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Evolution & Phylogenetic Analysis: Classroom Activities for Investigating Molecular & Morphological Concepts

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Evolution & Phylogenetic Analysis: Classroom Activities for Investigating Molecular & Morphological Concepts
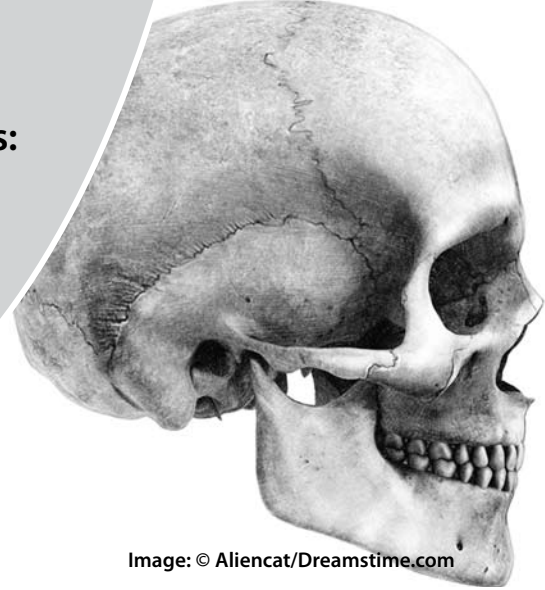
WILFRED A. FRANKLIN

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Abstract

In a flexible multisession laboratory, students investigate concepts of phylogenetic analysis at both the molecular and the morphological level. Students finish by conducting their own analysis on a collection of skeletons representing the major phyla of vertebrates, a collection of primate skulls, or a collection of hominid skulls.

Key Words: *Evolution; phylogenetic analysis; student inquiry; parsimony; convergence.*

"Nothing in biology makes sense except in the light of evolution." The title of Theodore Dobzhansky's 1973 essay captures the importance of evolution in today's biology curriculum. Given evolution's central role in modern biology, the absence of abundant curricula is surprising. After years of being unsatisfied with using fictitious paper cut-out creatures to demonstrate phylogenetic processes, I set out to demonstrate large-scale evolutionary patterns and phylogenetic processes by developing hands-on activities based on real organisms. Several good experiments using Wisconsin FastPlants (http://www.fastplants.org/activities.students.evolution.php), fruit flies (Kennington et al., 2003), or bacteria (McCarty & Marek, 1997; Weldon & Hossler, 2003) demonstrate natural selection through phenotypic changes in populations. Important evolutionary concepts are also covered by simulations and virtual activities (see "Understanding Evolution," http://evolution.berkeley.edu/evolibrary/home.php).

But as Bransford et al. (1999) demonstrated, learning retention and depth increase as active engagement increases. The importance of evolution as a unifying principle demands engaging activities based on real species and the exploration of real evolutionary questions. The activities described here begin to explore such questions. What is the evidence for or against current relationships between species? What kind of evidence do biologists use to develop phylogenetic trees? How does one discriminate between opposing theories in evolutionary biology and phylogenetic systematics? (For examples of three activities that tackle similar questions, see McMaster, 2004; Flory et al., 2005; Baum & Offner, 2008).

In designing my evolution lessons, I selected activities that utilize vertebrate skeletons and skulls of primates and hominids. Students begin

> *The importance of evolution as a unifying principle demands engaging activities based on real species and the exploration of real evolutionary questions.*

with a materials-based problem set that introduces them to concepts of phylogenetic analysis, including homology, convergence, parsimony, and ancestral versus derived characters. The project concludes with students conducting a phylogenetic study on a set of vertebrate skeletons, primate skulls, or hominid skulls and then giving presentations comparing their findings with published results.

I will discuss three separate activities that occur over the course of three consecutive 3-hour laboratory periods. Because of the modular nature of this lesson plan, the series of activities can be further broken down into any number of sessions to allow for various scheduling needs. These activities were originally designed for undergraduates in an introductory biology course, but several components have been used for 7th and 11th graders during an outreach program funded by Howard Hughes Medical Institute (HHMI) and sponsored by Bryn Mawr College (for suggested adaptations for various age groups, see Table 1). The outreach project is fully archived at http://www.brynmawr.edu/scienceresearch/FridaysinLabHomepage.html. The bone material is readily available from various teaching-supply companies, and Mesquite is a free, Web-based phylogenetic software package (http://mesquiteproject.org/mesquite/mesquite.html) that is easy to run from any computer with up-to-date Web browsers (for a materials list, see Figure 1).

○ Background

Before students begin activities 1 and 2, we discuss Darwin's idea of descent with modification and what that implies for the diversity of organisms on earth. If descent with modification is the mechanism of phylogeny, or the patterns of relationship among organisms, then similarities exist between organisms because they share a common ancestor. Based on the same principles that make siblings more similar than cousins, similarity between species can be used to make inferences about the evolutionary relationships between them. Similar characteristics are called "homologous characters" if the similarity is due to common ancestry. Not all similarities are homologous. Convergent evolution can result in similarities because selection pressures push widely divergent species into similar forms. Marsupial sugar gliders of Australia and placental flying squirrels of North America

Table 1. Suggested adaptations for three different class levels.

Middle School	High School	College
Omit molecular aspects of activities or add a lesson on DNA and/or protein structure.	Add a lesson on DNA and/or protein structure and allow for time to compare molecules as one would compare morphological structures in bone activities.	Use NCBI website to build DNA and protein alignments or ask more advanced students to build their own alignments with NCBI tools.
Omit character polarity terminology, instead emphasizing character change.	Omit polarity terminology, instead emphasizing character change, but challenge students to think about ancestral versus derived.	Treat character polarity as described in article.
Omit bone station on building a tree on hominid skulls or design a separate lesson/activity allowing for more guidance.	Allow for more time to explore bone station on hominid skulls.	Treat hominid bone station as described in article.
Omit activity 2 or expand it to form a teacher-guided activity instead of a take-home assignment. (*For an in-depth treatment of "tree-thinking," see Baum & Offner, 2008.*)	Expand activity 2 and make it a teacher-guided activity instead of a take-home assignment. (*For an in-depth treatment of "tree-thinking," see Baum & Offner, 2008.*)	Treat activity 2 as described in article.
Omit Powerpoint presentation or allow more time to help students develop presentations.	Allow more class time to help students develop presentations.	Treat activity 3 as described in article.

Figure 1. Materials list.

Limbs & miscellaneous bones
- Bony fish forelimb
- Cat forelimb
- Cow hindlimb
- Lemur skull
- Horse hindlimb
- Large bird hindlimb
- Lizard forelimb
- Manatee forelimb
- Rabbit forelimb

Vertebrate skeletons
- Bat
- Bird
- Cat
- Dog
- Human
- Monkey
- Opossum
- Salamander/Mudpuppy

Primates skulls
- Baboon
- Chimp

- Gibbon
- Gorilla
- Lemur
- Orangutan

Hominid skulls
- 2 *Homo sapiens*
- *Australopithecus robustus*
- *A. africanus*
- An infant *A. africanus*
- *H. heidelbergensis*
- *H. neaderthalensis*
- *H. erectus*

Software
- Mesquite Phylogenetic Package
- MS PowerPoint
- Cn3D

Miscellaneous
- Protractor
- Rulers
- String

are two of many examples of similarities in morphology that resulted from convergent evolution. Through the study of homologous characters – whether by comparing molecules like DNA, physical characteristics like anatomical traits, or fossil characters – phylogenetic trees can be made that reflect the relationships among organisms. Understanding and scrutinizing this process is how evolutionary theory advances.

Three important steps in phylogenetic analysis are (1) determining homology, (2) determining ancestral versus derived characteristics (i.e., character polarity), and (3) using parsimony as a criterion for judging between alternative trees. (Parsimony is addressed at the beginning of activity 3.)

Homology is determined by three lines of evidence: morphology, relative position in relation to other features, and embryological development of the feature of interest. When considering bones, relative position is, in part, a proxy for watching the development of that bone. Because bones, especially limb bones, can be morphologically variable as a result of adaptations, relative position is a useful tool to help determine homology. Two bones in different organisms may look significantly different but articulate with the exact same types of bones. This is a good indication of bone homology, even if the bones look vastly different, because change in the complex, interconnected developmental program for an entire limb is much less likely than the final shape of any one particular bone in that limb.

"Character polarity" is a term used to describe variation in homologous characters with regard to how they have changed through time. Imagine that a novel character has just evolved in a new species. As this species radiates and gives rise to other new species, the novel character can either stay the same or change. If the character changes and that variation is passed on to new species, the original form of the character is known as the "ancestral character state," whereas the new form is referred to as the "derived character state." For example, assume that a parent population of vertebrates had short limbs and gave rise to daughter species that had long limbs and short limbs. Short limbs would be considered the ancestral character state and long limbs would be considered derived. This information can then be used to reconstruct the evolutionary relationships among a group of extant species. Given that there is no way to look back in time to discover how characters – and, thus, species – have changed, fossils give the best evidence for determining character polarity. In lieu of fossils, out-group analysis can be used. Out-group analysis starts by finding a living species distantly related (but not too distantly) to the group of organisms in which the character polarity is under question. Assuming that the out-group also shared a common ancestor, but less recently, one can predict that the shared form of the characteristic is ancestral. For example, if the in-group of organisms under consideration has some members with long limbs and some with short limbs, the question becomes "which came first?" – that is to say,

which was the character state of the common ancestor? If the out-group has short limbs, it can be inferred that short limbs were the ancient state of the in-group. Why is that the case? It is more likely that short limbs evolved only once in a common ancestor and were passed down to both the in-group and the out-group, rather than evolving separately in both. If long limbs were assumed to be the ancestral trait of the in-group, then short limbs would have had to evolve once in the out-group and another time in some of the members of the in-group. This concept will be explored in more detail during the laboratory exercises.

○ Activity 1: Molecular & Morphological Character Homology & Polarity

In the first 3-hour session, students cycle through a series of stations that contain collections of bones and computers with DNA and polypeptide sequences set up for manipulation on the National Center for Bioinformatics (NCBI) Web site. If scheduling requires, this portion of the module can be broken into one session on morphological features and another on molecular features. Further, DNA and protein alignments can be printed from NCBI, and the time needed to learn to use the NCBI database can be avoided altogether. I use this activity as an opportunity for students to begin learning about the powerful tools and resources available through NCBI.

I prepare five stations of bones, but any number of stations could be set up, depending on the bones available and the amount of time allotted. There is a station with a collection of forelimbs, a station with ungulate and human hindlimbs, another with vertebrate skeletons of the major phyla, one with great ape skulls plus a human and a dog skull, and a final collection of hominid skulls (for a list of specimens at each station, see Figure 2). I include labeled figures and illustrations of all the specimens at all stations. The goal is for the students to use the figures and bones at the stations to answer a series of questions (for sample questions for each of the bone stations, see Figure 2).

The aim of the forelimb station is to demonstrate the wide range of variation in forelimb morphology and to challenge students to determine the homology between them. At the ungulate station, students are challenged to find homology in organisms with extreme adaptations and to compare ungulate "feet" to human feet. The goal at the vertebrate skeleton station is to use morphological evidence to support a hypothesis of evolutionary relationship based on overall similarity between the specimens. At the primate skull station, students are introduced to out-group analysis for determining character polarity by comparing the locations of the *foramen magnum* in a dog skull and in the various primate skulls. The final bone activity with hominid skulls challenges students to build a phylogenetic tree of the specimens, first using one character and then using several characters that they identify.

The first exercise on molecular homology compares three DNA sequences of varying degrees of similarity. Students go to the NCBI Web site and develop several alignments on a given sequence (for a detailed explanation of how to extract and analyze nucleotide

Figure 2. Sample questions from activity 1.

Limb Homology: Bat, rabbit, cat, lizard, fish, and manatee forelimbs
- From the evidence supplied at this activity and around the room at other activities, what bone or group of bones of the forelimb have had the most selective pressure? Explain your answer.
- What morphological qualities of the limb labeled Specimen A suggest a specialized function? What, if any, is the specialized function? [Specimen A is an unlabeled manatee limb.]
- Match the homologous bones in the rabbit, cat, lizard, fish, and specimen A to the labeled bone in the bat.

Ungulate Limbs: Horse, cow, flightless bird, and human hindlimbs
- The bone labeled #1 is homologous to which labeled bones in the other two limbs? To what bones on the human skeleton?
- In figure 3.2 and figure 3.3, phalanges are labeled with Roman numerals I–V and metarsals are labeled with Arabic numbers 1–5. Using that numbering system, list the bones that are completely lost in specimen A.
- Which bones are extremely reduced in specimen A?
- Of the three organisms (represented by the three limb specimens), which are the most closely related? Draw a phylogenetic tree representing your proposed relationships. (Hint: Start by writing down the taxa. Connect the two most closely related.)

Vertebrate Skeletons: Human, monkey, domestic cat, domestic dog, opossum, mudpuppy, and bird
- Which two specimens (A, B, C, D, E, F, or G – in blue tape) are the most closely related? In other words, which two share a common ancestor most recently?
- What evidence did you use to draw your conclusion? You may answer generally, but give at least one specific line of evidence.
- Is "number of vertebrae in the tail" a good character to use in establishing relationships between vertebrate taxa? Why or why not?

Character Polarity and Out-group Analysis: Baboon, gibbon, orangutan, chimp, gorilla, human, and lemur skulls
- Carefully pick up skulls and locate the foramen magnum (FM) on all specimens. It is the empty, round hole (or filled in with black casting) on the underside of the skull. What is the function of the FM?
- If the lemur (labeled E) is the most distantly related taxon among all the specimens, what do you think is the more ancestral character state, an FM located more medially toward the center of gravity or an FM located more distally farther away from the center of gravity?
- Considering that bipedalism is related to the location of the FM, do you think bipedalism is an ancestral or derived trait?
- List two other characters useful in analyzing skulls and identify the ancestral state for each.

Hominids and Constructing Trees: *Australopithecus africanus,* an infant *A. africanus, A. robustus, Homo erectus, H. heidelbergensis, H. neaderthalensis,* and *H. sapiens*
- Draw a phylogenetic tree of the 7 specimens based solely on the character "size of skull". Use the capital letters as taxon names.
- Do you think overall size is a useful character? Why or why not?
- Draw another tree based on your gut instincts (Gestalt) as to the real relationship between the taxa represented by these 7 specimens. What character(s) most influenced your tree's branching pattern?

sequences from GenBank, see Maier, 2001). This is a great way to introduce students to the site and some of the many tools available there, but it should be attempted only if time is allotted for leading students through the basic workings of the site. If the bones and molecular activities are to be completed in one 3-hour session, then the DNA alignments can be preprinted, thereby avoiding the steep learning curve. Figure 3 shows the preprinted alignments and the series of questions that require students to examine the meaning of molecular homology with regards to DNA. Alternatively, this molecular activity can be omitted if middle school students are unfamiliar

with DNA and protein structure. I found 11th graders well prepared for this exercise as long as enough time and teacher guidance is planned (for suggested adaptations, see Table 1).

The final exercise requires using the NCBI database and associated visualizing tools to explore issues of protein homology. The free visualizing software Cn3D should be preloaded on the computers to be used. The exercise begins with an alignment generated by Blastp from NCBI that shows the differences between two sequences. It is important to find two sequences that have crystal structure data in the NCBI database to allow for superimposed visualization of the two sequences. I used a conserved

Figure 3. DNA sequence alignments from NCBI used in activity 1. **(a)** A comparison between 2 DNA sequences that have been aligned by Blastn at the NCBI website. The gray highlighted region corresponds to the portion of sequence #1 that has putative homology with sequence #3 in the second alignment. **(b)** A comparison of DNA sequence #1 and a different sequence labeled #3. Considering these alignments, answer the following questions: How much sequence dissimilarity is allowable before homology is rejected? How does the length of a sequence affect the amount of dissimilarity that is allowable? Do the sequences in question code for genes, introns, exons, microsatellites, or junk? What type of information would help in determining DNA homology?
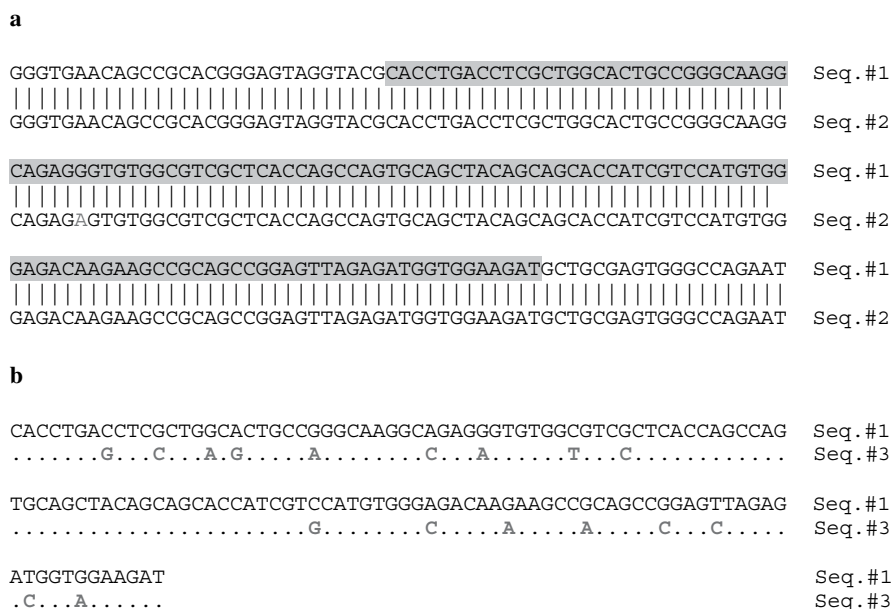
a

```
GGGTGAACAGCCGCACGGGAGTAGGTACGCACCTGACCTCGCTGGCACTGCCGGGCAAGG   Seq.#1
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GGGTGAACAGCCGCACGGGAGTAGGTACGCACCTGACCTCGCTGGCACTGCCGGGCAAGG   Seq.#2

CAGAGGGTGTGGCGTCGCTCACCAGCCAGTGCAGCTACAGCAGCACCATCGTCCATGTGG   Seq.#1
|||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
CAGAGAGTGTGGCGTCGCTCACCAGCCAGTGCAGCTACAGCAGCACCATCGTCCATGTGG   Seq.#2

GAGACAAGAAGCCGCAGCCGGAGTTAGAGATGGTGGAAGATGCTGCGAGTGGGCCAGAAT   Seq.#1
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GAGACAAGAAGCCGCAGCCGGAGTTAGAGATGGTGGAAGATGCTGCGAGTGGGCCAGAAT   Seq.#2
```

b

```
CACCTGACCTCGCTGGCACTGCCGGGCAAGGCAGAGGGTGTGGCGTCGCTCACCAGCCAG   Seq.#1
.......G...C...A.G.....A........C...A......T...C............   Seq.#3

TGCAGCTACAGCAGCACCATCGTCCATGTGGGGAGACAAGAAGCCGCAGCCGGAGTTAGAG   Seq.#1
.......................G........C.....A.....A.....C...C.....   Seq.#3

ATGGTGGAAGAT                                                   Seq.#1
.C...A......                                                   Seq.#3
```

Figure 4. Alignment of two amino acid sequences retrieved from a protein–protein Blast search at NCBI used in activity 1. **(a)** The starting amino acid sequence for a portion of the Period 2 protein, also the translation of the DNA sequence #1 from Figure 1. **(b)** 3PYP, the sequence retrieved from the database that has the most similarity with the target amino acid sequence. 1DRM A is the heme domain of a protein from the bacterium *Bradyrhizobium japonicum,* which has putative homology with 3PYP. These two proteins have been aligned side-by-side to aid in a pairwise comparison. The capital letters were determined by NCBI's protein homology algorithm to be the most chemically similar amino acids and have putative structural homology. Following the steps in Appendix A will allow for visualizing the superimposed 3-D crystalline models of 3PYP and 1DRM A. Then answer the following: From the pairwise comparison of AAs in both sequences, do you think these are homologous proteins? Why or why not? After seeing the 3-D models, do you think that 3PYP and 1DRM A are homologous proteins? Do you think they have a similar function? What evidence would lead you to believe that two amino acid sequences are homologous?
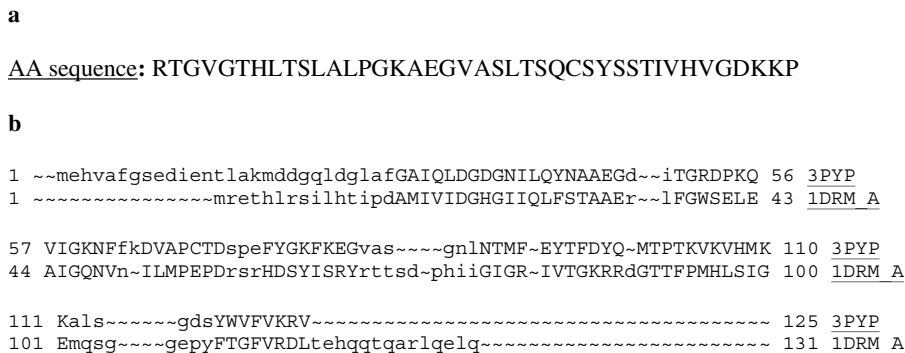
a

AA sequence**:** RTGVGTHLTSLALPGKAEGVASLTSQCSYSSTIVHVGDKKP

b

```
1 ~~mehvafgsedientlakmddgqldglafGAIQLDGDGNILQYNAAEGd~~iTGRDPKQ 56 3PYP
1 ~~~~~~~~~~~~~~~mrethlrsilhtipdAMIVIDGHGIIQLFSTAAEr~~lFGWSELE 43 1DRM_A

57 VIGKNFfkDVAPCTDspeFYGKFKEGvas~~~~gnlNTMF~EYTFDYQ~MTPTKVKVHMK 110 3PYP
44 AIGQNVn~ILMPEPDrsrHDSYISRYrttsd~phiiGIGR~IVTGKRRdGTTFPMHLSIG 100 1DRM_A

111 Kals~~~~~~gdsYWVFVKRV~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ 125 3PYP
101 Emqsg~~~~gepyFTGFVRDLtehqqtqarlqelq~~~~~~~~~~~~~~~~~~~~~~~~ 131 1DRM_A
```

Figure 5. Preclass assignment on building trees from character matrices used in activity 2.
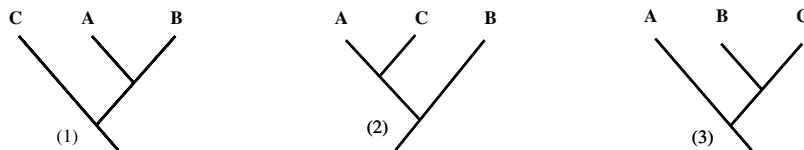
a Cytochrome-*c* amino acid sequence alignments:

```
Species A: a v l I f m f a
Species B: v v l I f p w d

Species A: a v l I f m f a
Species C: a v l g f m f a

Species B: v v l I f p w d
Species C: a v l g f m f a
```
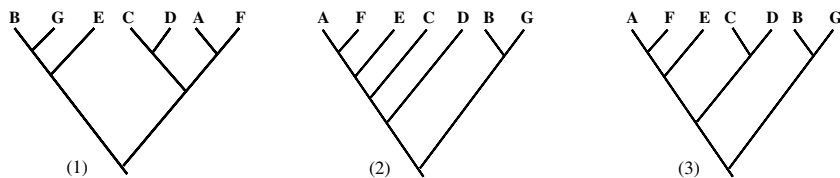
b Simple character matrix for cytochrome-*c* data:

c Possible trees from character matrix:

d Expanded character matrix and possible trees:

	A	B	C	D	E	F
B	14					
C	5	15				
D	5	15	2			
E	3	14	4	4		
F	1	14	5	5	3	
G	14	7	15	15	14	14

portion from the Period 2 gene associated with circadian rhythm, because we studied this gene and protein in a prior lab, adapted from an activity designed by HHMI (http://www.hhmi.org/biointeractive/). The peptide sequence alignment (Figure 4) shows little direct pairwise similarity, but the superimposed 3-D structures of the two sequences show significant overlap. The questions for this exercise probe the relationship between primary structure and final three-dimensional shapes of peptide sequences. Most students realize that because of some functional redundancy in amino acids, primary sequences can vary, whereas the final shape – and, hence, function – may be quite similar, especially if the proteins' active sites are similar. This leads to questions about distinguishing polypeptide homology versus convergence and the need to consider not only peptide sequence and final shape but also the DNA sequence coding for the peptide (for instruction on visualizing the crystal structure, see Appendix A).

Modifications of Activity 1

I have mentioned some minor modifications that allow for scheduling flexibility, but what about alternatives to the expensive (perhaps prohibitively so) use of large collections of bones? One option is to reduce the number of bone stations to match the available collection. If no bone specimens or resources to purchase them are available, the Web site eSkeletons (http://www.eskeletons.org/), developed at the University of Texas at Austin by John Kappelman and sponsored in part by the National Science Foundation, offers a virtual tool that allows comparisons of human and primate bones.

○ Activity 2: Phylogenetic Analysis with Amino Acid Data

This activity is an out-of-class assignment designed to introduce the concept of building and using character matrices to construct phylogenetic trees and is completed before the beginning of the second 3-hour session. The handout, which begins with a brief introduction, gives a three-way alignment of an abbreviated peptide sequence from the electron transport protein cytochrome *c* (Figure 5a). (Maier [2004] published a similar but far more in-depth activity using DNA–DNA hybridization data that is suitable for extending these concepts.) The activity here is simplified for use as an introductory, preclass assignment that will be extended during activity 3. From the given alignments, students are directed to fill in a character matrix (Figure 5b) of the number of differences between the respective aligned sequences and to choose a phylogenetic tree among several trees that reflect the data (Figure 5c). Next, students are given an expanded character matrix representing amino acid data from seven taxa and are again asked to choose a tree that reflects the data (Figure 5d). As students proceed with the worksheet, the taxa (horse, donkey, rabbit, birds, a moth, and a fly) are revealed, and students are ask to circle the taxonomic groups of animals, invertebrates, vertebrates, birds, and mammals. If the groups they have circled on the chosen tree do not form nested, monophyletic groups, they are asked to redo the problem. This exercise introduces students to the concept of character matrices, the way in which trees reflect those matrices, and the relationship between monophyletic groupings and taxonomy of organisms. At this stage, all that is required for students to complete their own phylogenetic analysis is the concept of parsimony.

○ Activity 3: Conducting a Phylogenetic Analysis on Morphological Data Using Parsimony

I begin the second 3-hour session with a class discussion of parsimony, an application of Occam's razor, which Campbell and Reece [2002] define as the search for the least complex explanation for an observed phenomenon. With regard to phylogenetic analysis, this means that trees with fewer character transformations are better theories of evolutionary relationships than trees that require more transformations.

Assuming descent with modification, characteristics are more likely to evolve only once and become passed on to descendants than the alternative, which would require traits to evolve more than once in independent lineages. This alternative can occur and is known as "convergence," but the phylogenetic tree with the fewest changes overall should be considered simpler and thus "better." I go over several examples with the class, one of which is an adaptation of Campbell and Reece's (2002) figure 25.16. This example compares two alternative trees of a lizard, bird, and mammal. The presence of a four-chambered heart is mapped onto the two alternative trees. The four-chambered heart appears only once in a tree with birds and mammals sharing the most recent common ancestor, whereas the trait occurs twice in the tree with lizards and birds sharing the most recent common ancestor. In this example, parsimony is misleading. During the class discussion, students recognize that parsimony will resolve the "best" tree only by considering many characters.

Once parsimony analysis has been discussed, teams of four students conduct a phylogenetic analysis on a group of organisms using skeletal characteristics. The student teams choose to work on a set of seven full skeletons of the major phyla of vertebrates, seven primate skulls, or seven hominid skulls (they can be the same specimens from activity 1). Students begin by generating a list of characteristics that vary across their study specimens (for an in-depth activity on quantifying a hominid skull, see Robertson, 2007). Next they identify a method of qualifying or quantifying the variation and define the character states that describe the variation (all they need is a ruler, a protractor, and a string for measuring circumferences). This becomes a simplified representation of the morphological differences in the various organisms, known as a "character matrix." Using Mesquite, student teams enter their data into a "matrix" file. Next, Mesquite generates a small subset of all possible trees that represent the most plausible phylogeny (on the basis of a defined selection criterion, like parsimony). Finally, students manipulate the trees with the simple drag-and-click interface designed to further investigate unresolved characters (e.g., those that have no clear best solution, possibly because of convergence). See Appendix B for instruction on using Mesquite.

○ Assessment Activity

Presentations by teams serve as a useful assessment option during the final 3-hour session. The assignment asks student teams to develop a presentation in Microsoft PowerPoint® outside of class to compare their findings with published data. This creates opportunities for students to learn about the most recent theories of evolution in their group of organisms, as well as to comment on the differences between the two sets of findings. Because most of the students' findings are very close to recently published work, this reinforces the robustness of the methods while also pointing to some of their limitations. In addition, the presentations offer an opportunity for peer-to-peer learning as the class hears about the other sets of organisms studied. Alternatively, if younger students are the audience, a separate class could be allotted to help students develop PowerPoint® presentations. Recently, I assigned a final one-page report on the merits and limitations of phylogenetic analysis. The assignment, which asks students to use details from the presentations to support their conclusions, compels each student to engage in their peers' presentations and adds an individual component to the PowerPoint® group assessment.

○ Discussion

Evolutionary processes, large-scale patterns, and the generation of evolutionary knowledge can be difficult for introductory students, and few hands-on activities exist to help. Evolution is made tangible and the level of student engagement enhanced by using real species and real artifacts, rather than simulations or virtual species (cf. Lemke, 1992). Skeletal artifacts from hominid specimens are particularly interesting to students. The topic of human evolution is rife with controversy, misconception, and mystery. If handling a replica of "Lucy's" skull can stimulate further questions about evolution in general, then this laboratory module has served its purpose. Furthermore, combining this module with labs on Wisconsin FastPlant selection (http://www.fastplants.org/activities.students.evolution.php) and reconstruction of fish phylogeny from protein electrophoresis data (Bio-Rad Laboratories, 2006) offers students broad exposure to many key concepts of evolution from many subdisciplines of biology.
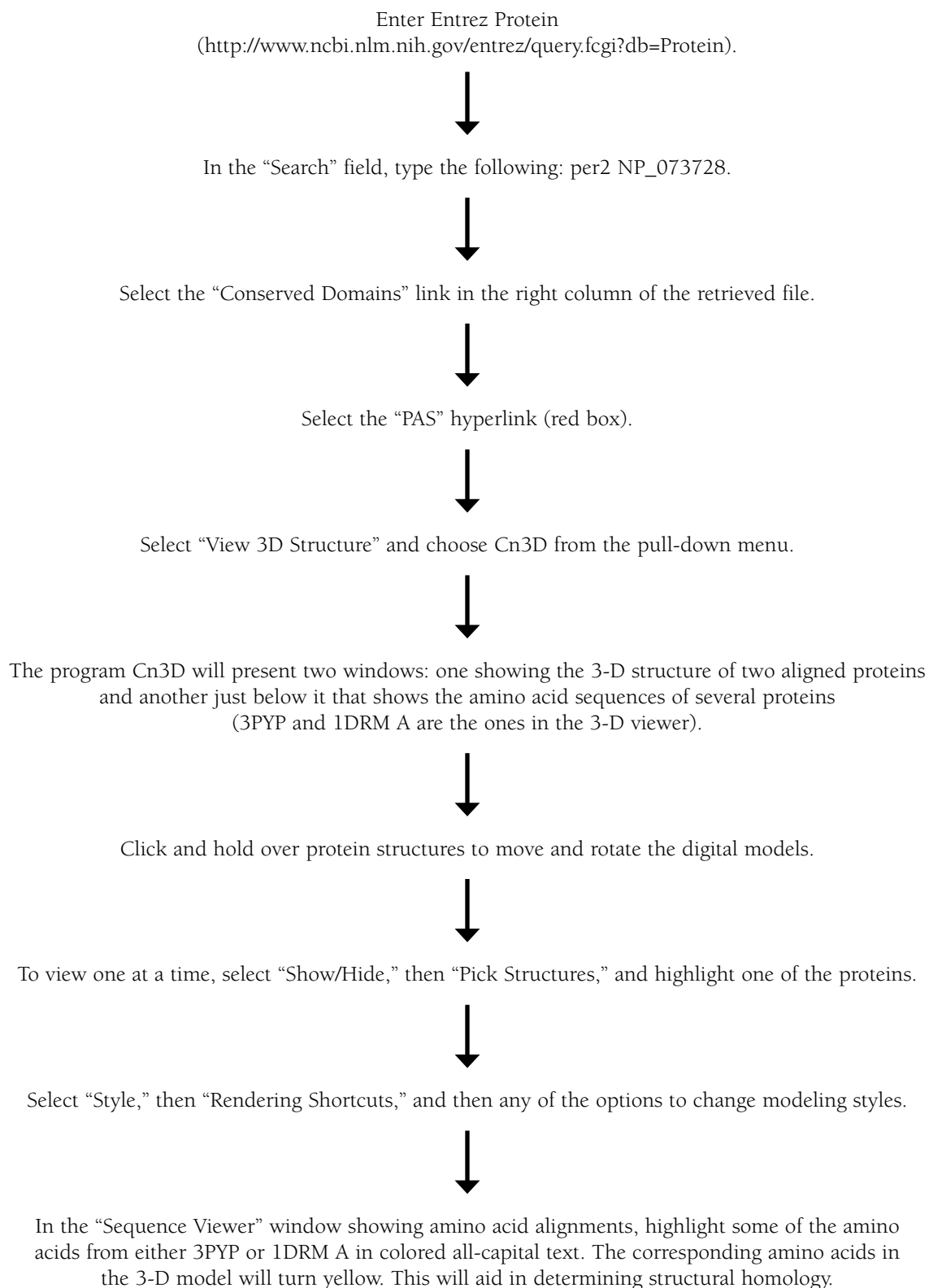
○ Acknowledgment

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Appendix A. Flow chart for visualizing protein crystal structure using Cn3D.

Enter Entrez Protein
(http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Protein).

↓

In the "Search" field, type the following: per2 NP_073728.

↓

Select the "Conserved Domains" link in the right column of the retrieved file.

↓

Select the "PAS" hyperlink (red box).

↓

Select "View 3D Structure" and choose Cn3D from the pull-down menu.

↓

The program Cn3D will present two windows: one showing the 3-D structure of two aligned proteins
and another just below it that shows the amino acid sequences of several proteins
(3PYP and 1DRM A are the ones in the 3-D viewer).

↓

Click and hold over protein structures to move and rotate the digital models.

↓

To view one at a time, select "Show/Hide," then "Pick Structures," and highlight one of the proteins.

↓

Select "Style," then "Rendering Shortcuts," and then any of the options to change modeling styles.

↓

In the "Sequence Viewer" window showing amino acid alignments, highlight some of the amino
acids from either 3PYP or 1DRM A in colored all-capital text. The corresponding amino acids in
the 3-D model will turn yellow. This will aid in determining structural homology.

Appendix B. Building & analyzing phylogenetic trees using Mesquite.

Setting Up a New File

1. Select **File** from the top menu bar and choose "New."
   a. Name and Save file to desktop.

2. In the next window, which will ask if you want to include new taxa:
   a. Check "Make Taxa Block."
   b. Name the taxa block.
   c. Designate the number of taxa.
   d. Deselect/uncheck "Show Tree Window."
   e. Check "Make Character Matrix."
   f. Press OK.

3. Name new character matrix:
   a. Designate the number of characters (type "5" or so; you can add more later).
   b. Select "Standard Categorical Data."
   c. Press OK.

Building a Character Matrix

1. Double click on taxon in rows to rename your taxa.

2. Double click in the empty cell below column numbers to name your characters. (Remember that characters are general descriptions such as "eye color" and that character states are, e.g., blue or brown eyes).

3. Enter your character states into the matrix.
   a. For example, Taxa 1 has blue, round eyes and Taxa 2 has brown, narrow eyes and you coded your characters as follows: 0 = blue, 1 = green, and 2 = brown for eye color; and 0 = round, 1 = narrow, 2 = oval, and 3 = square for shape. You would enter (0, 0) for Taxa 1 and (2, 1) for Taxa 2.
   b. Be sure to type up, in a Word document, a key to your characters and character states for use in your presentation.

4. You may add characters or taxa to your matrix by choosing **Matrix** on the menu bar, then selecting "Add Taxa" or "Add Characters."

Displaying & Analyzing Trees

1. Once your matrix is finished, select **Taxa & Trees** from the menu bar and choose "New Tree Window." Use "Simulated Trees" – OK. Then select "Uniform speciation (Yule)" and OK for "Tree Depth."

2. From Tree Window, select **Analysis** and choose "Tree Legend," then scroll down to "Tree Length" and select.

3. Press OK for "Current Parsimony Model" in next window.

4. Select **Analysis** from menu bar and choose "Trace Character History."

   a. Select "Stored Characters" in window prompt.
   b. Then select "Parsimony Ancestral States" in next window.

5. Click on the Forward and Back arrows in the "Trace Character" view box to see how your characters map onto the tree. As you move through the different characters' traces, take note of the characters that had to arise more than once (i.e., the color of the trace is on two or more separate branches). These are signs of multiple evolutionary events for the same character, which is not likely (not parsimonious). It is evidence of convergent evolution, which is much less likely (in most cases) than a single evolutionary event within a related group/clade. When you see this, try moving the branches together to make one clade of that particular character. Just click on the branch and, while holding the mouse button, drag to the branch you wish to attach it to. (See demonstration in class.)

6. When you swap branches notice the "Tree Length" value. To obtain the most parsimonious, and thus most likely, tree topography, you want to minimize the "Tree Length" value. The tree length reflects the number of evolutionary changes that need to occur in order to map all the character states onto any given tree (any given topography). The more convergences the longer the tree length, because one character state will have had to arise more than once. Keep swapping branches around until you find the lowest value for tree length and the tree pattern (topography) that reflects the evolutionary relationships that make the most "sense" in your group of organisms.

Altering Tree Color & Style

1. Go to **Drawing** on the menu bar and play with any of the color or style options that you feel enhance your tree, or use colors to highlight a particular character of taxa you may wish to talk about in your presentation.

Saving Trees for Your Presentation

1. Go to **File** on the menu bar, select "Save Tree as PDF," then name and locate the file where you can find it.

2. Save as many trees as you want to include in your PowerPoint presentation.

3. Current trees that you are working on may be lost when you close Mesquite. Be sure to capture PDF versions of all the trees you may want. You can always choose not to use them, but better to save many trees for your presentation.

Inserting Your Trees into PowerPoint

1. The PDF file of your saved trees will have a very large blank border. Consequently, if you try to insert the entire PDF file into PowerPoint, it will look awkward.

2. Therefore, open the PDF file, use the "Image Copy" tool to select only the tree parts of the file, and then paste into a PowerPoint slide.