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Using Phylogenetic Trees as an Investigative Tool in an Introductory Biology Course

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Although cladograms and phylogenetic trees are included in college biology textbooks more and more regularly, students frequently have little idea of how these structures are developed or of how they function as experimental tools for biologists. This workshop describes a laboratory exercise in which students are presented with experimental phylogenetically-based questions. For each question, students use morphological characters provided by the instructor to develop a cladogram. Students then use the cladogram as their hypothesis which they test by developing a phylogenetic tree using either a protein or nucleic acid database in Biology WorkBench.

Keywords: phylogenetics, cladogram, systematics

Introduction

Although cladograms and phylogenetic trees are included in college biology textbooks more and more regularly, students frequently have little idea of how these structures are developed or of how they function as experimental tools for biologists. Many laboratory exercises that incorporate phylogenetic trees and/or cladograms do little to further student understanding. This workshop presents a laboratory exercise designed for an introductory biology course. Students are given one of several experimental questions—some related to plants and some to animals—for which answers must be developed. Students first learn how to design a cladogram from a set of morphological characters. Then, students work in small groups to develop cladograms from morphological characters, provided by the instructor, that are particular to the organisms in their specific research question. Students use this cladogram as their hypothesis which they test by developing a phylogenetic tree using either a protein or nucleic acid database. Each group is responsible for preparing and presenting a 15-minute PowerPoint presentation of their experimental work.

The preparatory lesson for this exercise requires about an hour. Students could also do this part of the exercise prior to lab. The development of the problem-specific cladogram requires about an hour; the development of the phylogenetic tree requires about two more hours. Additional time is required for actual development and refinement of the PowerPoint presentation. This could easily be accomplished outside of scheduled lab time. The laboratory activity is completely computer-based; no additional materials are required. Optimally, each student group should have access to at least two computers at the same time.

Student Outline

In this laboratory exercise, you will first learn how to develop a cladogram from a set of morphological characters. Then you will be assigned a research question based on a particular group of organisms. You will develop a cladogram for that group of organisms which will serve as your hypothesis for the question. Finally, you will search a molecular data base for information that will enable you to develop a phylogenetic tree. You will use this tree to accept or reject your hypothesis.

Part I: Learning how to develop cladograms

You will be given a series of practice problems to learn how to develop a cladogram.

Part II: Solving a phylogenetics problem

In this activity, each lab group will work on its own problem. For your assigned problem, you will first develop a cladogram based on an analysis of morphological features that are characteristic to the taxon. This cladogram will serve as your hypothesis. Then to test your hypothesis you will develop a phylogenetic tree based on a molecular comparison of members of the taxon that you will carry out in Biology WorkBench.

Before coming to lab

Prepare the following (* denotes responsibilities that can be divided among members of your group):

- Do some background reading on the problem you will be solving. Some websites may be provided with the problem.*
- Select a genus to represent each family in your order (if necessary). Use the website(s) provided to find this information.*
- Do a Google Scholar search: your taxon plus phylogenetics. In the literature, find genes or proteins that other researchers have used to study your group of organisms. Print out the abstract for at least two articles. Highlight the gene/protein in each.*
- Set up an account to use Biology Workbench: (Everyone must do this). Make sure you're registering with Biology WorkBench 3.2, not the newer version.
  - Go to **http://workbench.sdsc.edu**
  - On the main page, you have two options: entering the program or signing up for a new account. Choose "Set up a free account."
  - Fill out the form and submit it.

Once you're in lab

Part II A: Explaining the problem

Share the information each individual group member has brought. Make sure the group clearly understands the basis of the problem before you proceed any further.

Part IIB: Developing a cladogram to serve as your hypothesis

1. Use the table of morphological characters to develop a Character Matrix for your taxa. To simplify the chart, use A, B, C, etc. for the taxonomic groups and 1,2,3, etc. for the characters (as you did in the practice problems). If the character is the same as in the outgroup (in other words, if it is ancestral), put a 0 in the appropriate space in the table; if the character is different from the outgroup (that is, if it is derived), put a 1 in the space.
2. Use the Character Matrix to develop a table of Shared Characters. Again, use A, B, C, etc. for the taxonomic groups and 1,2,3, etc. for the characters.
3. Use the Shared Characters table to develop a parsimonious cladogram. Once the cladogram is developed, change the letters to the names of the organisms. Keep the numbers to designate characters but develop a key to explain what each number represents. Make sure to use the derived trait in this key. Go to Paint to draw and fully label your cladogram. This diagram is your experimental hypothesis; therefore, format it so it clearly illustrates your suggested answer to the research question.

Part IIC: Developing a phylogenetic tree

To test your cladogram hypothesis, develop a phylogenetic tree, using the directions that follow.

1. Go to the Biology WorkBench website, **http://workbench.sdsc.edu**, and log on. You should already have signed up for an account before coming to class.
2. Biology WorkBench stores your data in chunks called Sessions. Store all the work related to your research question in a single session. You cannot enter data to the DEFAULT session.
3. Go to **Helper Applications > Session Tools > Start New Session > Run**. On the dialog screen, give your new session a name and click again on **Start New Session.**

Two sets of directions follow. Use the first if you are searching with a protein; use the second if you are searching with a gene.

If you are working with a protein

Gathering sequence data

1. Click on **Protein Tools > Ndjinn-Multiple Database Search > Run.**
2. When Ndjinn starts, you must first type in your search terms. There are two parts to the search terms: the specific taxon and the specific protein. The handout that explained your problem gave you the taxonomic information; your Google Scholar search provided you with the protein for your search. Enter your search terms in this form: taxon AND protein (note that AND must be in capital letters).
3. The second part of this step involves selecting the correct database. Scroll down the list of available databases until you find the most appropriate protein database for your experimental organisms. Click on the check box beside it, then scroll back up to the top of the window.
4. Once you have entered your search information and selected your database, choose **Search**. You will get various numbers of sequences, ranked in order of how closely they match your search criteria. Scan down the list until you find one that is the closest. Read them carefully, because they could be very close to each other. Click the check box next to the sequence, then scroll down until you see the button labeled **Import Sequence**. Select this, and the program should import the file and return you to the main page for Protein Tools.
5. Repeat steps 2-4 for each of the organisms in your search table. This part of the exercise takes time and patience. You may run into dead ends. It's most helpful to have several laptops in operation at the same time—one running Biology WorkBench and the other(s) checking out alternative genera to run through the program.
6. After you have imported all of the sequences, set up the designated outgroup for subsequent analyses. To do this, modify the file's name following these steps:
   - Find the sequence file for the outgroup's protein, and click on the file to check it. Under **Protein Tools > Edit Protein Sequence(s) > Run.**
   - There will be a box entitled **"Label:"** with the name of the file in it. Click in the box at the very beginning of the name of the file and add *zz* to the name of the file. For example, if the file name originally was *rabbit insulin*, the new name would be *zzrabbit insulin.*
   - Scroll down the screen until you see the **Save** button. Click it.
   - Return to the main page of the protein editing tools. A copy of the newly-renamed file will now be in the list of sequence files, along with the original file with the old name. Any time you want to use that particular sequence as the outgroup, just choose the version that has "zz" added to the name.

Aligning similar amino acid sequences

7. Once you have set the outgroup, align the imported sequences to find which amino acids are conserved and which vary between species. Click on the check boxes next to the sequences that you want to have included in the alignment. If you accidentally downloaded sequences you do not want to include, don't check them. Be sure that you do NOT check the original unedited file for the outgroup protein, but instead select the file for which you changed the name.

8. Scroll down the list of options on the Protein Tools page. Select the option **CLUSTALW**, which is a sequence aligning algorithm. Select **Run.**
9. The program shows a list confirming the sequences to be aligned, and gives you the option of changing how it will perform the alignment. Change the **Output Order** option from **Aligned** to **Input**. This forces the file you renamed with the prefix "zz" to be used as the outgroup. You can leave the other options at their default setting. Select **Submit.**
10. If the information in the sequence is what you wanted to see, select **Import Alignments**, and you will go directly to the **Alignment Tools** page. If for some reason the CLUSTALW report is not what you wanted, select **Return**. The program will delete the alignment without saving it and you can do it again.

Generating a parsimonious phylogenetic tree

11. If you are not already there, go to the page for **Alignment Tools**. Scroll through the list of options. Select **ProtPars**, check the box next to your group of aligned sequences, then select **Run.**
12. Again, you will have the option of setting several parameters. Make sure that the first one **Randomize order of sequences** is set to **No.** Leave the rest at their default setting, and click **Submit**.
13. The program will return in a few moments with one or more unrooted parsimonious trees. Each arm of the tree will have a number on it that indicates the number of changes that were necessary to arrive at the amino acid sequences represented on that particular branch point on the tree.
14. Cut-and-paste the tree to Microsoft Word and save it as a Word document. Replace the abbreviations with the taxonomic names of the organisms.

Generating an amino acid sequence comparison for the organisms involved

15. Click **Return**. Select **Alignment Tools > BOXSHADE**. The proteins that you want to sequence have already been checked off, so just click **Run**.
16. Change **Character Size** to **12** and **Residue Numbering** to Ruler. Click **Submit**.
17. Cut-and-paste the diagram to Microsoft Word and save it as a Word document. To interpret the alignment diagram, you must know the color code: green = completely conserved residues; yellow = identical residues; turquoise = similar residues; white = different residues.

If you are working with a nucleic acid

Gathering sequence data

1. Click on **Nucleic acid Tools > Ndjinn-Multiple Database Search > Run.**
2. When Ndjinn starts, you must first type in your search terms. There are two parts to the search terms: the specific taxon and the specific gene. The handout that explained your problem gave you the taxonomic information; your Google Scholar search provided you with the gene for your search. Enter your search terms in this form: taxon AND gene (note that AND must be in capital letters).
3. The second part of this step involves selecting the correct database. Scroll down the list of available databases until you find the most appropriate nucleic acid database for your experimental organisms. Click on the check box beside it, then scroll back up to the top of the window.
4. Once you have entered your search information and selected your database, choose **Search**. You will get various numbers of sequences, ranked in order of how closely they match your search criteria. Scan down the list until you find one that is the closest. Read them carefully, because they could be very close to each other. Click the check box next to the sequence, then scroll down until you see the button labeled **Import Sequence**. Select this, and the program should import the file and return you to the main page for Nucleic Acid Tools.
5. Repeat steps 2-4 for each of the organisms in your search table. This part of the exercise takes time and patience. You may run into dead ends. It's most helpful to have several laptops in operation at the same time—one running Biology WorkBench and the other(s) checking out alternative genera to run through the program.

6. After you have imported all of the sequences, set up the designated outgroup for subsequent analyses. To do this, modify the file's name following these steps:
   - Find the sequence file for the outgroup's nucleic acid, and click on the file to check it. Under **Nucleic acid Tools > Edit Protein Sequence(s) > Run**.
   - There will be a box entitled **"Label:"** with the name of the file in it. Click in the box at the very beginning of the name of the file and add *zz* to the name of the file. For example, if the file name originally was *rabbit insulin*, the new name would be *zzrabbit insulin*.
   - Scroll down the screen until you see the **Save** button. Click it.
   - Return to the main page of the nucleic acid editing tools. A copy of the newly-renamed file will now be in the list of sequence files, along with the original file with the old name. Any time you want to use that particular sequence as the outgroup, just choose the version that has "zz" added to the name.

Aligning similar nucleotide sequences

7. Once you have set the outgroup, align the imported sequences to find which nucleotides are conserved and which vary between species. Click on the check boxes next to the sequences that you want to have included in the alignment. If you accidentally downloaded sequences you do not want to include, don't check them. Be sure that you do NOT check the original unedited file for the outgroup gene, but instead select the file for which you changed the name.
8. Scroll down the list of options on the Nucleic Acid Tools page. Select the option **CLUSTALW**, which is a sequence aligning algorithm. Select **Run**.
9. The program shows a list confirming the sequences to be aligned, and gives you the option of changing how it will perform the alignment. Change the **Output Order** option from **Aligned** to **Input**. This forces the file you renamed with the prefix "zz" to be used as the outgroup. You can leave the other options at their default setting. Select **Submit.**
10. If the information in the sequence is what you wanted to see, select **Import Alignments**, and you will go directly to the **Alignment Tools** page. If for some reason the CLUSTALW report is not what you wanted, select **Return**. The program will delete the alignment without saving it and you can do it again.

Generating a parsimonious phylogenetic tree

11. If you are not already there, go to the page for **Alignment Tools**. Scroll through the list of options. Select **DNAPars**, check the box next to your group of aligned sequences, then select **Run**.
12. Again, you will have the option of setting several parameters. Make sure that the first one **Randomize order of sequences** is set to **No**. Leave the rest at their default setting, and click **Submit.**
13. The program will return in a few moments with one or more unrooted parsimonious trees. Each arm of the tree will have a number on it that indicates the number of changes that were necessary to arrive at the nucleotide sequences represented on that particular branch point on the tree.
14. Cut-and-paste the tree to Microsoft Word and save it as a Word document. Replace the abbreviations with the taxonomic names of the organisms.

Generating a nucleotide sequence comparison for the organisms involved

15. Click **Return**. Select **Alignment Tools > BOXSHADE**. The genes that you want to sequence have already been checked off, so just click **Run**.
16. Change **Character Size** to **12** and **Residue Numbering** to Ruler. Click **Submit**.
17. Cut-and-paste the diagram to Microsoft Word and save it as a Word document. To interpret the alignment diagram, you must know the color code: green = completely conserved residues; yellow = identical residues; turquoise = similar residues; white = different residues.

Assignment

Your group is responsible for a 15-minute PowerPoint presentation of the work you carried out in Part II of this laboratory exercise. At minimum, the presentation **must** include:

- An explanation of the research question with which you were presented
- Background information about the taxon of organisms with which you are working, including specific morphological features that you used to develop the cladogram
- The Shared Character Table (the font will be very small; that's OK)
- A description of the procedures you followed to answer the research question (both parts: developing the cladogram and developing the phylogenetic tree)
- The cladogram based on morphology (your hypothesis)
- The amino acid alignment diagram OR the nucleotide alignment diagram. (Although you generated this alignment diagram after you produced the phylogenetic tree, remember that the alignment diagram represents what enabled the tree to be produced. Therefore, it must precede the tree in your results.)
- The phylogenetic tree obtained from Biology WorkBench
- A phylogenetic tree pertaining to the same taxon of organisms that you found in the literature
- An explanation of whether your phylogenetic tree supports your hypothesis; an explanation of how well the phylogenetic tree from the literature matches the tree you produced in Biology WorkBench.
- References (including the *url* for each figure or diagram you obtained from the Internet)
- Additional information you think is either necessary for an understanding of the problem or interesting to the listener

You must also submit a hard copy of a slide handout (4 slides/page), including speaker notes that you have added to each slide.

Notes for the Instructor

Ideally, students should have learned background information about phylogenetics prior to beginning this laboratory exercise. Many introductory biology textbooks devote a chapter to explaining the design of phylogenetic trees. In *Life, the Science of Biology* (Sadava, Hillis, Heller, Berenbarum, 2010), for example, Chapter 22 covers this concept and could be assigned as pre-lab reading assignment. Several reputable websites also address this concept, for example, Understanding Evolution, at **http://evolution.berkeley.edu/evolibrary/article/phylogenetics_02**. Baum and Offner (2008) in The American Biology Teacher also provides some helpful background.

The terms cladogram, phylogenetic tree, phylogram, and dendrogram are used interchangeably in the literature; they all refer in some way to the representation of evolutionary relationships among a group of organisms. For the purposes of this laboratory exercise, a distinction is made between a cladogram and a phylogenetic tree. A cladogram is used as a hypothesis about the evolutionary relationships based on morphology; a phylogenetic tree is the actual evolutionary history based on molecular information.

In this laboratory exercise, students are presented with a variety of research questions that they must solve. Three of these were adapted from an ABLE workshop (Johnson, 2005). In the first part of the laboratory session, students are taught how to develop cladograms from a table of morphological characters.

The sample problems included in this workshop (see Appendix) were adapted from an ABLE workshop (Kosinski, 2006). It is crucial that the instructor become familiar with developing sample cladograms prior to the laboratory session. Several modifications of the Kosinski exercise are necessary. In the Kosinski exercise, X is used to indicate the derived state, and a blank space indicates the ancestral state (as shown below).

Table 5. Character matrix for Species A-F. An X indicates the derived state of the character.

	3	4	5	6	8	11	12	15	16	17	18	20
A					*X*							
B	*X*	*X*		*X*	*X*	*X*						
C			*X*	*X*	*X*	*X*						*X*
D					*X*		*X*					
E			*X*	*X*	*X*	*X*		*X*	*X*		*X*	*X*
F			*X*	*X*	*X*	*X*				*X*		*X*

To make the sample problems more closely match what the students will be doing later in the lab exercise, reformat the Kosinski character matrices so there is a letter in each space in the table, as shown as follows.

	3	4	5	6	8	11	12	15	16	17	18	20
A	*Y*	*Y*	*Y*	*Y*	*X*	*Y*	*Y*	*Y*	*Y*	*Y*	*Y*	*Y*
B	*X*	*X*	*Y*	*X*	*X*	*X*	*Y*	*Y*	*Y*	*Y*	*Y*	*Y*
C	*Y*	*Y*	*X*	*X*	*X*	*X*	*Y*	*Y*	*Y*	*Y*	*Y*	*X*
D	*Y*	*Y*	*Y*	*Y*	*X*	*Y*	*X*	*Y*	*Y*	*Y*	*Y*	*Y*
E	*Y*	*Y*	*X*	*X*	*X*	*X*	*Y*	*X*	*X*	*Y*	*X*	*X*
F	*Y*	*Y*	*X*	*X*	*X*	*X*	*Y*	*Y*	*Y*	*X*	*Y*	*X*

Then, in an additional organizational table (not included in Kosinski) require the students to re-write the character trait information in the conventional 0/1 format, where 0=ancestral trait and 1=derived trait.

When the students are developing their practice cladograms and the cladogram for their particular experimental problems, I have them use large (~24 x 20 in) dry-erase boards. This enables me to see more clearly what they are doing and makes their corrections less onerous. The answers to the experimental cladograms are given at the end.

In the second part of the laboratory session, students use Biology WorkBench 3.2 (**http://workbench.sdsc.edu**) to search amino acid and/or nucleotide databases and develop a phylogenetic tree. The website is run by the San Diego Supercomputer Center. Other databases which the instructor is familiar could certainly be used instead. Although Biology WorkBench is relatively easy to use and the directions, adapted from an ABLE workshop (Johnson, 2005), are quite explicit, it is crucial that the instructor become familiar with the website prior to the laboratory session. Be aware that there is a "new and improved" version of Biology WorkBench called SWAMI. The directions that are included in this workshop do not apply to SWAMI.

There are several points to watch out for in the Biology WorkBench portion of the experiment. Make sure students enter AND as all capital letters. Although using the common names of organisms may produce some hits, it doesn't work as well as using taxonomic designations. Make sure students select the appropriate database and not one that is guaranteed to produce no hits: a plant database when their search involves animals, for instance. On occasion, students select a protein/gene for which there is no information in the database for one particular taxon. It is up to the instructor to determine how essential it is that students find a sequence for each organism. Generally, if I have observed that students have been working consistently throughout the laboratory period, I accept their phylogenetic tree even if it is missing a branch or two. Phylogenetic trees have not been provided since they are dependent on the particular gene/protein and the taxa used by students in their search.

Instructors can also use this exercise as an opportunity to teach students how to prepare a professional PowerPoint presentation. In particular, specific guidelines regarding slide composition, font choice and font size are important to include.

Acknowledgements

Parts of this workshop, the directions for designing a cladogram and the directions for using Biology WorkBench, are modified versions of two earlier ABLE workshops (Kosinski, 2006; Johnson, 2005). The main objective of this workshop, however—providing students with an opportunity to use phylogenetics in an actual experimental setting—is original (as far as the author can tell).

Dan Johnson and Bob Kosinski for presenting engaging workshops at ABLE.

Literature Cited

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Appendix

Sample Problems

The Caryophyllales (Tables A1 & A2, Fig. A1)

Several members of the Caryophyllales group of plants have evolved to live in extremely arid places. Two characteristics in particular help them survive in arid or desert conditions—formation of succulent leaves and changing from C3 photosynthesis to CAM photosynthesis.

The question for which you will develop a hypothesis is: Did these two adaptations evolve more than once in this group of plants?

These are the taxa for which you are responsible:

Aizoaceae
Cactaceae
Caryophyllaceae
Phytolacaceae
Polygonum
Portulacaceae
Simmondsiaceae

Use these websites to help with this exercise:

http://tolweb.org/Caryophyllales/20703
http://www.phytoimages.siu.edu/taxpage/0/order/Caryophyllales.html

In working on this problem, don't focus on learning plant anatomy. Here are some terms you might not know:

Crystals: some plants secrete sharp calcium or other crystals into the leaves to deter herbivores.

Plant metabolism: Some plants absorb the carbon dioxide needed for photosynthesis immediately before they use it to produce glucose. These are referred to as C3 plants. Other plants absorb carbon dioxide and temporarily store it bound to an intracellular molecule; these are referred to as CAM plants.

Traces: when buds or scales form at the nodes (leaf junctions) of a plant, they create scars on the stem called traces. The arrangement of traces can be seen under a microscope.

Xylem & Phloem: the xylem carries water and soil minerals from roots to shoots. Phloem carries sugars and water from leaves to storage areas, often in the roots. The arrangement of these tubes is very consistent within a related group of plants.

Table A1. Morphological features of certain Caryophyllales.

	Polygonum (outgroup)	Phyto-lacaceae	Cactaceae	Caryo-phyllaceae	Portula-caceae	Aizoacaceae	Simmond-siaceae
# traces/ node	3	1	1	1	1	1	1
Crystals in leaves	Absent	Present	Absent	Absent	Absent	Present	Absent
Leaves	Not succu-lent	Not succu-lent	Succulent	Not succu-lent	Succulent	Not succu-lent	Not succu-lent
Embryo shape	Straight	Curved	Curved	Curved	Curved	Curved	Straight
Flower pig-ment	Anthocya-nins	Betalains	Betalains	Anthocya-nins	Betalains	Betalains	Anthocya-nins
Photosyn-thesis	C3	C3	CAM	C3	CAM	C3	C3
Xylem & phloem ar-rangement	Single ring of each type	Concentric rings	Concentric rings	Concentric rings	Concentric rings	Concentric rings	Concentric rings
Stamen #	<10	>20	>20	<10	>20	>20	<10

Table A2. Morphological features of certain Caryophyallales designated by conventional method.

	Polygonum (outgroup)	Phyto-lacaceae	Cactaceae	Caryo-phyllaceae	Portulaca-ceae	Aizoacaceae	Simmond-siaceae
# traces/ node	0	1	1	1	1	1	1
Crystals in leaves	0	1	0	0	0	1	0
Leaves	0	0	1	0	1	0	0
Embryo shape	0	1	1	1	1	1	0
Flower pigment	0	1	1	0	1	1	0
Photosyn-thesis	0	0	1	0	1	0	0
Xylem & phloem arrangement	0	1	1	1	1	1	1
Stamen #	0	1	1	0	1	1	0

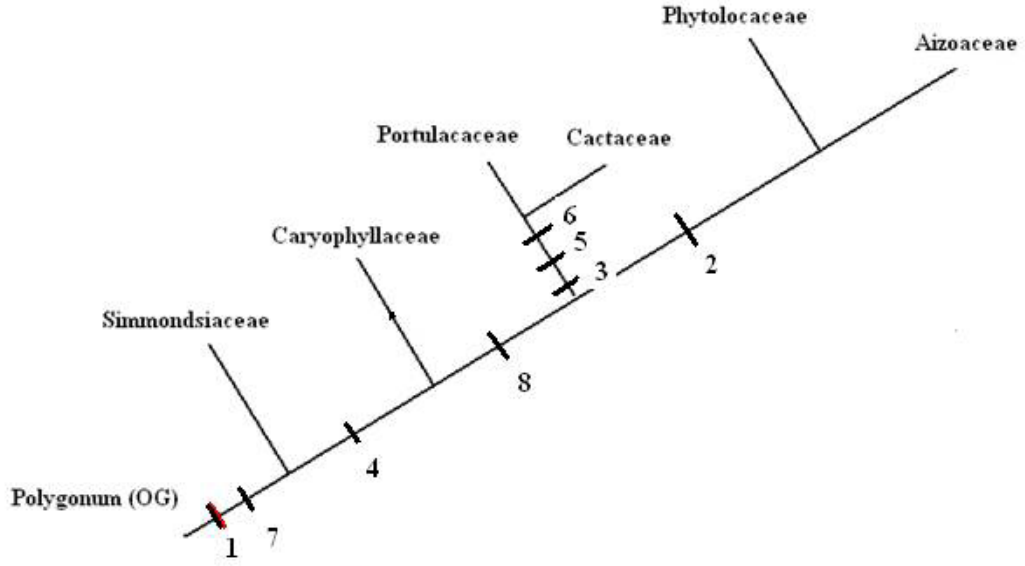

Figure A1. Cladogram of certain Caryophyllalles based on designated morphological characteristics.

The Fagales (Tables A3 & A4, Fig. A2)

Members of Fagales represent some of the most important temperate deciduous or evergreen trees of both hemispheres, including oaks, beeches, walnuts, hickories, and birches.

The question for which you will develop a hypothesis is: Did allelopathy evolve more than once in this group of plants?

These are the taxa for which you are responsible:

Betulaceae
Casuarinaceae
Fagaceae
Juglandaceae
Myricaceae

Use the websites provided here to help with this exercise:

http://tolweb.org/Fagales/21027
http://www.phytoimages.siu.edu/taxpage/0/order/Fagales.html

In working on this problem, don't focus on learning plant anatomy. Here are some terms you might not know:

Cupule: a cup-shaped anatomical structure, like that holding an acorn.
Hairs: often leaves, stems, or other structures are covered with coarse or fine hairs.
Nectaries: the nectar-producing glands at the base of stamens.
Pollen types: pollen grains have many different features that are visible under a microscope. Trisulcate grains have 3 rows or furrows in them. Triporate grains have three rounded indentations.
Sepal: the (usually) green leaf-like cup structure surrounding the base of a flower.
Gametophyte: the embryonic plant precursor within a seed.
Ovule: the structure that develops into a seed.

Table A3. Morphological features of certain Fagales.

	Cucurbitaceae (outgroup)	Myricaceae	Casuarinaceae	Fagaceae	Betulaceae	Juglandaceae
Nectaries	Present	Absent	Absent	Absent	Absent	Absent
Gametophytes/ ovule	One	Many	Many	One	Many	Many
Aromatic glands	No	Yes	No	No	No	Yes
Pollen surface texture	Smooth	Tiny spines in rows	Tiny spines in rows	Smooth	Tiny spines in rows	Tiny spines in rows
Sepals and petals	Reduced	Normal	Normal	Normal	Normal	Normal
Production of allelochemical	No	No	Yes	No	No	Yes
Cupule	Smooth	Smooth	Smooth	Scaly	Smooth	Smooth
Hair type	Non–glandular, unbranched	Glandular, branched	Glandular, branched	Glandular, branched	Glandular, branched	Glandular, branched
Pollen type	Trisulcate	Trisulcate	Triporate	Trisulcate	Triporate	Trisulcate

Table A4. Morphological features of certain Fagales designated by conventional method.

	Cucurbitaceae (outgroup)	Myricaceae	Casuarinaceae	Fagaceae	Betulaceae	Juglandaceae
Nectaries	0	1	1	1	1	1
Gametophytes/ ovule	0	1	1	0	1	1
Aromatic glands	0	1	0	0	0	1
Pollen surface texture	0	1	1	0	1	1
Sepals and petals	0	1	1	1	1	1
Production of allelochemical	0	0	1	0	0	1
Cupule	0	0	0	1	0	0
Hair type	0	1	1	1	1	1
Pollen type	0	0	1	0	1	0

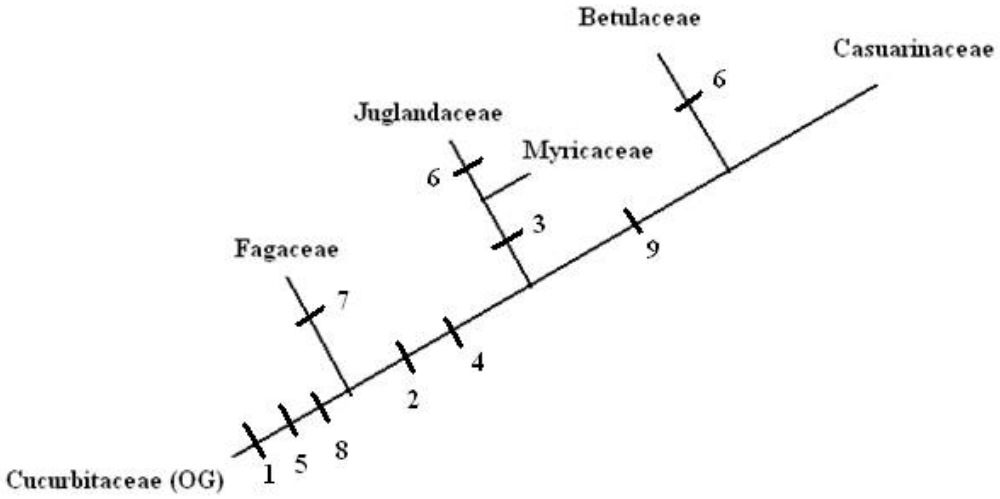

Figure A2. Cladogram of certain Fagales based on designated morphological characteristics.

Flightless Birds (Tables A5 & A6, Fig. A3)

Living birds are divided into two major groups, Paleognathae—flightless birds—and Neognathae—birds that can fly. *Paleognath* is a word derived from the ancient Greek for "old jaws," in reference to the skeletal anatomy of the palate, which is described as more primitive and reptilian than that in other birds. Flightless birds, which include fewer than 1% of all bird species, are also divided into two groups, the truly flightless ratites and tinamous that can fly for only very short distances.

The question for which you will develop a hypothesis is: How can the evolution of Paleognathae be explained by the break-up of Pangaea?

These are the taxa for which you are responsible:

Aepyornithidae
Apterygidae
Casuariinae
Dinornithidae
Dromiceinae
Rheinae
Struthioniae
Tinamidae

Use the websites provided here to help with this exercise:

http://en.wikipedia.org/wiki/Palaeognathae
http://www.oucom.ohiou.edu/dbms-witmer/Downloads/1993_Baumel_&_Witmer_NAA-2_Osteologia.pdf

Table A5. Morphological features of certain flightless birds.

	Tinamou (outgroup)	**Apterygidae**	**Dinornithidae**	**Aepyornithidae**	**Struthioniae**	**Rheinae**	**Casuariinae**	**Dromiceinae**
Zygomatic process	Not elongated	Elongated	Elongated	Elongated	Elongated	Elongated	Elongated	Elongated
Zygomatic process: base	No knob	No knob	No knob	No knob	No knob	No knob	Smooth knob	Smooth knob
Pila otica near the ear	Not slender or sharply defined	Not slender or sharply defined	Not slender or sharply defined	Not slender or sharply defined	Not slender or sharply defined	Slender and sharply defined	Slender and sharply defined	Slender and sharply defined
Lumbar vertebrae processes	Not broad or fused	Not broad or fused	Not broad or fused	Not broad or fused	Not broad or fused	Not broad or fused	Broad and fused with each other	Broad and fused with each other
Sternum	Large keel	No keel	No keel	No keel	No keel	No keel	No keel	No keel
Sternum	Long to square in shape	Flattened and wide	Flattened and wide	Long to square in shape	Long to square in shape	Long to square in shape	Long to square in shape	Long to square in shape
Sternum: coracoid sulci	Moderately separated	Widely separated	Widely separated	Moderately separated	Moderately separated	Moderately separated	Moderately separated	Moderately separated
Humerus: head of humerus	Not separated from tubercle	Not separated from tubercle	Not separated from tubercle	Not separated from tubercle	Not separated from tubercle	Not separated from tubercle	Separated from tubercle	Separated from tubercle
Pelvis	Broad	Narrow	Narrow	Narrow	Narrow	Narrow	Narrow	Narrow
Ilium: length of wings relative to the acetabulum	Wing behind < wing in front	Wing behind < wing in front	Wing behind < wing in front	Wing behind < wing in front	Wing behind > wing in front	Wing behind > wing in front	Wing behind > wing in front	Wing behind > wing in front

Ischium: wings	Not narrow or slender	Not narrow or slender	Not narrow or slender	Not narrow or slender	Narrow and slender	Narrow and slender	Narrow and slender	Narrow and slender
Femur: comparison of lateral and medial condyles	Medial > lateral	Medial > lateral	Medial > lateral	Lateral > medial	Lateral > medial	Lateral > medial	Lateral > medial	Lateral > medial
Tibiotarsus: medial condyle	Does not project rostrally	Projects rostrally and is flanked by depression	Projects rostrally and is flanked by depression	Does not project rostrally	Does not project rostrally	Does not project rostrally	Does not project rostrally	Does not project rostrally
Foot	4 digits	4 digits	4 digits	3 digits	3 digits	3 digits	3 digits	3 digits

Table A6. Morphological features of certain flightless birds designated by conventional method.

	Tinamou (outgroup)	**Apterygidae**	**Dinornithidae**	**Aepyornithidae**	**Struthioniae**	**Rheinae**	**Casuariinae**	**Dromiceinae**
Zygomatic process	0	1	1	1	1	1	1	1
Zygomatic base	0	0	0	0	0	0	1	1
Pila otica near the ear	0	0	0	0	0	1	1	1
Lumbar processes	0	0	0	0	0	0	1	1
Sternum	0	1	1	1	1	1	1	1
Sternum	0	1	1	0	0	0	0	0
Sternum: coracoid sulci	0	1	1	0	0	0	0	0
Head of humerus	0	0	0	0	0	0	1	0
Pelvis	0	1	1	1	1	1	1	1
Length of wings relative to acetabulum	0	0	0	0	1	1	1	1
Ischium: wings	0	0	0	0	1	1	1	1
Femur: comparison of lateral and medial condyles	0	0	0	1	1	1	1	1
Tibiotarsus: medial condyle	0	1	1	0	0	0	0	0
Foot	0	0	0	1	1	1	1	1

Femur: comparison of lateral and medial condyles	0	0	0	1	1	1	1	1
Tibiotarsus: medial condyle	0	1	1	0	0	0	0	0

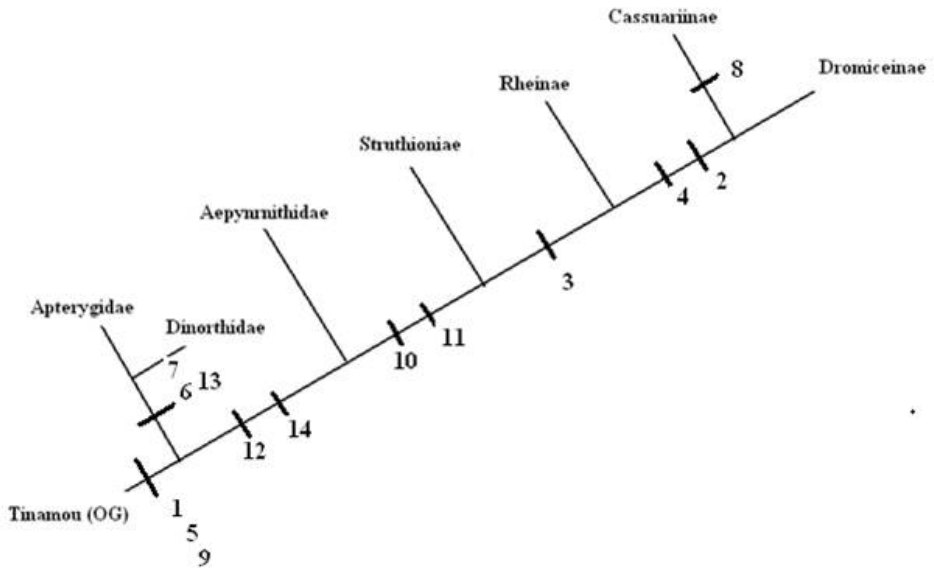

Figure A3. Cladogram of certain flightless birds based on designated morphological characteristics.

Cetaceans (Tables A7 & A8, Fig. A4)

Cetaceans—whales, dolphins, and porpoises—are mammals that live, eat, reproduce, and rest in the water. Their ancestors were land mammals. More than 50 million years ago, these ancestors evolved physical characteristics that allowed them to live successfully in the water. There are two suborders of Cetaceans. Mysticeti, or baleen whales, are filter feeders; Odontoceti, or toothed whales, are active predators.

The question for which you will develop a hypothesis is: Did filter feeding evolve more than once among cetaceans? Use the websites provided to help you with this exercise.

http://tolweb.org/Cetacea/15977
http://www.acsonline.org/factpack/index.html
http://en.wikipedia.org/wiki/Cetaceans

These are the taxa for which you are responsible:

Balaenidae
Balaenopteridae
Delphinidae
Eschrichtiidae
Monodontidae
Neobalaenidae
Physeteridae
Ziphiidae

In working on this problem, don't focus on learning cetacean anatomy. Here are some terms you might not know:

Fluke—either of the two horizontally flattened divisions of the tail of a whale

Blubber--thick layer of fat between the skin and the muscle layers of whales and other marine mammals, from which an oil is obtained.

Melon--fatty organ found in the forehead of all toothed whales and believed to be used in echolocation.

Baleen—fibrous yet elastic structure that enables some whales to filter food from the water for ingestion. Baleen is composed of keratin and grows in long thin plates with brush-like, frayed edges that hang down from the animal's upper jaw.

Olfactory nerve—one of the 12 cranial nerves; instrumental in the sense of smell

Spermaceti organ –made of two large oil-filled sacs. Contact with air the oil partially solidifies turning it white and giving it a semen like appearance, hence the name spermaceti oil The organ is used by whales to transmit and receive ultrasonic waves.

Temporal fossa—shallow depression on the side of the skull bounded by the temporal lines and terminating below the level of the zygomatic arch.

Tympanic bulla—a thin-walled bony capsule that houses an extension of the cavity of the middle ear, the tympanic cavity.

Table A7. Morphological features of certain Cetaceans.

	Artiodactyla (outgroup)	**Balaenopteridae**	**Balaenidae**	**Neobalaenidae**	**Eschrichtiidae**	**Physeteridae**	**Ziphiidae**	**Monodontidae**	**Delphinidae**
Tail	Not terminating in flukes	Terminating in flukes	Terminating in flukes	Terminating in flukes	Terminating in flukes	Terminating in flukes	Terminating in flukes	Terminating in flukes	Terminating in flukes
External hind limbs	Present	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Front limbs	Similar in structure to hind limbs	Modified into flippers	Modified into flippers	Modified into flippers	Modified into flippers	Modified into flippers	Modified into flippers	Modified into flippers	Modified into flippers
Blubber	Absent	Present	Present	Present	Present	Present	Present	Present	Present
Melon	Absent	Present	Present	Present	Present	Present	Present	Present	Present
Baleen	Absent	Present	Present	Present	Present	Absent	Absent	Absent	Absent
Olfactory nerve	Present	Present	Present	Present	Present	Absent	Absent	Absent	Absent

Number of nostrils	2	2	2	2	2	1 (blow-hole)	1 (blow-hole)	1 (blow-hole)	1 (blow-hole)
Sperma-ceti organ	Absent	Absent	Absent	Absent	Absent	Present	Absent	Absent	Absent
Nasal passages	Separate	Separate	Separate	Separate	Separate	Separate	Confluent	Confluent	Confluent
Temporal fossa	Not roofed over by maxillae	Not roofed over by maxillae	Not roofed over by maxillae	Not roofed over by maxillae	Not roofed over by maxillae	Not roofed over by maxillae	Roofed over by maxillae	Roofed over by maxillae	Roofed over by maxillae
Tympanic bulla	Not reduced in size	Not reduced in size	Not reduced in size	Not reduced in size	Not reduced in size	Not reduced in size	Not reduced in size	Moderate-ly reduced in size	Moderate-ly reduced in size
Vestibular sac	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Present	Present
Right side of vestibu-lar air sac	Not hypertro-phied	Not hypertro-phied	Not hypertro-phied	Not hypertro-phied	Not hypertro-phied	Not hypertro-phied	Hypertro-phied	Not hypertro-phied	Not hyper-trophied
Facial plane	Concave	Concave	Concave	Concave	Concave	Concave	Concave	Flat or convex	Concave
Bridle pig-mentation pattern on head	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Present

Table A8. Morphological features of certain Cetaceans designated by conventional method.

	Artiodac-tyla (out-group)	**Balae-nopteri-dae**	**Balaeni-dae**	**Neoba-laenidae**	**Eschrich-tiidae**	**Physe-teridae**	**Ziphiidae**	**Mon-odontidae**	**Delphini-dae**
(out-group)	Balaenop-teridae	Balaeni-dae	Neobalae-nidae	Eschrich-tiidae	Physeteri-dae	Ziphiidae	Monodon-tidae	Delphini-dae	
Tail	0	1	1	1	1	1	1	1	1
External hind limbs	0	1	1	1	1	1	1	1	1
Front limbs	0	1	1	1	1	1	1	1	1
Blubber	0	1	1	1	1	1	1	1	1
Melon	0	1	1	1	1	1	1	1	1
Baleen	0	1	1	1	1	0	0	0	0
Olfactory nerve	0	1	1	1	1	0	0	0	0
Number of nostrils	0	0	0	0	0	1	1	1	1
Sperma-ceti organ	0	0	0	0	0	1	0	0	0
Nasal pas-sages	0	0	0	0	0	0	1	1	1
Temporal fossa	0	0	0	0	0	0	1	1	1

Tympanic bulla	0	0	0	0	0	0	0	1	1
Vestibular sac	0	0	0	0	0	0	0	1	1
Right side of vestibular air sac	0	0	0	0	0	0	1	0	0
Facial plane	0	0	0	0	0	0	0	1	0
Bridle pigmentation pattern on head	0	0	0	0	0	0	0	0	1

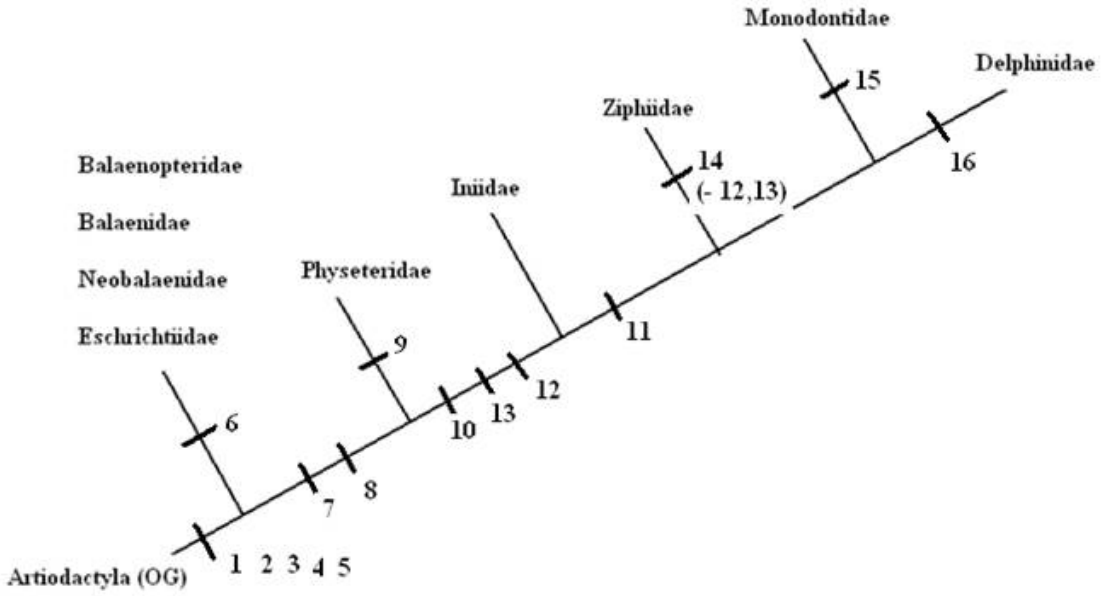

Figure A4. Cladogram of certain cetaceans based on designated morphological characteristics.

About the Author

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