

Competitive Phylogenetics: A Laboratory Exercise

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ABSTRACT

This exercise demonstrates the principle of parsimony in constructing cladograms. Although it is designed using mammalian cranial characters, the activity could be adapted for characters from any group of organisms. Students score categorical traits on skulls and record the data in a spreadsheet. Using the Mesquite software package, students generate arbitrary cladograms and measure tree length. They then move taxa around to reduce tree length. The exercise can become competitive when students report out on tree lengths and try to achieve shorter trees than their peers. The resulting cladograms can be compared with a published mammalian phylogeny. The exercise illustrates phylogenetics, the principle of parsimony, and hypothesis testing using morphological data.

Key Words: Phylogeny; morphology; parsimony; clade; cladistics; polytomy; synapomorphy; Occam's razor; homoplasy; homology; node; terminal node; branch; transition; root; natural taxon; sister group; out group.

Morphological traits can be used to readily generate phylogenetic data sets by undergraduate and high school students exploring evolutionary biology. Traits can be scored without specialized equipment and used to produce phylogenies that can be evaluated side-by-side with published phylogenies. The exercise takes students through the observation, data-collection, interpretation, and hypothesis-testing stages. There is an ample literature on the topic, and students should have no difficulty finding primary sources for laboratory reports.

○ The Principle of Parsimony

Selecting the phylogenetic tree that requires the fewest steps illustrates the broader scientific principle of parsimony: When choosing among competing hypotheses, one should select the simplest explanation that accurately describes the data. In this exercise, students make assumptions about transitions in character states. By minimizing the number of assumptions made, they will generate a shorter, more parsimonious phylogeny.

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○ Specimen Selection

Students should be supplied with a collection of mammal skulls or replicas. Providing some taxa likely to form clades nested within the larger phylogeny enhances the activity (Table 1). For example, the house cat and bobcat should fall out as sister groups nested within the carnivore clade. Similarly, the muskrat, beaver, and gray squirrel should group together. Skulls are distributed to pairs of students to score characters. The skulls in Table 1 reflect what specimens were available, but this lab could be implemented using whatever skulls are at hand. Specimens with lower mandibles increase the number of useful characters. Specimens with a nearly full complement of teeth are valuable, particularly with rodents and lagomorphs, where the characteristics of the incisors provided informative data. Online images can be used to fill in missing information from incomplete specimens.

○ Character Scoring

Students should come up with a list of qualitative characters that could be scored from the skulls, subject to the following constraints:

- Each trait should occur in at least two but not all of the skulls. Canine teeth, for example, occur in several species but not all. A noninformative character is included in Table 1 to illustrate this idea. Rostral fenestration occurs only in the rabbit specimen (Table 1). Because this character is not shared with other taxa in the matrix, it does not help to group rabbits with other taxa.
- Continuous traits should be expressed as fractions of skull size and reduced to categories. For example, snout length can be expressed as greater or less than one-third of skull length, resulting in two character states (long snouts = 1 and short snouts = 0).
- Discrete traits such as the number of postcanine teeth are permitted but treated as categories by the software.

Table 1. Example of data used to generate the trees in Figure 2. Quantitative traits such as the number of postcanine teeth are treated as categorical traits in the software if used as described in this exercise. Some characters, such as “Mandibular Condyle High,” were determined in relation to specific landmarks on the skulls. Tooth counts refer to one side of upper jaw.

	Upper Incisors	Postcanine Teeth	Incisive Foramina	Lower Mandible Foramina	Incisor Color Layers	Canine Teeth	Mandibular Condyle High	Infra-orbital Foramen Front	Sagittal Crest on Parietal	Post-orbital Process Forward	Snout > 1/3 Skull	Molar Roots in Eye Socket	Post-orbital Processes Connected	Foramen Magnum > 1/4 Skull Width	Rostral Fenestration
Raccoon	3	6	1	4	1	1	0	1	1	1	0	1	0	1	0
Pig	3	7	1	4	1	1	1	0	0	0	1	0	0	0	0
Dog	3	6	2	4	1	1	0	1	1	0	0	0	0	0	0
Otter	3	5	2	4	1	1	0	1	0	1	0	1	0	0	0
Rabbit	2	6	3	1	1	0	1	0	1	0	1	1	0	0	1
Mink	3	4	2	3	1	1	0	1	1	1	0	0	0	0	0
Chimpanzee	2	5	2	1	1	1	1	0	1	1	0	0	1	0	0
Beaver	1	4	3	1	2	0	1	0	1	0	0	1	0	1	0
Bobcat	3	3	2	2	1	1	0	1	0	1	0	0	0	0	0
Squirrel	1	4	3	2	2	0	1	0	0	0	1	0	0	0	0
Moose	0	6	3	1	0	0	1	1	1	0	1	0	1	0	0
Human	2	5	1	1	1	1	1	1	0	1	0	0	1	0	0
Muskrat	1	3	3	1	2	0	1	0	0	0	1	0	0	1	0
House cat	3	4	1	2	1	1	0	1	0	1	0	0	0	0	0

- Use as many characters as you can and, if at all possible, use more traits than the number of specimens.
- Trait scores must be single numerical digits.

○ Examples of Traits

Any trait that can be reduced to a small number of categories should be considered.

Canine Teeth. These are present in primates and carnivores, for example, and would be scored as 1; they are absent in rodents and would be scored as 0.

Number of Upper Incisors. By convention, we count on just one side of the jaw: so, for humans and chimps, the score would be 2; for beavers and other rodents, it would be 1; for dogs and cats, it would be 3; for moose and deer, it would be 0, because they lack upper incisors.

Incisive Foramen Length. The incisive foramen is an opening in the palate. This could be scored as a fraction of the palate length and then grouped into categories. Rodents and rabbits have long incisive foramina; humans, house cats, and pigs have short foramina; and chimpanzees, dogs, otters, and bobcats have foramina of intermediate length.

○ Achieving Consensus

Scoring traits is not always straightforward, and discrepancies between scores can occur. Such conflicts can be resolved by rotating

specimens among students. Each trait should be independently scored three times for each skull. When the three scorings do not agree, the skull should be rescored for the trait in question. This has the added advantage that students work with a larger number of specimens. For example, we found that the incisive foramen scores did not match and learned that some students assumed that the single opening on our replica human skull was a manufacturing defect. An online photograph of a real skull confirmed that there was a single central opening. The scored traits should be saved as a spreadsheet matrix with species listed vertically and traits listed horizontally (Table 1).

○ Generating Trees

Mesquite phylogenetic software (Maddison & Maddison, 2011) is available at <http://mesquiteproject.org>. Once the software is installed, use the followed simple steps to input data:

- Select “File/New” and save your file.
- In the “New File Options” window, enter the number of taxa (14 in our example); check “Make character matrix” and “OK”.
- Enter the number of characters (15 in our example); select “Standard categorical data”.
- The column and row headings and data matrix can be pasted or typed into Mesquite.

- Use “1” for character present, and “0” for character absent. Traits such as number of incisors on one side of the jaw could have more than 2 states: 1, 2, or 3.

A generic tree (Figure 1) can be generated using the following steps:

- Use the Mesquite menus as follows: “Taxa&trees/New tree window/with trees from source”.
- Select “Default tree” and “OK”.

The tree produced will not reflect the data; rather, it serves as a starting point to be modified by student investigators (Figure 1).

Students should use Mesquite to measure and record starting tree length as follows:

- Select the “Tree Window showing Default Trees” tab and “Analysis/Values for current tree”.

- Select “Tree length” followed by “OK”.
- Select “Stored Matrices” followed by “OK”.

To print trees, look under the “File” menu for “Save tree as PDF” and then print the PDF file.

○ Explaining Tree Length

Tree length is the number of changes in character state needed to explain the data. Figure 2A represents a hypothetical relationship among five organisms. It took a total of five evolutionary events to produce this tree. Figure 2B offers a better explanation because it explains the evolution of the same traits in fewer steps. This illustrates the principle of parsimony: we should use the simplest explanation rather than invoking a more complex series of events. Importantly, increasing the number of specimens or characters will automatically make the tree longer; comparisons of tree length should only be made between trees for a single data matrix.

○ Generating the Most Parsimonious Tree

One can drag and drop tree branches in Mesquite, and tree length is instantly recalculated. Students might drag the moose branch next to the pig branch to see if that grouping reduces tree length. A number of approaches can be used: Students may simply drag and drop branches and keep changes that reduce tree length. A more pragmatic approach would be to group taxa on the basis of similarities in the data matrix. An example of a modified tree is provided to illustrate the result of the process (Figure 1B).

At intervals through the tree generation process, students should report out on their tree length. This can generate competition as students work harder to outdo their classmates. Importantly, this process is intended to generate the most reasonable explanation of the student-generated data. Adding more data would very likely result in a slightly different, and potentially better, tree. There are no wrong answers, and student trees are very likely to disagree with one or another of the published hypotheses. Such discrepancies can generate discussion about the limitations of data sets, process of science, and accrual of new knowledge.

○ Testing Hypotheses

The Tree of Life project (Maddison & Schulz, 2007) provides a general picture of placental mammal phylogeny (<http://tolweb.org/Eutheria/15997/1997.01.01>). Meredith et al.’s (2011) mammalian phylogeny can serve as a comparison

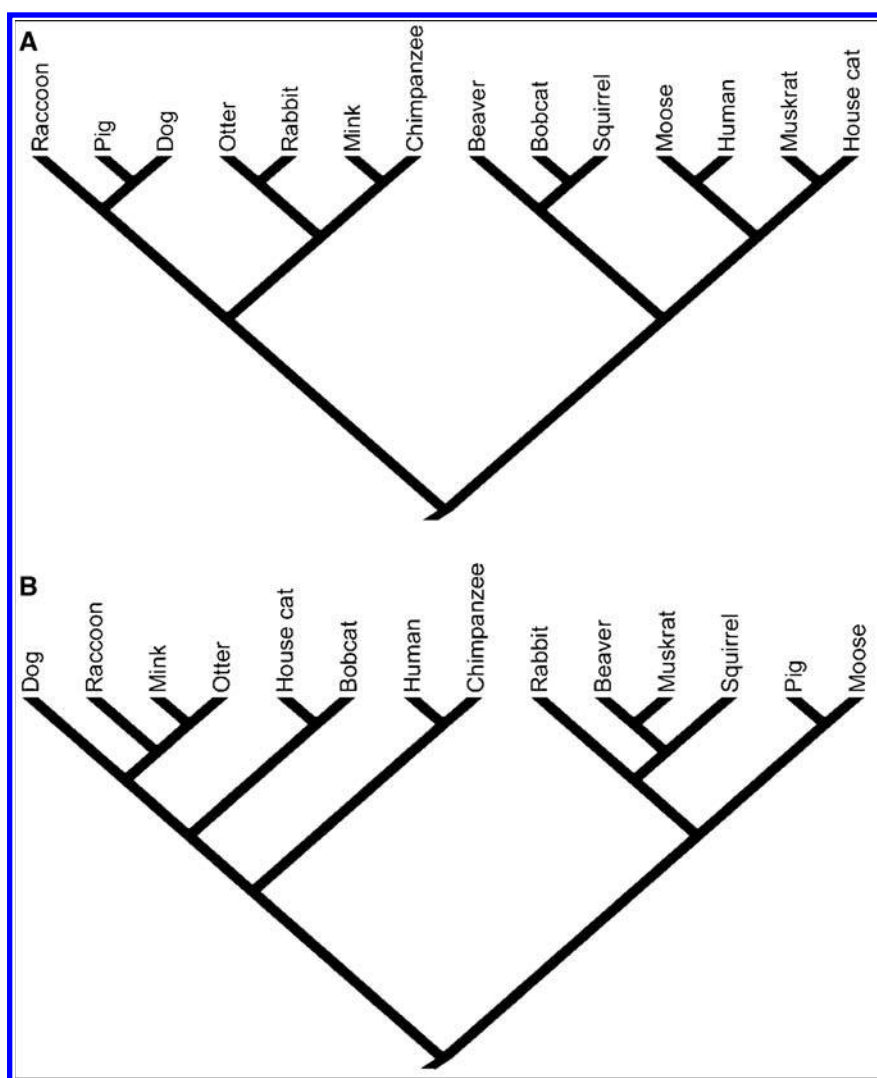


Figure 1. (A) A generic phylogeny generated by the Mesquite software package. The order of the taxa is identical to the order in the data matrix and in no way reflects the evolutionary relationships of the organisms. Tree length = 74. (B) A phylogeny that requires fewer transitions to explain the data. This tree was generated by dragging and dropping branches from the starting tree in Figure 2A. For this more parsimonious phylogeny, tree length = 49.

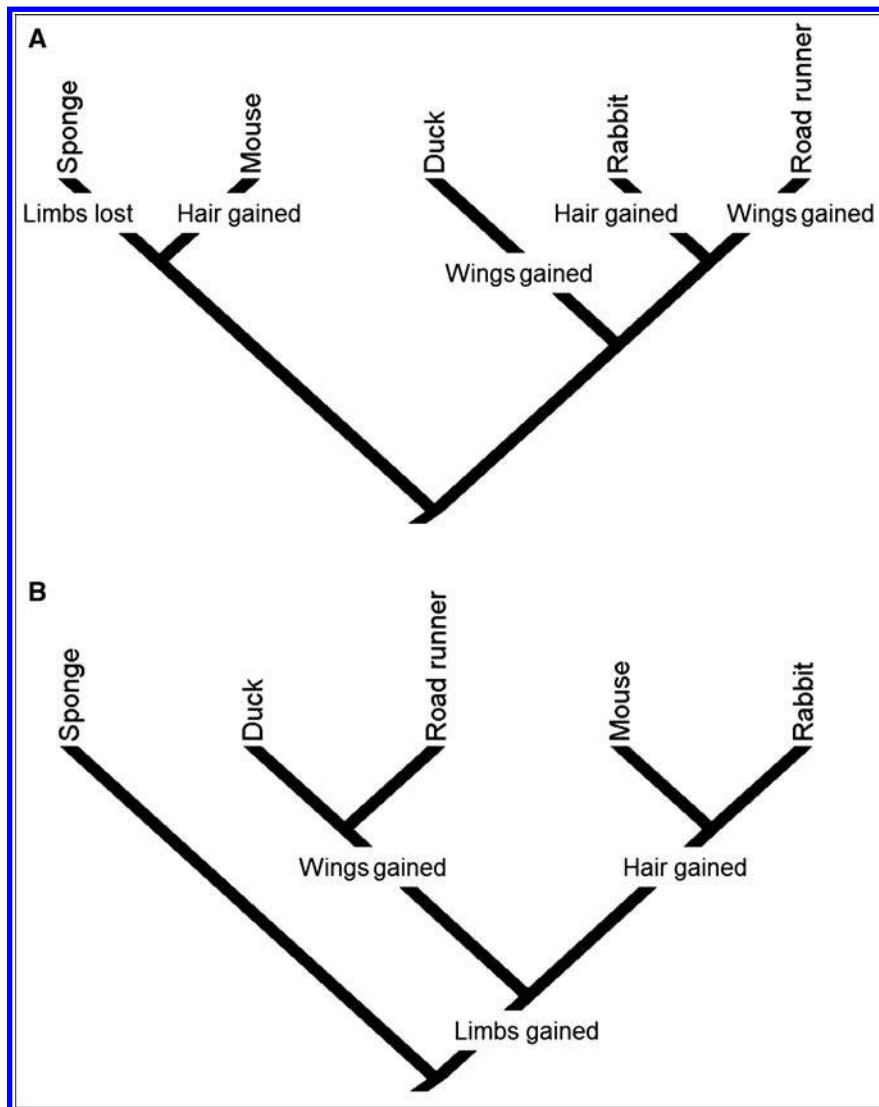


Figure 2. Simple phylogenies illustrating tree length calculation using three characters: limbs, wings, and hair. (A) A tree that uses five transitions, or changes of character state, to explain the data; tree length = 5. (B) A shorter and therefore more parsimonious tree that explains the same data with just three transitions.

for the main branches deep in the student tree. Groupings near the branch tips can be examined by working through the Integrated Taxonomic Information System (ITIS) site <http://www.itis.gov/>. ITIS places house cats and bobcats in the same subfamily, and we should therefore expect them to group together on our tree. Each branch of a student tree can be tested as a hypothesis. The hypotheses that each proposed branch is identical to the relationship proposed by Meredith et al. (2011) or ITIS can be tested. In our example (Figure 1B), the human–chimpanzee pairing is supported by ITIS, but the raccoon–otter–mink grouping is not. ITIS lists mink and otters in Mustelidae and raccoons in Procyonidae. The muskrat is similarly misplaced. With each of these discrepancies, if we redraw the tree to match the ITIS grouping, we get a slightly longer tree. Again, this provides fodder for discussing the nature of reconstructing phylogenies and limitations of data sets.

○ Alternatives to Hands-on Specimens

In the absence of a skull collection, this exercise can be completed using high-quality images of skulls or other specimens. The Digital Morphology site (<http://www.digimorph.org/>) at the University of Texas at Austin is an excellent source of images. Printed lateral and dorsal views can be used for measurements, and presence–absence characters can be scored from the three-dimensional images on the site. This site can also be used as a supplementary resource when specimens lack lower jaws.

○ Skull Identification & Anatomical Guides

Wiehe has provided an online version of his (1978) key to identify North American specimens. Elbroch (2006) is an essential in-depth reference for the North American species. Labeled human skull diagrams, cat dissection guides (e.g., Wingerd, 2006), and online images are useful for identifying cranial bones. Labeled dog skull images can be found at <http://anatomy.wikispaces.com/>.

○ Modifications

By using fewer specimens, this exercise could be completed in one laboratory meeting. It is possible to run a simplified exercise using spreadsheet sort functions. Strictly presence–absence traits should be coded as 1's and 0's in a spreadsheet. Sorting by column totals will group the most widespread and likely ancestral traits together. A sequential row sort, first by most widespread trait and then by decreasingly less common traits, will roughly bring more identical species closer together. For example, if Table 1 was sorted by presence of canine teeth, then the rabbit, rodents, and moose would group together.

This exercise uses few of Mesquite's features. The software is powerful, and the possibilities of expanding this exercise are broad. Mesquite integrates with other phylogenetic software packages and can be used to evaluate trees generated by different approaches.

References

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Articles specific to the terminology used in this exercise:

http://evolution.berkeley.edu/evolibrary/article/phylogenetics_01

http://evolution.berkeley.edu/evolibrary/article/evo_05

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Phylogenetic terminology resource:

<http://evolution.berkeley.edu>

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