

MOLECULAR SEQUENCES & PRIMATE EVOLUTION

Adapted from C.E. Nelson and M.K. Nickels. 2001

BACKGROUND AND TEACHER PREPARATION

General Introduction

This lesson can be presented as a stand-alone activity, serving as one of many examples demonstrating the close relationship of humans with primates in general, and apes in particular, OR as part of a series of activities providing a concert of independent but congruent lines of evidence for the unique pattern of nested hierarchy exhibited by life forms in general, and primates in particular. The other activities can be found on this site, entitled: "Hominoid Cranial Comparison", and "Comparison of Hominoid Chromosomes."

These three activities (one using amino acid sequence data, one using skull comparisons, and one comparing chromosomes) illustrate three different aspects of human biology and underscore the immense pedagogical value and appeal of emphasizing humans as a central example when discussing the evidence for evolution in biology courses. The combined use of skeletal morphological data with cytological and biochemical data is especially effective with students. All three activities are intended to provide students the opportunity to utilize *real* data, some of it self-determined.

A significant, but often unrecognized result of doing these activities is that it shows why taxonomic classification in biology differs from the categorization of other objects (e.g. furniture, vehicles, hardware [nails, screws, bolts, etc.], miscellaneous junk, etc.) often done in biology classes to illustrate classification. The classification of such human artifacts can be based on different arbitrary criteria, and usually produce very different results depending on the criteria used. In striking contrast, organisms naturally tend to cluster into the same, non-arbitrary, hierarchically-nested groupings, regardless of the sorting criteria, only because of their mutually-shared evolutionary ancestry, due to the congruence across different sets of biological data. For this reason, the authors argue that such classifications of non-biological objects should be *avoided* in teaching biological classification, UNLESS this distinction is made crystal clear. [See the "Nuts & Bolts" lesson on the ENSI site, in which Nickels and Nelson provide the structure and materials for students to explore these facts <<http://www.indiana.edu/~ensiweb/lessons/cl.intro.html>>. These authors have also published an article presenting this material in *The American Biology Teacher*, May 2005, pages 283-289: "Beware of Nuts & Bolts: Putting Evolution into the Teaching of Biological Classification." Ed.]

SYNOPSIS

Students compare differences in amino acids in the beta hemoglobin from representative primates, complete a matrix of those differences, and from these data, construct and interpret cladograms as they reflect relationships and timing of divergence.

MAIN CONCEPT

Modern apes and humans evolved from a common ancestor.

ASSOCIATED CONCEPTS

1. The groups-within-groups hierarchical pattern of Linnaean classification is a result of both extinction and branching from common descent.
2. The evidence that humans have evolved from non-humans is as strong as or stronger than that for evolution within most other groups.
3. Molecular comparisons of different groups generally reveal degrees and patterns of relationship consistent with that revealed by other kinds of comparisons (anatomy, fossils, embryology, cytology, etc.)
4. Cladistics is helpful for showing common ancestry and sequence of evolution between taxa.

MOLECULAR SEQUENCES & PRIMATE EVOLUTION

Adapted from Nelson, C.E. and M.K. Nickels. 2001. Using humans as a central example in teaching undergraduate biology labs. *Tested Studies for Laboratory Teaching*. 22:332-365. Association for Biology Laboratory Education. Online access at <<http://www.zoo.toronto.edu/able/volumes/volume22.htm#19>>

Authors' Introduction for the Primate Molecular Sequence Comparison Lab

This activity has two goals. First, it takes students through the transformations from sequence data to cladograms and molecular clocks. This hands-on analysis greatly facilitates the students' understanding of these concepts. Second, it applies these concepts to the relationships of humans with other primates. Using primates, and emphasizing the great apes (including humans), helps students focus on what is otherwise a major difficulty for many—understanding the strength of the evidence that humans have indeed evolved. Using this activity in conjunction with the hominoid skull exercise and the chromosome comparisons will substantially enhance the effectiveness of each activity in addressing the latter goal. Note that the molecular sequence activity can be done either in a laboratory setting or in small groups in a lecture room.

Materials and Notes for the Instructor for the Sequence Lab (Including expected student results)

Materials, Time and Procedures: The only materials needed are the handouts. Time: One to two 45-55 minute periods, depending on the amount of analysis you want to have the students engage in. (Some or all of the analyses can be done outside of the classroom as homework.)

Discussion of the activities, concepts and results will foster both deeper and more enthusiastic learning. Ideally, students work in groups of 2-5. Option: have different teams count different comparison sets and report to you to; record consensus on overhead. Some classes will benefit from noting the species ID key earlier in the exercise. However, its current placement allows them to analyze the similarities among the sequences without interference from preconceived ideas about the relationships. For that reason, it sometimes may be helpful to withhold Cladistic Tree B until the students have completed Cladistic Tree A. Tree B could be shown on an overhead, made a separate handout or given as a part of a take home exercise.

Comments on Individual Sections

Section A:

1. Species I, same species. Would expect no differences. Diagonal values compare a sequence with itself (thus no differences are possible).. None.
2. See KEY to Worksheet A.
3. See KEY to Worksheet A.

Section B: [May be rather challenging to some; this could be an optional section.]

4. We would have to move the columns and rows into whatever order made the numbers show the simplest patterns within columns and across rows. The first two columns would contain the two most similar species. The third column would contain the species that was most similar to the first two, etc. And, yes, it gets more complicated if you add several species from each group.

Section C:

5. The two most similar species share all 146 amino acids (i.e., NO differences between species I and II). The two least similar species share $146 - 33 = 113$ (for species VII with VIII).
6. Patterns are a stepwise decrease along each row (least clear between columns V and VI) and relative uniformity within each column. This suggests that every species to the left of a given species is approximately equally divergent in sequence (and hence evolutionarily) from that species. This is counter-intuitive as most students expect a ladder-like gradient, with gradually decreasing differences between closer and closer relatives.. See Section D for more.

Section D:

7. See KEY Worksheet A.
8. Prosimians are the least similar to the others and are about equally divergent from each of the others species (i.e., the pattern is NOT ladder-like with gorillas half way to humans from gibbons, etc.).
9. Gorillas are equally distant from humans and from chimps. Gibbons are almost exactly equally distant from chimps, humans and gorillas (as expected if these three shared common ancestry after the gibbon lineage diverged. Humans therefore fall *within* the Great Ape clade. Thus, humans have to be viewed as great apes IF clades are to be monophyletic and not paraphyletic (see e.g., Freeman and Herron, 2000, *Evolutionary Analysis*, for terminology). A clade is a group of organisms that share a common ancestor.

Section E:

10. The two most similar species are chimpanzees and humans. They go on the two shortest branches of Tree A (in the middle of the tree). It makes no biological difference which one goes on the left—and indeed the form of Tree A is designed to emphasize that fact (thereby allowing a contrast with the conventional, more human-focused presentation in Tree B, later.)
11. Gorillas are next, then the gibbon. The similarities in values for the Gibbon when compared with each of the Great Apes suggest that these three are all about equally distant from Gibbons, presumably because they had the same, shared common ancestors for a while after their lineage diverged from that of the gibbons.
12. See KEY. The left to right order in the matrix is the same as the top to base order of branching.

Section F:

13. **#33** is V (valine) in all species except the Old-World monkey, which has L (leucine). The straight-forward interpretation is the incorporation of a new mutation to Leucine in the OW monkey lineage after it branched from the ape lineage.
14. African apes (including humans) differ from Gibbons at positions **#87** and **#125**. In both cases the change is from Q (glutamine), found widely across the lower primates at these two positions, to T (threonine) at 87 and P (proline) at 125.
15. The most parsimonious interpretation (simplest, fewest changes) is that the two mutations occurred in the great ape lineage before any of the three species diverged from the others. **#50** and **#104** both require either convergent substitutions or back-mutation and the data here does not allow these two alternatives to be differentiated (the DNA sequences might allow an unambiguous interpretation). Be sure and ask WHY the more parsimonious interpretations should be preferred.

Section G: Worksheet B

16. The age data on Tree B are new. The biological information in trees A and B is identical. The sequence of branching nodes (1-6) between species is the same for both; tree B was formed from tree A simply by rotating each node horizontally 180° (reversing left to right).

Section H:

17. See KEY for Worksheet B. The general relationship is more changes over longer times since separation. This pattern is the basis for the idea of molecular clocks--total mutations increase fairly regularly with time.
18. However, nothing makes the clocks tick exactly at the same rate (the extra mutation in the gorilla, for example). With more time, convergences and back-mutations begin to reduce the differences to fewer than expected from a simple clock. This acts to slow the rise in the curve with time. This is especially evident in the comparisons of apes and monkeys first with the lemur and then with the horse.
19. See KEY for Worksheet B.
20. The differences with the horse are much fewer than the 1.5 times the differences from the lemur (the expectation from a linear clock, given the differences in divergence times).
21. Hopefully, students will say something to the effect that the lesson helped to see that humans show very close [evolutionary] relationship to the apes, that they must share a common ancestor, and possibly that chimps seem more closely related to humans than they are to gorillas. Some might even conclude that the great apes and humans should be placed in the same clade! If the students have experienced the similar results with the skulls and chromosome comparisons for hominoids, they should recognize how these multiple independent lines of evidence all point to these same conclusions.

Extensions and Additional Comments

Deeper mastery of the concepts and more confirmation of the strength of the evidence for human evolution (as well as for evolution generally) can be achieved by searching out and comparing other molecules. Recent, especially helpful guides to doing this include:

R.P. Hershberger. 2000. What I could teach Darwin using "Darwin 2000," an interactive web site for student research into the evolution of genes and proteins. *Tested Studies for Laboratory Teaching* 21:4-32 Association for Biology Laboratory Education. See also his site www.bioactivesite.com

R.Moss.1999. The molecular evidence for evolution. *Journal of College Science Teaching* 29:111-113. Also features student use of public, on-line databases.

S.D. Brewer. 1997. Constructing Student Problems in Phylogenetic Tree Construction. Paper presented at the Annual Meeting of the American Educational Research Association (Chicago, IL, March 24-28, 1997).EDRS DOCUMENT LINK:
<http://orders.edrs.com/members/sp.cfm?AN=ED407227>

See also the lessons, tutorials and advanced resources directly available through the "Biology Workbench" homepage <http://glycine.ncsa.uiuc.edu/educwb/index2.html>

In addition, check the tutorial on the ENSI site which will take the student step-by-step through the process of selecting a molecule to compare, the organisms for which to compare the amino acid sequences, how to run an online comparison application, and how to build a comparison matrix from those data: "Tutorial: Using Online Molecular Databases to Examine Evolutionary Relationships" at <http://www.indiana.edu/~ensiweb/lessons/p.tut.db.html> This tutorial utilizes the ExPASy, Swiss-Prot, and Lalign sources.

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