confidence interval for each group, we considered the groups comparable for the purposes of this assessment. We identified three questions on exam one that specifically address the issue of phylogenetics and the deduction of evolutionary relationships (described in *Experimental Methods*). Importantly, both groups were taught this material by the same instructor, and both groups worked from the same textbook, from which these three questions derived (*Biology, 7th ed.* (Campbell and Reece, 2005). Figure 5A shows that, despite scoring lower on the exam overall, the experimental section slightly outperformed the control group on all three of these select exam questions. Although these differences are not dramatic, they are consistent, especially when considering that phylogeny was just one concept on an exam covering four weeks' worth of material.

Finally, we performed a third mode of assessment aimed at inferring student perceptions regarding evolution. As part of an ongoing assessment project regarding teaching the process and nature of science, we utilized pre- and postsurveys to scrutinize student perceptions regarding the scientific theory of evolution by natural selection, how those perceptions are affected by learning more about the theory in a formal biology course, and what role, if any, this activity plays in the alteration of those perceptions. For this inquiry, we used the same control and experimental groups described above. At the beginning of the semester, both groups were given a survey instrument previously validated to reveal student perceptions regarding evolution and natural selection. Then, both groups were surveyed again 2 wk after the first examination, which was thus 1 mo after the execution of this experimental laboratory activity. More detail regarding the composition and scoring of the survey instrument is included in the *Experimental Methods* section. Briefly, all survey responses were scaled 1–5 and calculations were performed to yield a "percent change" value for each question, with a positive value indicating an increase in acceptance of the scientific theory of evolution by natural selection.

As seen in Figure 5B, a noticeable difference between the two groups was observed. In the control group, depending on the particular question, group responses sometimes reflected slightly increased acceptance of evolution and sometimes indicated slightly decreased acceptance of evolution. The experimental group, however, responded to instruction about evolution in a dramatically more consistent manner. Regardless of the question, the average scores on all ques-
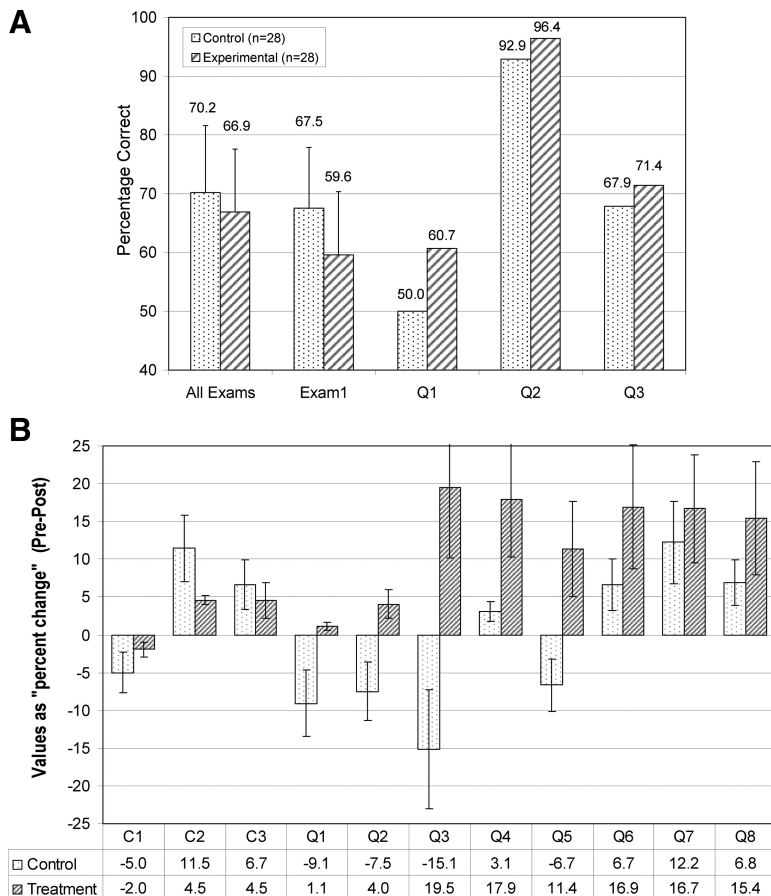
A

Legend:
- Control (n=28)
- Experimental (n=28)

Y-axis: Percentage Correct

Values (Control, Experimental):
- All Exams: 70.2, 66.9
- Exam1: 67.5, 59.6
- Q1: 50.0, 60.7
- Q2: 92.9, 96.4
- Q3: 67.9, 71.4

B

Y-axis: Values as "percent change" (Pre-Post)

	C1	C2	C3	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8
Control	-5.0	11.5	6.7	-9.1	-7.5	-15.1	3.1	-6.7	6.7	12.2	6.8
Treatment	-2.0	4.5	4.5	1.1	4.0	19.5	17.9	11.4	16.9	16.7	15.4

Figure 5. (A) Performance of the control and experimental sections of students on all course exams, the first exam, both with error bars indicating the 95% confidence interval, together with three specific exam questions that explicitly test comprehension of phylogeny and natural selection. (B) Percent change of average student responses to eight questions on a pre- and posttest survey measuring acceptance of the modern theory of evolution by natural selection. Positive values indicate an overall group change toward more acceptance of modern evolutionary theory, while negative values indicate change toward less acceptance. The text of the survey questions and a description of the calculations are found in the *Experimental Methods*.

tions concerning the acceptance of evolution showed an upward deflection, indicating that, as a whole, the group more consistently came to accept the scientific validity of modern evolutionary theory. This provides support for a breakthrough study (Lombrozo *et al.*, 2008) that found that student perceptions and acceptance of the theory of evolution are directly impacted by their understanding of the nature and process of science and research.

CONCLUSIONS

The inquiry-based student activity described herein is a novel approach toward the instruction of the practice of molecular phylogeny and systematics. Such approaches are strongly mandated, both because of recent threats to proper biology education in our country due to poor understanding of evolutionary theory (Miller *et al.*, 2006; Ayala, 2008) and because this approach has been shown to be more effective than traditional approaches to teaching (O'Hara, 1997; Robbins and Roy, 2007; Lombrozo *et al.*, 2008). Although the skills that are required and reinforced by this group exercise are part and parcel of most any introductory biology curriculum, these activities may also be applicable to students in biology courses at the nonmajor and even secondary education levels. No advanced quantitative skills are necessary, nor is a high-level understanding of molecular biology or

evolutionary theory. In fact, these exercises are designed to help enlighten these very concepts to students.

Educators who use educational innovations involving student-centered learning modalities have often encountered student resistance (Giroux, 2001). This has been specifically noted in various inquiry-based methods in science education (Anderson, 2002; Hofstein and Lunetta, 2004) and in efforts to explicitly teach the process and nature of science (McComas *et al.*, 2006). Indeed, in our implementation of these activities, we encountered some initial resistance among our students. This is not surprising, given that introductory science students are often accustomed to being given precise experimental protocols and being told exactly how to proceed in their laboratory courses. Thus, the resistance and confusion we observed was generally limited to the first initiation of the activity as students are instructed to examine the DNA sequences in activity one. During this period, we consider it crucial that the instructor not give in and simply walk them through the activity. One of they key features of our educational approach is that students must actively consider the data, contrive different possible methods of analysis, and decide on the strategy they think is best. That there may be a multitude of approaches used by a given class of students is a strength of inquiry-based learning, helping students learn to think for themselves regarding the interpretation of data (Hanauer *et al.*, 2006).

By completing these exercises, students will mimic the scientific process engaged by contemporary biologists. First of all, the technique of comparative genomics is at the forefront of evolutionary biology, anthropology, structural and molecular biology, and even medical genetics. The first activity provides students with a familiarity of concepts and techniques that they are likely to read or hear about in reports of scientific discoveries in scientific journals and the popular press. Second, students also execute several distinct comparisons, with completely different sources of data, in an effort to explore a single concept: the descent of humankind from primate ancestors. This underscores the scientific practice of pursuing multiple lines of evidence when approaching unresolved scientific questions. Third, the collaborative, cooperative nature of science is illustrated because students are encouraged to work in small groups but also collaborate with other groups. Fourth and perhaps most important, in these exercises, students are not working toward a preconceived conclusion, using a predetermined series of steps, only to reveal something that they probably already learned about as a "known fact." Instead, students are encouraged to use their prior scientific knowledge, design their own approaches, draw their own unique conclusions, and identify the data that support those conclusions. Such pedagogical approaches have been shown in a variety of contexts to facilitate significant gains not just in content learning but in the understanding and internalization of broad concepts.

In addition, by withholding the identities of the species in question, students who may have been resistant to the concept of human evolution from primate ancestors are encouraged to let their guard down and work freely on the project at hand. While this aspect of the exercise is by no means required, it is our hypothesis that this could help break through the psychological resistance that some students have to the biological understanding of human origins. We are bolstered in that belief by our survey results, which reveal that, on average, students who explore the concept of phylogeny in this manner are more likely to make gains in their acceptance of modern evolutionary theory than those who complete a more traditional laboratory exercise. We hope that this laboratory exercise will inspire further such approaches and that the arsenal of process-oriented inquiry-based tools for teaching evolutionary theory will continue to grow. In so doing, we can help reverse some of the disturbing trends regarding public acceptance of evolutionary theory, as well as help to educate more budding young scientists about the true nature, process, and practice of science.

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