Biology 4415
Evolution

**LABORATORY EXERCISE:
CLADISTICS III**

The last lab and the accompanying lectures should have given you an in-depth introduction to cladistics: what a cladogram means, how to draw one up, and how to read one. But you may be asking by now: *why* is this important? What is a cladogram good for?

In fact, cladistics is becoming increasingly applied in a wide range of fields. Here's a sampling:

• **Coevolution**.Suppose two lineages of organisms live in close contact—for instance, hosts and parasites, or hosts and pathogens. We might expect that the cladograms for the parasites should parallel the cladograms for the parasites. As the host lineage speciates, the parasites or pathogens should have speciated too. This often is in fact the case. To give a well-studied example, the cladogram of primates and the cladogram of the pinworm nematodes that parasitize them show identical patterns: closely related primates usually have closely related parasites.

If we create cladograms for host and parasite taxa and they don't match, there must have been a *host switch*—at least one parasite or pathogen lineage must have been able to jump to a new host. That leads to the question: what features of the parasite or pathogen make that host switching possible? How often has host switching happened? Under what circumstances does it happen? can it happen again? This has applications in everything from AIDS research to public health to veterinary medicine and agriculture.

• **Biogeography and Ecology.** We can compare cladograms drawn up for organisms with the geologic history of their habitats. We might expect that the cladogram for a set of organisms will match the geologic history: two closely related species, for instance, should inhabit areas that were most recently separated. In the same way, we can look at the relationships between species and their habitats. In fact, this is what we're going to do in lab today.

We might also predict that closely related species should have very similar habitats. If they do not, that leads to the question of why not: what made it possible for certain species to colonize habitats that are unlike those of their closest relatives?[1]

[1] This is a horrible oversimplification of a complex field.For an older but still useful treatment, see: Brooks, D. R., and McLennan, D. A. 1991. *Phylogeny, Ecology, and Behavior*. University of Chicago Press, Chicago.

• **Conservation Biology.** In order to conserve a disappearing species, it is necessary to preserve as much of the species' genetic diversity as possible. Unfortunately, it is not always possible to save every population of an endangered species. Cladistics is coming into increasing use to identify which populations of a species are most distinctive, and therefore merit particular attention from conservation biologists. To give a simplified example: saving ten populations of one species that are all closely related to each other is less useful than saving ten populations that are more widely dispersed through the species's total history. Also, a cladistic analysis sometimes uncovers *cryptic species*—separate biological species that are normally difficult to distinguish by morphology.

• **Other Uses.** Epidemiologists have used cladistics and rough molecular-clock dates to gauge how rapidly an epidemic is spreading.[2] Geneticists and anthropologists have used cladistics to examine the origins of crop plants and livestock. Molecular biologists use cladistics and similar algorithms to trace duplications and functional switches in genes, and to search databases for molecular sequences that are related to a known sequence. Linguists have used cladistics to reconstruct the history of languages and of texts.

Today's lab involves creating a cladogram based on molecular data, and then using it to examine the geographic distribution, ecology, and behavior of the organisms in question. The organisms are *anoles*, species of lizard in the genus *Anolis*. Anoles are sometimes called "chamaeleons", and some species can change their skin coloration to match the background the way true chamaeleons do. However, anoles are not true chamaeleons, but rather are more closely related to true iguanas. One species of anole, *Anolis carolinensis*, is native to the southeastern United States (including southern Arkansas), and several more species have been introduced into Florida by humans. However, there are about three hundred species of anole in all, over half of which are native to the islands of the Caribbean. Like the Galápagos finches, Hawaiian honeycreepers, and Lake Victoria cichlid fish, Caribbean anoles make up what is known as a *species flock*—a group of closely related species representing an evolutionary radiation.[3] Because of their ecological diversity and distribution patterns, Caribbean anoles have been studied intensively.[4]

Most Caribbean anole species are unique to individual islands, and the smaller islands typically have only one or two species. The larger islands each have many more: Cuba has 52 anole species; Hispaniola (i.e. Haiti and the Dominican Republic) has 37, Jamaica has 10, and Puerto Rico has 7.

On the larger islands, the species of anole form ecological groupings, or *ecomorphs,* that are repeated on each island. All four islands have at least one *crown giant* anole species—a different species on each island—with a large body size and large toe pads, living high in trees. All four have at least one *trunk/canopy* anole species, with large toe

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[2] Holmes, E. C. et al. 1996. Using phylogenetic trees to reconstruct the history of infectious disease epidemics. *New Uses for New Phylogenies* (P. H. Harvey et al., eds.) Oxford University Press. 169-186.
[3] Losos, J. B. and de Queiroz, K. 1997. Darwin's lizards. *Natural History* 106 (12/97): 34-39.
[4] Roughgarden, J. 1995. *Anolis Lizards of the Caribbean: Ecology, Evolution and Plate Tectonics.* Oxford University Press, New York and Oxford.

pads and an enhanced ability to change color and travel through tree canopies. All four have at least one *twig* species, with a short, slender body and short legs adapted to life on narrow branches; and all four have a *trunk/ground* species living on low tree trunks, with long hind limbs and a sit-and-wait foraging strategy. Three of the four have *grass/bush* species dwelling in underbrush, with long legs and body—for some reason, there is no grass/bush ecomorph on Jamaica. (Which raises the interesting questions: Did a grass/bush form never evolve on Jamaica? If not, why not, eve though there is appropriate habitat on Jamaica? Or did such a form recently go extinct?) Cuba and Hispaniola have *trunk* ecomorph species with flattened bodies, living on tree trunks—Puerto Rico and Jamaica have none. Interestingly, western Cuba is home to a number of ecomorphs that aren't found anywhere else in the Caribbean—for instance, there's an aquatic ecomorph species in western Cuba that catches fish. There are streams with fish on all four islands—why is western Cuba the only known place with an aquatic anole?

It is currently thought that each of these ecomorphs arose convergently on each island. If that is the case, we might expect, for instance, that the Cuban crown giant species will not be closely related to the crown giants from Hispaniola, Puerto Rico, or Jamaica. However, it's possible that the same ecomorphs on different islands evolved from a single common ancestor that somehow got dispersed

The situation is more complicated than this, because larger islands have several species that are "subtypes" of each ecomorph—for instance, moist and dry forests on the same island will have different species in the same ecomorph. What is more, larger islands were divided up into smaller islands at times when sea level was higher than today; this means that speciation has probably happened on once-separated parts of each island. Just to take one example: on Cuba, you find four species of "twig" ecomorphs: the giant *Anolis chamaeleonides* in moist forests all over the island, giant *A. barbatus* in moist forests in the west, giant *A. porcus* in moist forests in the east, and the dwarf *A. angusticeps* in drier forests all over the island. Even worse, some of the islands that are now separated by ocean were joined when sea level was lower. . . The upshot is that quite a lot of vicariance, dispersal, and ecological isolation have been acting for at least fifteen million years (the age of the oldest known fossil anoles from the Caribbean). Our goal is to untangle some of this history.

MATERIALS:

***Anolis* sequence data (mitochondrial NADH dehydrogenase, subunit 2)**

PROCEDURES:

1. Read the article "Darwin's Lizards" from *Natural History* magazine, by Jonathan Losos and Kevin de Queiroz, to get some background on Caribbean *Anolis*.

2. Download the data file **anolis.NEXUS**.[5] This is a set of gene sequences (mitochondrial NADH dehydrogenase, subunit 2) from thirty-seven species of lizards. (See the table on

[5] Data from T. Jackman, A. Larson, K. DeQueiroz and J. B. Losos, acquired from GenBank, **http://www.ncbi.nlm.nih.gov/** .

the last page for the complete species list.) Thirty-five of these lizards are anoles. The other two are outside of the genus *Anolis* but are thought to be related to anoles, and will be used as outgroups.

3. Go to the website **http://www.phylogeny.fr/** and select the option "One Click". Upload **anolis.nexus** and click **Submit.** Save the tree that you get and include it in your writeup.

4. Plot the geographic and ecologic distributions of your species on your tree. (It may be helpful to use highlighter markers of different colors: highlight all Cuban species in yellow and all Hispaniolan species in pink, or something like that. It may also be helpful to plot the geographic distribution on one copy of your tree, and the ecological distribution on another.)

5. Turn in a full lab report—introduction, procedure, data (just the trees), and discussion. In your discussion, give a general reconstruction of how Caribbean anoles evolved, and answer the following questions:

- Cuba and Hispaniola were both formed from the fusion of two or three separate islands. Is this reflected in your tree? How?
- Lowered sea level would once have connected several of the islands. However, there is a deep trench between the southern Antilles (including Grenada and St. Lucia) and the northern Antilles (including St. Kitts, Antigua, and St. Croix). (See the map on the last page.) Is this reflected in your tree? How?
- Which of the major islands seems to have received the most colonists from other islands?
- Does one ecotype of lizards seem to be more likely to disperse than others? In other words, did (for example) most of the crown anoles evolve in place, and most of the grass anoles disperse to other islands?
- How radical do evolutionary changes in ecotype seem to be? Are there cases where a clade of mostly grass anoles gives rise to a crown anole, or are inferred ecological niche shifts less drastic?
- There are about 150 anole species in mainland South America (only one could be included here). There's only one that is native to North America. How likely is it that the North American anole, *Anolis carolinensis*, evolved from ancestors that dispersed to North America via the Isthmus of Panama? What would the cladogram look like if that hypothesis were true? If it's not true, then where did the North American anole come from?

TABLE 1: Species of anole considered in this analysis.

Scientific Name	Common Name	Range	Ecomorph
Anolis equestris	Knight anole	Cuba (E)	crown
Anolis luteogularis	Yellow-throated anole	Cuba (W)	crown
Anolis angusticeps	Cuban twig anole	Cuba (E, W)	twig
Anolis loysiana	Spiny anole	Cuba (E, W)	midtrunk
Anolis sagrei	Cuban brown anole	Cuba (E, W)	trunk/ground
Anolis alutaceus	Blue-eyed anole	Cuba (E)	grass/bush
Anolis vanadicus	Escambray anole	Cuba (W)	grass/bush
Anolis ophiolepis	Snake-scaled anole	Cuba (E, W)	ground/rock
Anolis vermiculatus	Cuban stream anole	Cuba (W)	aquatic
Anolis bartschi	Bartsch's anole	Cuba (W)	rock
Anolis pumilis	Pygmy anole	Cuba (W)	leaf litter
Anolis barahonae	Baraona anole	Hispaniola	crown
Anolis insolitus	Central twig anole	Hispaniola	twig
Anolis christophei	Big-fanned trunk anole	Hispaniola	midtrunk
Anolis distichus	Gracile anole	Hispaniola	midtrunk
Anolis marcanoi	Red-fanned anole	Hispaniola	trunk/ground
Anolis olssoni	Desert grass anole	Hispaniola	grass/bush
Anolis garmani	Jamaican giant anole	Jamaica	crown
Anolis grahami	Graham's anole	Jamaica	trunk/canopy
Anolis valencienni	Jamaican twig anole	Jamaica	twig
Anolis lineatopus	Jamaican gray anole	Jamaica	trunk/ground
Anolis cuvieri	Cuvier's anole	Puerto Rico	crown
Anolis stratulus	Spotted anole	Puerto Rico	trunk/canopy
Anolis occultus	Puerto Rico twig anole	Puerto Rico	twig
Anolis cristatellus	Crested anole	Puerto Rico	trunk/ground
Anolis krugi	Olive bush anole	Puerto Rico	bush/grass
Anolis aeneus	Grenada blue anole	Grenada	large
Anolis richardi	Grenada tree anole	Grenada	small
Anolis luciae	St. Lucia anole	St. Lucia	
Anolis lineatus	Lined anole	Curacao	
Anolis wattsi	Leeward bush anole	Antigua	
Anolis bimaculatus	Leeward tree anole	St. Kitts	
Anolis acutus	St. Croix anole	St. Croix	
Anolis carolinensis	Green anole	North America	
Anolis agassizi	Agassiz's anole	South America	
Diplolaemus darwinii	Darwin's lizard	South America	
Polychrus acutirostris	Monkey lizard	South America	

Map of the Caribbean islands, with the relevant islands labeled.