USING DNA TO EXPLORE LIZARD PHYLOGENY

INTRODUCTION

The more than 700 islands of the Caribbean are home to about 150 species of anoles, a closely related group of lizards (genus *Anolis*) that occupy diverse habitats and niches. Research on these lizards is enriching our understanding of evolutionary processes, such as adaptation by natural selection, evolution, and the formation of new species—and it is helping to illuminate how and why there are so many different kinds of living organisms on Earth.

Figure 1: Diverse anoles share common features. *Anolis cristatellus* is a common anole species found in Puerto Rico. It has a colorful flap of skin under its throat that it uses to communicate. Anole species live in diverse habitats and vary greatly in size and other obvious physical features such as leg and tail length. (Photo courtesy of Luke Mahler, University of California, Davis.)

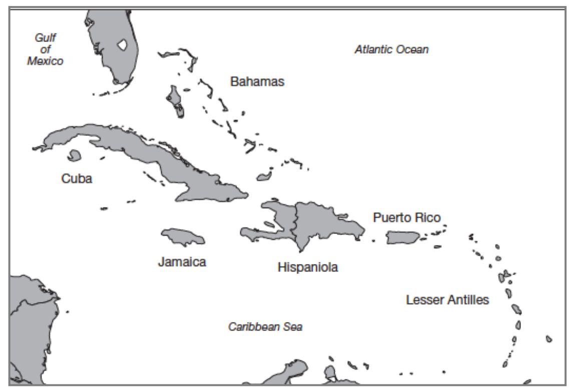


Figure 2: Caribbean islands are home to the anoles. The anole species featured in the film live on the islands of Cuba, Jamaica, Hispaniola (which comprises Haiti and the Dominican Republic), and Puerto Rico. (Reproduced with permission from Losos, J. *Lizards in an Evolutionary Tree*. UC Press, 2011.)

MATERIALS

- A set of lizard cards that accompanies this document
- Access to a computer that is connected to the internet
- HHMI Short Film *Lizards in an Evolutionary Tree*
- (Optional) A computer with the freely available ClustalX program installed.

PROCEDURE

Part 1: Identifying Ecomorphs and Exploring Anole Adaptive Radiation

1. Examine the photographs of 16 species of anole lizards and sort them into as many groups as you want according to how they appear in the photographs. Explain how you grouped the lizards and your rationale for the various groupings. If you picked a body feature, speculate about the advantages or disadvantages of such a body feature in the environment that species occupies.

2. Watch the first part of the film (up until 09:48, the end of the experiment on a small rocky island). Now that you have more information about the anoles, revise your groupings if you wish. Explain any changes you made.

3. 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. The film discusses four ecomorphs. Did your groupings match the ecomorphs described in the film? Why or why not?

4. Using the diagram on the left and the information from the film, choose one ecomorph and explain how the characteristics of that body type are adaptations to that ecomorph's particular habitat.

5. Explain how the different ecomorphs are an example of adaptive radiation.

6. Develop a hypothesis about why similar ecomorph classes can be found on many of the different islands in the Caribbean.

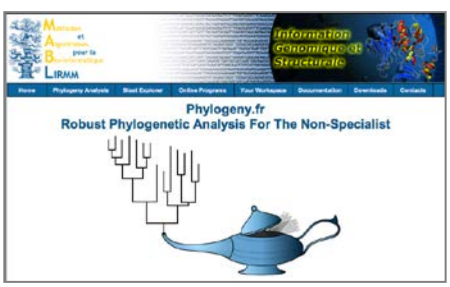
Part 2: Generating a Phylogeny from DNA Sequences and Revealing Evolutionary Relationships of Anoles

How can you explain why similar ecomorphs can be found on each of the different Caribbean islands?

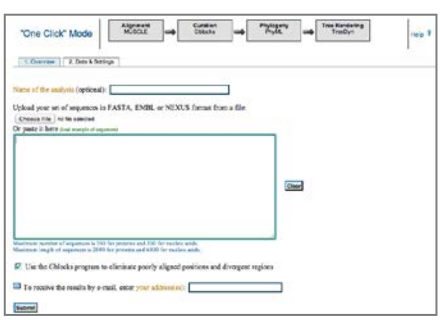
Hypothesis 1: Each ecomorph evolved once and migrated across the narrow channels and spread to the other islands. Or perhaps the islands were contiguous in the past when the ecomorphs evolved and then subsequently become separated. You would expect to find that species belonging to each ecomorph would be more closely related to each other than to species belonging to other ecomorphs.

Hypothesis 2: The ecomorphs evolved repeatedly and independently on each of the islands and thus are examples of convergent evolution. (Convergent evolution is when different groups of organisms independently evolve similar traits by adapting to similar environments or ecological niches.) In this case, you would expect the anoles on each island to be more closely related to one another than they would be to species on other islands.

1. 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 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.
2. Open www.phylogeny.fr in a browser. Phylogeny.fr is an easy-to-use website for sequence alignment and phylogeny generation. The entry page looks like this:



3. Select the "One Click" link from the home page of www.phylogeny.fr. This page will open:



4. 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.
5. Click "Submit."
6. The website will generate a phylogenetic tree. The red numbers in the phylogeny result are bootstrapping values and denote how confident the program is about the specific branch pattern. A 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, you should not worry about it too much.
7. Print two copies of the phylogenetic tree. (Your instructor might provide the trees instead.)
8. Obtain the list of lizard species from your GTA, and color the phylogenetic trees to get a visual picture of how the species evolved.

   a. 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. Include a legend.

   b. Color each branch similarly according to the ecomorph. Include a legend.

ANALYSIS QUESTIONS

Examine the phylogenetic trees you colored and answer the following questions.

1. What general patterns do you see in the tree?
   a. Colored by island:

   b. Colored by ecomorph:

2. Do species from the same ecomorph group together on the tree? Provide evidence to support your answer.

3. Do species from the same island group together on the tree? Provide evidence to support your answer.

4. Based on the trees you colored, develop a claim about whether the species that belong to the same ecomorphs or the species that live on the same islands are more closely related to each other. Justify your claim with several pieces of evidence from the colored phylogenetic trees.

5. Let's review the two hypotheses discussed earlier. One hypothesis is that each ecomorph evolved once, and the species belonging to the same ecomorph are closely related to one another. An alternative hypothesis is that the ecomorphs evolved repeatedly and independently on each of the islands. Which hypothesis is supported by the DNA analysis shown in the tree? Justify your answer and explain your reasoning.

6. Examine the relationship between *Anolis sheplani* and *Anolis angusticeps*. Does it obey the hypothesis you provided in the question above? Explain your answer. Develop a possible hypothesis that could explain this relationship.

7. Watch the rest of the film. Identify and explain any similarities and differences between the phylogeny shown in the film and the phylogeny you made in this activity.