



## Biodiversity and Evolutionary Trees

### OVERVIEW

In this activity, students use DNA sequences from marine mollusks to construct phylogenetic trees. This activity uses mollusks as model organisms to introduce phylogeny and the importance of evolutionary relationships in modern taxonomy.

In Part 1, students learn how DNA can be used to study evolutionary relationships. Part 2 introduces an online software ([MAFFT](#)) for generating DNA sequence alignments and phylogenetic trees. In Parts 3 and 4, students build and explore phylogenetic trees of different mollusks. In the optional inquiry-based extension, students apply the techniques they've learned to explore evolutionary relationships among species of their choosing.

This activity was designed to accompany the Click & Learn [Sorting Seashells](#), in which students construct an evolutionary tree of the same mollusk species based on morphological characteristics. Consider having students build the tree in the Click & Learn first, then use this activity to explore whether that tree is supported by DNA evidence.

Additional information related to pedagogy and implementation can be found on [this resource's webpage](#), including suggested audience, estimated time, and curriculum connections.

### KEY CONCEPTS

- Phylogeny is based on evolutionary relationships.
- A phylogenetic tree can be built by repeatedly identifying both the most dissimilar members and the most similar members in a group of organisms.
- DNA sequences can be used in the study of phylogenetics.

### STUDENT LEARNING TARGETS

- Use an online software to align DNA sequences and build phylogenetic trees.
- Interpret simple phylogenetic trees.
- Use phylogenetic trees to compare hypotheses about evolutionary relationships among groups of organisms.
- Explain how DNA sequences can be used to study evolutionary relationships.

### PRIOR KNOWLEDGE

Students should have a basic understanding of:

- the classification of species or organisms
- the structure of DNA and mutations
- interpreting phylogenetic trees

### MATERIALS

- copies of the "Student Handout"
- access to the online software [MAFFT](#)
- DNA sequence files (test.txt, molluscs1.txt, molluscs2.txt), available in the "Sequence Files" ZIP file

## TEACHING TIPS

- For students who need extra language support, including English Language Learners:
  - Create a “word wall” to help students learn and use key vocabulary from the activity, including terms that may be unfamiliar in a scientific context. These terms could include mollusk, evolutionary, relationship, ancestor, alignment.
  - Encourage students to work together, discuss their answers, and ask additional questions as they work. Talking through ideas helps students develop stronger language skills.
  - Assist students with the reading, especially in Part 1, as needed.
- There are many different methods for generating phylogenetic trees from DNA sequences, and different methods may give different results. You may wish to have students try this activity with other algorithms or software. Explain that the results may not always agree and will depend on the underlying assumptions and limitations of each method.
- As a potential extension, follow up this activity with the Click & Learn [Creating Phylogenetic Trees from DNA Sequences](#), which has more information about evolutionary relationships, DNA sequence alignment, and phylogenetic trees.

## PROCEDURE

1. In advance, try the activity using your school’s computer system to ensure that students can access the online software and text files.
2. At the start of the activity, activate students’ prior knowledge and build engagement. For example:
  - a. Tell students that the shells in the activity are from marine mollusks of the Philippines. Show where the Philippines are on a map of the world if needed.
  - b. Point out that the Indo–West Pacific region, including the Philippines, is rich in marine biodiversity. Show students [“Surrounded by the Ocean,”](#) a 12-minute mini-documentary of Philippines biodiversity, or the 2-minute video clip [“Philippines Biodiversity.”](#)
  - c. Introduce students to some interesting mollusks, such as the cone snails featured in the activity. Cone snails are venomous and carnivorous; some hunt fish, others hunt snails or worms. You can highlight the feeding behavior of cone snails using [“Cone Snail Strikes a Fish,”](#) a 2-minute video clip showing a fish-hunting species, or [“Cone Snails: Versatile Hunters,”](#) a 13-minute video on cone snails.
  - d. Have students do the Click & Learn [Sorting Seashells](#), in which students construct an evolutionary tree of mollusk species based on morphological characteristics. After students build the tree in the Click & Learn, they can then use this activity to explore whether that tree is supported by DNA evidence.
    - i. Note *COI* gene sequences for five of the species used in the Click & Learn were not available, so this activity substitutes some sequences with ones from closely related species. Substitutions were as follows: *Neritina pulligera* was substituted for *Neritina communis*, *Distorsio reticularis* for *Distorsio anus*, *Bursa granularis* for *Bursa nobilis*, *Mitra lens* for *Imbricaria conularis*, and *Pecten jacobaeus* for *Pecten pallium*.
3. Have students complete **Parts 1–4** in the “Student Worksheet.”
  - a. Group or pair students and encourage them to discuss ideas as they go along.
  - b. For Parts 2–4, remind students to choose “UPGMA” as their clustering method in Simple Phylogeny. Students that choose a different clustering method may get different results.
    - i. Clustering methods like UPGMA are algorithms for generating clusters. We recommend using UPGMA because it produces a tree with a root, which is what we want. If students are curious, they

could try generating a tree with the other clustering method, “Neighbor-joining,” and see the difference.

4. Optionally, have students complete the **extension** at the end of the “Student Worksheet,” either individually or in groups.
  - a. You could also repeat the extension using different genes. For phylogenetic comparisons to work, the gene should be conserved among different species you are interested in — similar enough to be compared, yet different enough to give enough variability.
  - b. You could try genes for actin, myosin, or ubiquitin. If you restrict the range of species, even the gene for hemoglobin might work.

## ANSWER KEY

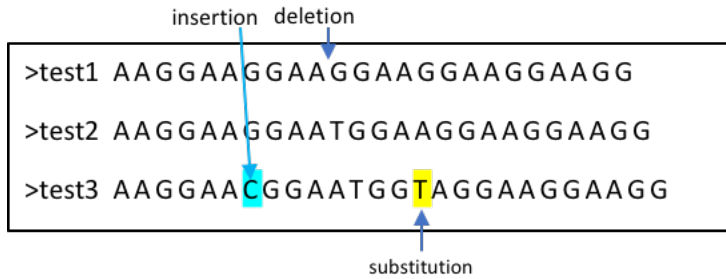
### PART 1: Comparing DNA Sequences to Determine Evolutionary Relationships

1. What is a “morphological characteristic”? Give an example of a morphological characteristic in mollusks.  
***A morphological characteristic is a physical or structural trait. Examples in mollusks include shell size, shape, color, etc. (These and other examples are shown in the [Sorting Seashells Click & Learn.](#))***
2. What might be some advantages of using DNA sequences, rather than morphological characteristics, to determine evolutionary relationships?  
***Answers will vary. Students may suggest that morphological data are less precise or less reliable, are difficult to observe for some organisms, might depend on developmental stage, might be misleading due to similar structures in unrelated organisms, etc. (As an extension, you could have students discuss potential disadvantages or assumptions involved in using DNA sequences — for example, assuming constant rates of evolution.)***
3. Why would a *COI* sequence *not* be a good choice for studying variations within the *same* species?  
***The COI gene is not a good choice for studying variations within the same species (or even among species that have recently speciated) because their COI sequences would be too similar.***
4. *COI* sequences in plants have a much lower mutation rate than those in animals. Based on this information, why might *COI* sequences be less useful for determining evolutionary relationships among plant species than among animal species?  
***Due to the low mutation rate, there may not be enough variation among plant COI sequences for determining evolutionary relationships.***
5. What type of animal did the *COI* sequence in Figure 1 come from? (*Hint*: Look at the information before the DNA sequence.)  
***According to the information at the beginning of Figure 1, the sequence came from a dog (scientific name *Canis lupus familiaris*).***

### PART 2: Generating DNA Sequence Alignments and Phylogenetic Trees

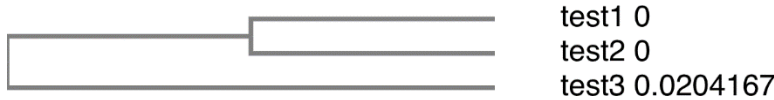
6. Would you expect organisms that are *more* closely related to have more or fewer differences between sequences? Explain your answer.  
***More closely related organisms probably have fewer differences between sequences. This is because there has been less time for mutations to accumulate between more closely related species compared to less closely related species.***

7. Based on your MAFFT results, label the differences in sequences in Figure 2 as deletions, insertions, or substitutions.



8. Which two sequences do you think are from the *most* closely related organisms, and why?  
***There is one difference between test1 and test2, two differences between test2 and test3, and three differences between test1 and test3. Since test1 and test2 have the fewest differences, their organisms are probably more closely related to each other than to the test3 organism.***

9. Sketch or provide a copy of the phylogenetic tree that was generated.



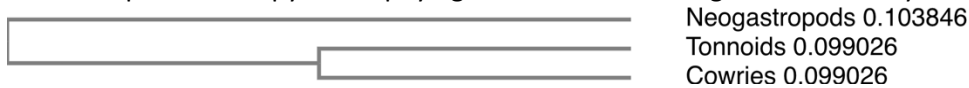
10. Based on this tree, would you revise your answer to Question 8? Why or why not?  
***Answers will vary. Since test1 and test2 are on the same branch of the tree, it suggests their organisms are more closely related to each other than to the test3 organism.***

**PART 3: Phylogenetic Trees of Neogastropods, Tonnoids, and Cowries**

11. According to the phylogenetic tree in Figure 3, which two groups of snails are most closely related?  
***Tonnoids and cowries***

12. Based on Figure 3, how might the *COI* gene sequences of these three groups vary? What would you expect to see in terms of similarities and differences?  
***We would expect the tonnoid and cowrie sequences to be more similar to each other than to the neogastropod sequence.***

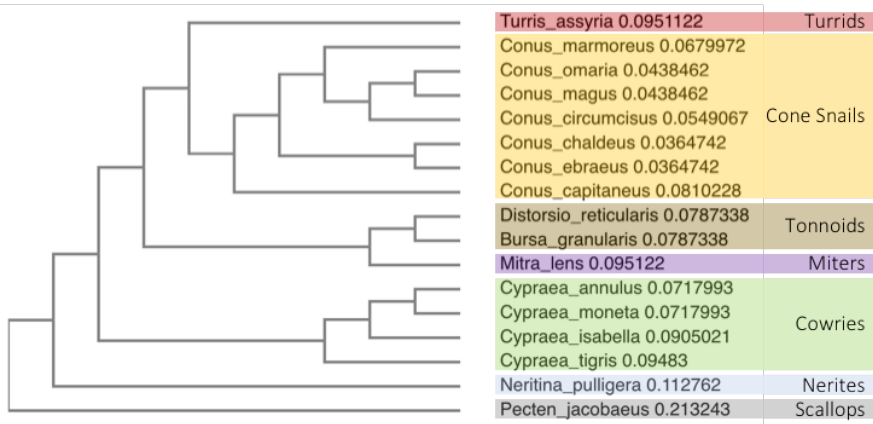
13. Sketch or provide a copy of the phylogenetic tree that was generated after your alignment.



14. Do your results support or contradict the evolutionary relationships proposed in Figure 3? Support your answer using evidence from the phylogenetic tree you generated.  
***The results support the relationships in Figure 3. Both the tree in Figure 3 and the phylogenetic tree based on DNA have tonnoids and cowries on the same branch and neogastropods on a different branch, suggesting that tonnoids and cowries are more closely related to each other than to neogastropods.***

**PART 4: Phylogenetic Trees of More Mollusks**

15. Sketch or provide a copy of the phylogenetic tree that was generated after your alignment. Label or color-code the species according to the groups shown in Table 1.  
***An example is shown below. Make sure students choose “UPGMA” as their clustering method in Simple Phylogeny, or they may get a different result.***



16. According to the phylogenetic tree you generated, which mollusks are *most* closely related to miters?  
**Tonnoids (Distorsio reticularis and Bursa granularis)**
17. According to the phylogenetic tree you generated, are turrids more closely related to miters or to cone snails?  
**Cone snails**
18. Do the DNA sequences you examined support all the evolutionary relationships proposed in Figure 5? Why or why not?  
**No, the DNA sequences do not support all the relationships in Figure 5. We can see this by comparing the tree generated from DNA sequences (Question 15) to the tree in Figure 5.**
- Both trees have scallops as the first outgroup and nerites as the second outgroup. However, Figure 5 indicates that miters are more closely related to the turrids and cone snails, and that the tonnoids and cowries are more closely related to each other than to the miters, turrids, and cone snails. In contrast, the tree based on genetic data suggests that the miters are more closely related to the tonnoids and that the miters and tonnoids are more closely related to the cone snails than to the cowries.**
19. Imagine showing what you've been working on to a friend or family member who is not familiar with the science. Explain what a phylogenetic tree is, the information that is used to make it, and its purpose, in a way that your friend or family member could understand.  
**Answers will vary; an example is as follows: A phylogenetic tree is a diagram that shows the evolutionary relationships among organisms. The tree can be made by comparing similarities and differences in the organisms' characteristics or DNA. (DNA is preferred.) The purpose of a phylogenetic tree is to propose hypothetical evolutionary relationships among organisms, so that we can examine how closely related species/groups are and how they might have evolved.**

#### EXTENSION: Build Your Own Phylogenetic Tree

**Student answers will vary. Be open to a range of reasonable responses.**

#### CREDITS

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