

USING DNA TO EXPLORE LIZARD PHYLOGENY

OVERVIEW

This lesson serves as a supplement to the film *Lizards in an Evolutionary Tree*. The film focuses on a closely related group of lizards called anoles (genus *Anolis*) that live in the Caribbean islands. They have evolved to occupy different niches in the environment, and researchers are finding surprising insights into evolutionary processes, such as adaptation by natural selection, convergent evolution, and the formation of new species. Please refer to the In-Depth Guide (http://media.hhmi.org/biointeractive/activities/lizard/IDG_Lizards.pdf) for more information about the anole lizards and the film.

In this activity, students explore the evolutionary relationships of the Caribbean anole lizards. In part 1, students examine the diverse appearance of the lizards and group them into categories according to different body types. Differences between the species illustrate adaptive radiation; students can generate hypotheses about possible reasons why different adaptations have evolved and infer evolutionary relationships among lizards based on their adaptations. In part 2, mitochondrial DNA sequence data from these same species of lizards are used to generate a phylogenetic tree to reveal their true evolutionary relationships. The students need to reconcile why the evolutionary tree obtained in part 2 is different from the original grouping proposed in part 1.

Students should watch the film in stages at different points in the activity, or afterward.

KEY CONCEPTS

- A. An adaptation is a structure or function that confers greater ability to survive and reproduce in a particular environment.
- B. Adaptive radiation occurs when an ancestral species diversifies into many descendant species that occupy different environmental niches.
- C. Different groups of organisms can independently evolve similar traits by adapting to similar environments or ecological niches in a phenomenon known as convergent evolution.
- D. DNA sequence comparisons among different populations and species allow scientists to determine the relatedness of different species.

CURRICULUM CONNECTIONS

Curriculum	Standards
NGSS (April 2013)	MS-LS1-5, MS-LS4-2, MS-LS4-4, MS-LS4.A, MS-LS4.B, MA-LS4.C, HS-LS4-1, HS-LS4-2, HS-LS4-3, HS-LS4-4, HS-LS4-5, HS-LS4.B, HS-LS4.C, HS-LS4.D
AP (2012–13)	1.A.1, 1.A.2, 1.A.4, 1.B.2, 1.C.1, 1.C.2, 1.C.3
IB (2009)	5.1, 5.4, D2, D5

TEXTBOOK CONNECTIONS

Textbook	Chapter Sections
Miller and Levine, <i>Biology</i> (2010 ed.)	16.1, 16.3, 16.4, 17.2, 17.3, 17.4, 18.2, 19.2
Reece <i>et al.</i> , <i>Campbell Biology</i> (9th ed.)	22.2, 22.3, 23.3, 23.4, 24.1, 24.2, 24.4, 26.2, 26.3

TIME REQUIREMENTS

Allowing time for discussion, and viewing the film, this activity will take two 50 minute periods for an AP biology class. Allow for more time if you need to cover basics of DNA alignment in greater detail.

PRIOR KNOWLEDGE

- A basic understanding of natural selection, evolution, and adaptation
- Familiarity with the concept that organisms fill specific niches in their environments
- Understand that DNA sequence changes during evolution, and closely related species have similar DNA sequences
- A basic understanding of phylogenetic trees, especially the concept that closely related species are located close together on adjacent or nearby branches of a phylogenetic tree.

MATERIALS

- A set of lizard cards that accompanies this document
- Access to a computer that is connected to the internet
- (Optional) A computer with the freely available ClustalX program installed.

BACKGROUND SUMMARY (FOR MORE DETAIL, SEE THE IN-DEPTH GUIDE)

Different Morphologies of Anole Lizards



Figure 1: Caribbean islands are home to the anoles. The anole species featured in the film and this activity live on the islands of Cuba, Jamaica, Hispaniola (which comprises Haiti and the Dominican Republic), and Puerto Rico. (Reproduced with permission from Losos, J. *Lizards in an Evolutionary Tree*. UC Press, 2009).

Species of Caribbean anoles can be categorized into six groups according to their body characteristics (morphology) and the ecological niches they occupy. The groups are referred to as ecological morphotypes, or ecomorphs.

Table 1 below lists the six anole ecomorphs found in the Caribbean islands and their body features. The film discusses only four ecomorphs: the crown-giant, trunk-ground, twig, and grass-bush anoles. In this activity, we have representatives from the trunk-crown, trunk-ground, twig, and grass-bush anoles, shaded in the table.

Table 1: Six Ecomorphs of Anole Lizards Found in the Caribbean Islands.

Ecomorph	Body length	Limb length	Toe pad size	Tail length	Color	Habitat
Crown-giant	130-191 mm	Short	Large	Long	Usually green	High trunks and branches
Trunk-crown	44-84 mm	Short	Very large	Long	Green	Trunks, branches, and leaves
Trunk	40-58 mm	Intermediate	Intermediate	Short	Gray	Trunks
Twig	41-80 mm	Very short	Small	Short	Gray	Narrow twigs
Trunk-ground	55-79 mm	Long	Intermediate	Long	Brown	Lower trunk and ground
Grass-bush	33-51 mm	Long	Intermediate	Very long	Brown	Bush and grasses

The body features of the ecomorphs are adaptations to their particular niches. (See Figure 2 for an illustration of the habitats of different ecomorphs.) In the film, the long-legged trunk-ground anoles are shown to move faster on the ground than the short-legged twig anoles. The long-legged adaptation helps them not only catch prey on the ground but also avoid predators. However, on twigs, the twig anoles can move easily with their short legs, while the trunk-ground anoles are clumsy. The grass-bush anoles are small and have long legs and strikingly long tails that help them balance on thin branches and blades of grass, and the trunk-crown anoles, with their large toe pads, are adapted to living on leaf surfaces in the canopy.

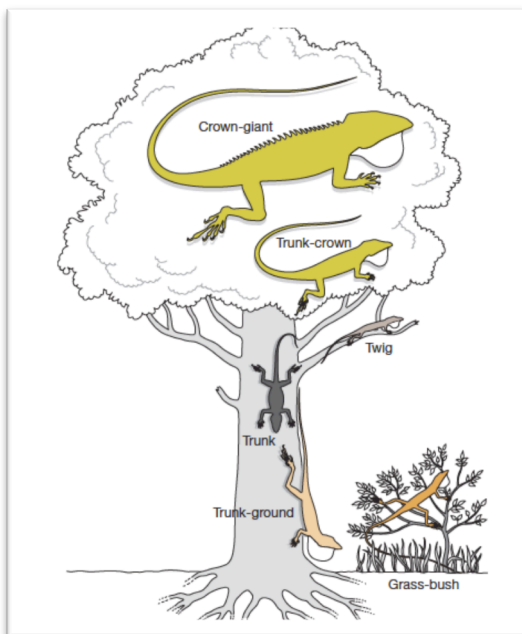


Figure 2: Anoles occupy a variety of ecological niches. Different types of anole lizards have evolved adaptations that enable them to be successful in different ecological niches—different parts of trees, grasses, and bushes. The figure shows the six ecomorphs of anole lizards found in the Caribbean islands in their habitats. (Reproduced with permission from Losos, J. *Lizards in an Evolutionary Tree*. UC Press, 2009).

Convergent Evolution Among Anole Lizards

There are different explanations for why similar ecomorph classes can be found on each of the different islands.

One hypothesis is that each ecomorph evolved once, and the species belonging to the same ecomorph are closely related to one another. Under this scenario, one possibility is that the islands were contiguous in the past when the ecomorphs evolved, and subsequent plate tectonics separated the islands. Another possibility is that the ecomorphs all evolved on the same island, then they all migrated over the narrow channels and established themselves in each of the islands. In these scenarios, you would predict that DNA analysis would reveal, for example, that the twig anole species on all of the islands are more closely related to one another than they are to the grass-bush, trunk-ground, and trunk-crown anoles on the same island.

An alternative hypothesis is that the ecomorphs evolved repeatedly and independently on each of the islands. A prediction based on this hypothesis is that the anoles on each island would be more closely related to one another than they would be to species on other islands belonging to their ecomorph.

When scientists examined DNA sequences from dozens of species of Caribbean anoles, they found that in general, species on the same island tend to be more closely related to one another than to species with similar body types found on different islands. This suggests that the same adaptations evolved independently in different anole populations on each of the islands, an example of convergent evolution.

SUGGESTED PROCEDURE

Students can work individually or in small groups of perhaps between two and four, depending on your classroom. When the procedure calls for a discussion, you can interact with each individual student group, or hold a whole-classroom discussion depending on the dynamics of your classroom.

Part 1: Identifying the Ecomorphs and Exploring Anole Adaptive Radiation

1. At this point, students have not seen the film yet.
2. Let teams of students examine photographs of 16 species of anole lizards and sort them according to how they appear in the photographs into as many groups as they want.
Students can use any clues in the photographs to group the lizards as they see fit. They may use features of the ecomorphs, including color, thickness or length of legs, body shape, tail length, or size. They may even group them by the habitat pictured, such as branches, tree trunks, or grass.
3. Ask the teams to explain how they grouped the lizards and their rationale for the various groupings. If they pick a body feature, ask them to speculate about the advantages or disadvantages of such a body feature in the environment they occupy.
4. Watch the first part of the film (up until 09:48, the end of the experiment on a small rocky island).
5. Students can revise their groupings if they wish.
6. Reveal the four ecomorphs. The 16 species represent the following four ecomorphs. Refer to Table 1 for more descriptions of ecomorphs. Also the first page of the lizard picture file can be used as a master reference.

Trunk-crown: green, foot pads

Twig: short legs, pencil-like morphology

Grass-bush: Slender limbs, body, and long thin tail

Trunk-ground: Robust and chunky appearance, with long legs

7. Discuss the body features of the ecomorphs and how they are adapted to the environment they live in, and the concept of adaptive radiation. For example, the large toe pads of the trunk-crown anoles allow them to adhere

to leaves and let them move about freely among the leaves of the tree canopy. The short legs of the twig lizards are adaptations to living on thin twigs. Refer to the Different Morphologies of Anole Lizards section in the background.

Part 2: Generating a Phylogeny from DNA Sequences and Revealing Evolutionary Relationships of Anoles.

1. Refer to the *Convergent Evolution Among Anole Lizards* section. Discuss with the students different hypothetical relationships among the anole lizards as described in the film. See Figure 3 below.

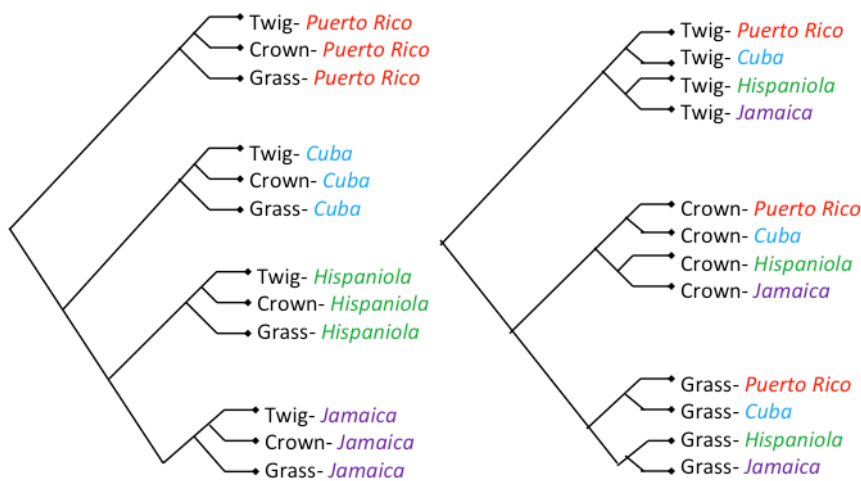


Figure 3: Possible relationships of anole lizards. Two extreme possibilities of the relationships of the Caribbean anole lizards represented by phylogenetic trees. Left: Species living on the same island are closely related to one another. Right: Species belonging to the same ecomorph are closely related to one another.

2. (Optional) If your students have never been exposed to generating and interpreting phylogenies from DNA sequences, you may want to use the following resources from BioInteractive:
 - a. Follow the procedure outlined in *Introduction to DNA Sequence Alignment Using ClustalX*.
<http://media.hhmi.org/biointeractive/activities/lizard/Sequence-Alignment-Introduction.pdf>
 - b. Have your students explore the Click and Learn *Creating Phylogenetic Trees from DNA Sequences*.
<http://www.hhmi.org/biointeractive/creating-phylogenetic-trees-dna-sequences>
3. Download the Anolis DNA sequences TXT file (<http://media.hhmi.org/biointeractive/activities/lizard/Anolis-DNA-sequences.txt>). This file includes the DNA sequences of the 16 *Anolis* species in the photographs. The sequence used is a stretch of mitochondrial DNA that includes the NADH dehydrogenase subunit 2 (ND2) gene and five tRNA genes. These genes are conserved enough among animal species that they can be recognized in two distantly related species and compared, yet they are also variable enough that each species has a unique sequence, and that sequence can be compared to that of closely related species to infer evolutionary relationships. Genes such as these are used in DNA fingerprinting of species.

In addition to the anoles, one unrelated lizard species, *Leiocephalus barahonensis* is included as an outgroup. This species is included to “root” the tree—to add a node that is most like the distant relative to represent the ancestral group from which the species radiated.

4. Open www.phylogeny.fr in a browser. Phylogeny.fr is an easy-to-use website for sequence alignment and phylogeny generation.

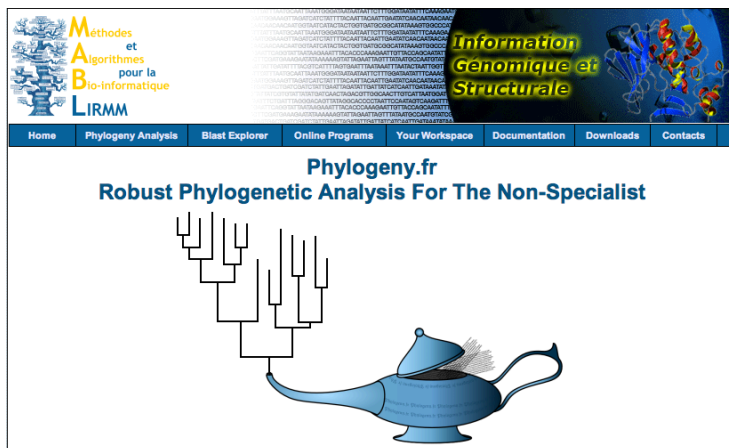


Figure 4: Phylogeny.fr homepage.
This is the entry page for www.phylogeny.fr.

5. Select “One Click” link from the home page of www.phylogeny.fr. This page will open.

"One Click" Mode

Alignment
MUSCLE

→

Curation
Gblocks

→

Phylogeny
PhyML

→

Tree Rendering
TreeDyn

[Help ?](#)

1. Overview

2. Data & Settings

Name of the analysis (optional):

Upload your set of sequences in FASTA, EMBL or NEXUS format from a file:

no file selected

Or paste it here (load example of sequences)

Maximum number of sequences is 200 for proteins and 200 for nucleic acids.
Maximum length of sequences is 2000 for proteins and 6000 for nucleic acids.

☒ Use the Gblocks program to eliminate poorly aligned positions and divergent regions

☐ To receive the results by e-mail, enter your address(es):

Figure 5: One-click mode page at Phylogeny.fr

6. At this point, you can either
 - a. Upload the sequence file by clicking “Choose File” and following the instructions; or
 - b. Open the sequence file with a text editor, copy all, and paste it in the box indicated.
 Then click “Submit”.

7. The website will generate a phylogenetic tree that can be saved in a variety of formats. Because of the nature of the phylogenetic analysis that involves random resampling, each student may get a slightly different tree, but the overall pattern should be mostly similar.

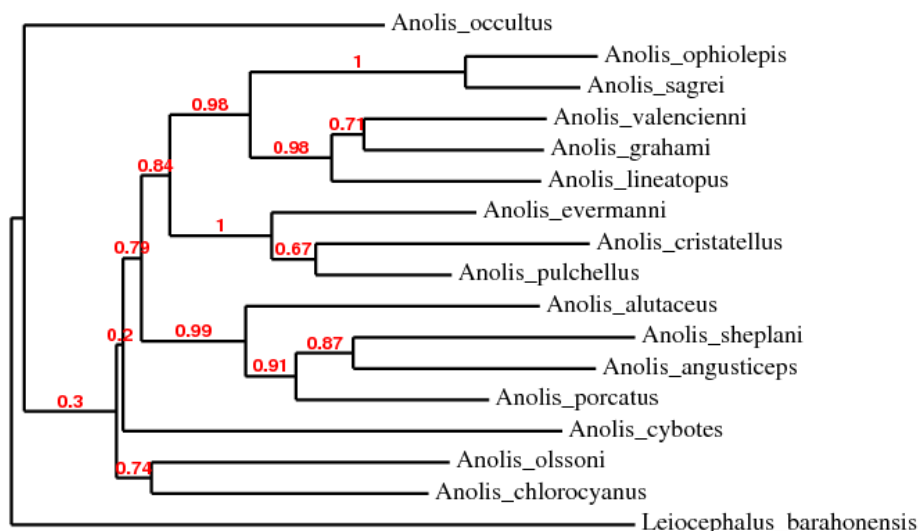


Figure 6: Example phylogeny from www.phylogeny.fr. Students' phylogeny should be similar, but there may be small differences.

The red numbers in the phylogeny result are bootstrapping values, and denote how confident the program is about the specific branch pattern. The value of 1 indicates high confidence; 0 indicates no confidence. Values less than 0.5 are generally regarded as uncertain. However, because the purpose of this activity is to illustrate the principle of DNA sequence comparison, students should not worry about it too much.

8. Print out two copies of the phylogenetic tree per group of students and have them use the list of lizard species that can be found in this document to color the trees in the following ways:
 - a. On one copy, color each branch according to the island of origin (Cuba, Hispaniola, Jamaica, or Puerto Rico). If both branches at a node are the same color, color the root branch and continue left to the next node.

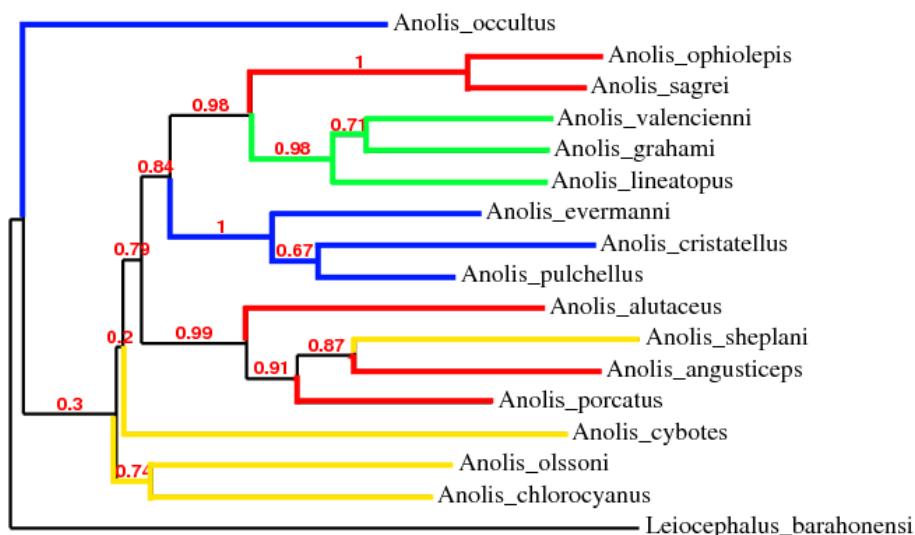


Figure 7: Phylogeny colored in according to geographical distribution.

Red = Cuba, Green = Jamaica, Blue = Puerto Rico, Yellow = Hispaniola.

All species from Jamaica cluster together, and many species from Puerto Rico cluster together. Species from Cuba and Hispaniola.

- b. On the other copy, color each branch according to the ecomorph similarly.

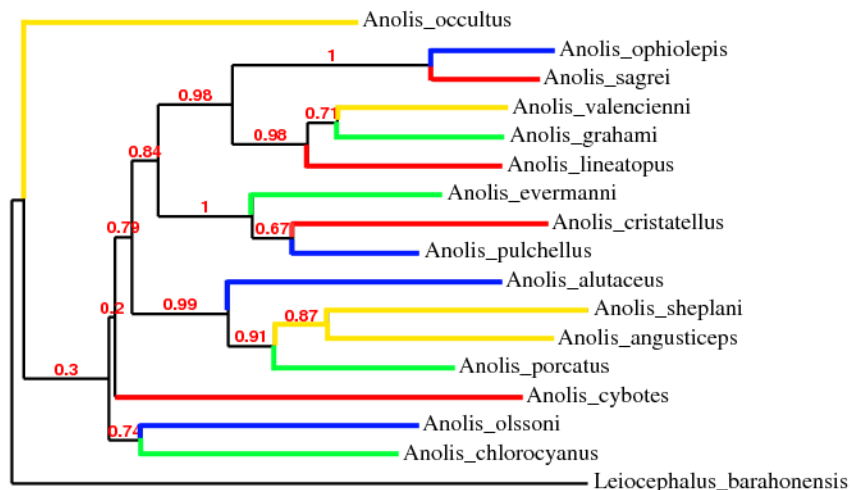


Figure 8: Phylogeny colored in according to ecomorphs.

Red = Trunk-ground,
Green = Trunk-crown,
Blue = Grass-bush,
Yellow = Twig.

Except for two twig species, none of the ecomorphs cluster together, meaning they are not closely related.

9. Examine the phylogenetic tree and discuss the following points:
 - a. What general patterns do you see in the tree?
 - b. Do species from the same ecomorph group together on a branch?
 - c. Do species from the same island group together on a branch?

In general, the phylogenies should show that species from the same island are more closely related than are species from the same ecomorph group. For example, the three Jamaican species should cluster together, as will three of the four Puerto Rican species. The Cuban and Hispaniola species also cluster, but the pattern is more complicated. These pieces of evidence suggest that most of the lizards on each island evolved independently, in a process of convergent evolution, but there are exceptions.

An example of this exception is *Anolis sheplani*, a Hispaniola twig species, which is closely related to another twig species from Cuba, *Anolis angusticeps*, in a branch that is otherwise all Cuban. One could hypothesize that *A. sheplani* evolved after Hispaniola was colonized by a twig ecomorph species from Cuba.

10. Watch the rest of the film.
11. Ask the students whether their results agree with what's shown in the film. What are the similarities and differences?
12. Students may ask whether there are examples of convergent evolution in groups other than the anoles. Perhaps the students can be enticed to volunteer if they know anything that looks like a shark. They could list tuna, dolphins, manatees, and ichthyosaurs. Wings in vertebrates are another classic example: birds, bats, pterosaurs. Reduced or absent limbs is another: snakes, caecilians, sirens, slow worms, worm lizards.
13. (Optional) Download the *Reference-Phylogeny* (<http://media.hhmi.org/biointeractive/activities/lizard/Reference-Phylogeny.pdf>) document and refer to the phylogenetic tree. This is one of the phylogenies published in a scientific journal. Find the 16 species used in this activity, and see if their phylogeny differs from the tree generated by the student groups.

Some reasons for differences: The paper uses many more species, which can generate different patterns; the paper also uses different calculations to align the sequences and generate phylogenies.

LIST OF LIZARD SPECIES

Species name	Island	Ecomorph
<i>Anolis alutaceus</i>	Cuba	Grass-bush
<i>Anolis angusticeps</i>	Cuba	Twig
<i>Anolis chlorocyanus</i>	Hispaniola	Trunk-crown
<i>Anolis cristatellus</i>	Puerto Rico	Trunk-ground
<i>Anolis cybotes</i>	Hispaniola	Trunk-ground
<i>Anolis evermanni</i>	Puerto Rico	Trunk-crown
<i>Anolis grahami</i>	Jamaica	Trunk-crown
<i>Anolis lineatopus</i>	Jamaica	Trunk-ground
<i>Anolis occultus</i>	Puerto Rico	Twig
<i>Anolis olssoni</i>	Hispaniola	Grass-bush
<i>Anolis ophiolepis</i>	Cuba	Grass-bush
<i>Anolis porcatus</i>	Cuba	Trunk-crown
<i>Anolis pulchellus</i>	Puerto Rico	Grass-bush
<i>Anolis sagrei</i>	Cuba	Trunk-ground
<i>Anolis shelpani</i>	Hispaniola	Twig
<i>Anolis valencienni</i>	Jamaica	Twig
<i>Leiocephalus barahonensis</i>		Outgroup

RELATED CLASSROOM RESOURCES

Other Resources for the film (<http://www.hhmi.org/biointeractive/classroom-activities-origin-species-lizards-evolutionary-tree>) Check this page to see other resources developed for the film.

Sorting Seashells (<http://www.hhmi.org/biointeractive/sorting-seashells>): In this Click and Learn, students explore principles of taxonomy by sorting seashells according to their morphological characteristics and constructing an evolutionary tree.

Classroom Activities: Biodiversity and Evolutionary Trees (<http://www.hhmi.org/biointeractive/classroom-activities-biodiversity-and-evolutionary-trees>): This is a companion classroom resource to accompany the Click and Learn above. It also includes a DNA sequence comparison activity of the seashells. In the seashell activity, phylogeny generated by examining morphology closely aligns with that generated from DNA, so it is a good contrast to the case of the anole lizards where the two do not agree.

Creating Phylogenetic Trees from DNA Sequences (<http://www.hhmi.org/biointeractive/creating-phylogenetic-trees-dna-sequences>): This Click and Learn explains how DNA sequences can be used to generate such trees, and how to interpret them.

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