LAB #2: Genetic drift and phylogenetics of anoles

Adapted from an evolution game developed at Franklin and Marshall College

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BEFORE LAB

- **Read the Introduction and skim the lab exercises below.**
- **Read Sadava et al., chapter 20.2, 21.1**

BRING TO LAB

Pencil and paper, calculator may help but app is available on laptops

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OBJECTIVES

1. Understand basic population genetic terms; gene vs genotype, allele frequency vs genotype frequency
2. Understand what Hardy-Weinberg proportions are and how to calculate them.
3. Learn how phylogenies show relatedness defined by shared common ancestry, and are themselves hypotheses and tools to answer evolutionary questions.

INTRODUCTION

It's incredible to think that Mendel inferred the inheritance of traits not by looking at sequences, understanding the chemistry of DNA, or even knowing where information was stored in the body, but by making inferences based on the frequencies of offspring differing in traits from each other and their parents. In the 20th century early geneticists extended Mendelian genetics to describe the distribution of alleles and genotypes in populations. A **population** is a group of N individuals belonging to the same species living close enough together that all members can mate and exchange genes. This is contrast to a **species**, which is the larger group of all potentially interbreeding individuals (that might be separated in space or time and not able to breed for that reason).

Alleles, the alternate forms a gene for a single trait can take, occur in given frequencies in any population. Those frequencies can change over time, which is one definition of **evolution**. If that change occurs by chance (some individuals die without mating, some **heterozygotes** only pass on one version of the two alternate alleles they carry) it is called **genetic drift**. If it's due to differential average reproductive success to individuals with different alleles, it's called evolution by **natural selection**. Mutation and gene flow also can have strong effects on allele frequencies, but today we will work on selection and drift.

The Gene pool is the total number of all alleles in a population, but it's usually easier to look at a single locus at a time. For diploid organisms like us, there will always be $2N$ alleles in the gene pool for a given locus, because each individual has two alleles. In the example in Figure 20.10 in Sadava *et al*. there are two population with 200 individuals each, and the same allele frequencies of 0.55 for allele A and 0.45 for allele a. In this case the gene pool is 220 A alleles and 180 a alleles. Each individual has two alleles that defines their **genotype** for that locus. Note that for any single **allele frequency** there may be many different **genotype frequencies** in any given population.

Changes in allele frequencies are important because if enough changes become fixed between two populations, they may become reproductively isolated and form two separate species. Geographic isolation makes it easier for both forces in the first part of this lab, selection and drift, to make populations different enough from each other that speciation has occurred. Islands provide the geographic isolation that makes speciation much much eaiser than in **sympatry**. The second part of this lab looks at how isolated species can colonize new niches in similar ways.

OVERVIEW

In the first part of this lab we will simulate a population of fish in a lake, each student is a fish, each fish has two traits that we will represent with a card that shows its genotype. Your fish will exchange genes with another fish in the population, produce offspring, and then die. In fact there are fish in the wild that die shortly after spawning, like salmon, in addition to many species that reproduce many times over their lifetimes. We will then evolve as a population under selection and drift. In the second part of the lab we will build a phylogeny of the anoles found on different islands and use the patterns of shared ancestry to ask whether different ecomorphs evolved once and dispersed, or several times from similar ancestors.

Activity 1: Characterizing a Population

Each student should have at their bench an index card that describes the genotype of an individual fish. On the cards you will see three characteristics:

mating type: There are two mating types, **XX** and **XY**. Individuals can only exchange genes with individuals that have a different mating type.

blue locus: This locus controls one trait in your individual (such as fin shape). There are two kinds of alleles (light blue and dark blue) in the population at this locus, and your individual may be homozygous for one or the other, or heterozygous.

green locus: This locus controls another trait in your individual (mouth size). As with the blue locus, there are two kinds of alleles (light green and dark green).

Class exercise

Count the alleles at each locus in your class population, and answer the questions below. You will need these data for subsequent activities.

Q: What is the population size?____

Q: How many total alleles are there for the blue locus?_____________________________

Q: What is the relationship between population size and number of alleles?_______________

____

Q: What are the frequencies of each allele type? A frequency is a proportion of the total. The sum of all frequencies equals one. (Complete Table 1, columns labeled 1 only.)

Table 1. Record of allele frequencies for activities 1, 3, 4, 5, and 6.

	Actual frequencies blue locus					**Actual** frequencies green locus				
	1	**3**	**4**	**5**	**6**	**1**	**3**	**4**	**5**	**6**
light allele										
dark allele										
mutant 1										
mutant 2										
mutant 3										

Q: What are the frequencies of each genotype? (Complete Table 2, columns labeled 1 only.)

Table 2. Genotype frequencies for activities 1 and 3.

	Actual frequencies blue locus		**Actual** frequencies green locus	
	1	**3**	**1**	**3**
homozygous light genotype				
heterozygous genotype				
homozygous dark genotype				

Activity 2: Hardy-Weinberg Proportions

Hardy-Weinberg is an example of a null model. A **null model** is a prediction based on randomization that will match the natural world if the null hypothesis is true, if there is not some hypothesized force or process causing a regular pattern in the world. Hardy-Weinberg proportions are the *frequencies of genotypes that we expect* in a population with a given set of allele frequencies. In other words, Hardy-Weinberg genotype frequencies are what we expect if genotypes are assembled by choosing alleles completely at random from the gene pool and placing them in pairs. As it happens Hardy-Weinberg is a null model for evolution. The null hypothesis is that evolution is not taking place in a population. Any of the forces that drive evolution will also drive the actual genotype frequencies in a population away from H-W equilibrium. The mathematical miracle of Hardy-Weinberg is that evolution is a process that takes time, several generations, and may be difficult to observe. Genotype frequencies, however, can be sampled in a single moment in time. Hardy-Weinberg is a way of using genotype frequencies measured at one instant in time to make inferences about evolution, which takes place over many generations. If, at any locus, a population is not in Hardy-Weinberg proportions, this is evidence of evolution.

What genotype frequencies do we expect in a population? In a population with only two kinds of alleles at a locus, the frequency of one allele can be symbolized by $\boldsymbol{p}$, and the frequency of the other can be symbolized by $\boldsymbol{q}$. It is always true that $\boldsymbol{p} + \boldsymbol{q} = 1$. The probability that any individual has one copy of the first allele is $\boldsymbol{p}$. The probability that that individual has two copies of the first allele is $\boldsymbol{p}^2$. This is because the probability that two events occur *simultaneously* is the product of their independent probabilities. By the same logic, the probability that an individual has two copies of the second allele is $\boldsymbol{q}^2$.

What is the probability that an individual has one of each allele? At first you might say $\boldsymbol{pq}$. That is the product of their independent probabilities. But there is another way that an individual can have one of each allele, that is, by $\boldsymbol{qp}$. Thus there are two ways of being heterozygote, $\boldsymbol{pq}$ or $\boldsymbol{qp}$. The probability that two events occur *alternatively* is the sum of their independent probabilities. Thus, the chance that an individual is heterozygous is $\boldsymbol{pq} + \boldsymbol{qp} = \boldsymbol{2pq}$. Thus, we expect the genotype frequencies in a population to be $\boldsymbol{p}^2$, $\boldsymbol{2pq}$, and $\boldsymbol{q}^2$. The sum of these three frequencies equals one because they represent 100% of the population.

Lab group exercise

Work with the students at your lab bench (group of three or four students) to fill in the following table.

Q: What are the Hardy-Weinberg proportions of each genotype? (Complete Table 3.)

Table 3. Predicted genotype frequencies from activity 2.

	Expected frequencies blue locus	**Expected** frequencies green locus
homozygous light genotype		
heterozygous genotype		
homozygous dark genotype		

Compare the expected genotype frequencies with your actual genotype frequencies for the blue locus and for the green locus.
Q: Is the blue locus actually in Hardy-Weinberg proportions?______________________________
Q: Is the green locus actually in Hardy-Weinberg proportions?_____________________________

Class discussion

Q: What does it mean if a locus is in Hardy-Weinberg proportions?_________________________
____
Q: Can one locus be in Hardy-Weinberg proportions, and the other not?______________________
Q: Why or why not?____
____

Activity 3: The Next Generation and Genetic Drift

Before we begin, venture a guess to the following questions:

Q: Do allele frequencies change from one generation to the next?__________________________
Q: Do genotype frequencies change from one generation to the next?________________________

Procedure

Take your fish to one of the spawning grounds, and find someone who is carrying a fish with a different mating type. If you can't find anyone, wait out this turn until the second round. Failure to find a mate is common in many species.

1. Determine the genotype of the first offspring.
- Write down the genotype of your parental fish before you begin (Table 4)
- Take the two alleles from the blue locus of your fish and put each allele in a different one of your fists. The person carrying your fish's mate should blindly select one of your fists. The allele they choose will be the first allele of the first offspring.
- You should do the same with the alleles of your fish's mate to select the second allele of the first offspring.
- You now have two selected alleles that represent the blue locus genotype of your first offspring.
- On your gene exchange worksheet (Table 4) write in the new blue-locus genotype of the first offspring.
- Replace the alleles from your fists into your fish's genotype card to restore it to its original parental state (refer to your written notes if necessary).
- Repeat the same procedure for the green locus alleles.
- Determine the mating type of the first offspring using the same procedure.

2. Determine the genotype of the second offspring. Use the same method as above.
3. Allow your parental fish to die.
- Remove the alleles and mating type labels from the genotype cards.
- Put these alleles and labels into the various containers around the room that hold the extras.

4. Allow your fishes' offspring to be born by filling your empty genotype cards with their new identities.
- Note that your fish card's new genotype may have a different mating type and different alleles than it had in the preceding generation.
- Use the necessary alleles from the allele containers to make the new genotypes.
- Your new fish is now a member of the next generation.

When the whole class has reached the new generation, return to the spawning grounds with your new fish, and find it a new mate. You may not choose a mate carried by someone with whom you have exchanged genes in the previous generation. This will avoid inbreeding. (If you did not avoid this, your fish might mate with a brother or sister. Non-random mating of this sort is an evolutionary process in its own right. We will not consider it in this lab.) Repeat the cycle through the second and third generations.

Class calculation

Q: At the end of three generations, what are the new frequencies of each allele? (Complete Table 1, columns labeled 3 only.)
Q: What are the frequencies of each genotype? (Complete Table 2, columns labeled 3 only.)

Class discussion

Q: Have the allele frequencies changed at the blue locus?________________________________
Q: Have the allele frequencies changed at the green locus?________________________________
Q: Why?____
Q: Have the genotype frequencies changed at the blue locus?______________________________
Q: Have the genotype frequencies changed at the green locus?_____________________________
Q: Is there a difference in how the genotype frequencies have changed at the two loci?_________
Q: Why?____
Q: The number of successful breeding individuals is the **effective population size**. Is the effective population size equal to the total population size?______________________________

Activity 4: Mutation and Selection

Until now, the different alleles have had no distinctive effect on **fitness**. That is to say that they did not differ in their influence on the survival or reproductive success of the fish that carried them. Now, a new allele will arise in your population (by mutation) that enhances fitness. The new allele, "mutant B1," will be introduced at the blue locus. Remember that the blue locus has alleles that influence fin shape. Offspring with the new "mutant B1" allele have a new fin shape that enables them to evade predators more effectively. These offspring will have a much-improved chance of surviving their first few minutes of life compared to new offspring without this allele.

Q: What do you predict will happen to the new mutant B1 allele in the fish population over three generations?____

A new allele will arise at the green locus too, "mutant G1." Recall that the green locus influences mouth size, and this new mutation changes mouth size by making the gape wider. Because the lake is full of nutritious food sources for the fish, this new mutation does not increase fish fitness. The fishes without this mutation can get all the food resources that they need, even though their mouths are smaller. Thus, this mutation is considered **neutral**.

Q: What do you predict will happen to the new mutant G1 allele in the fish population over three generations?____

Procedure

As before, your individuals will undergo three bouts of gene exchange, but the methods are slightly different, so please follow these new instructions.

Take your individual to one of the spawning grounds, and find someone who is carrying an individual with a different mating type. If you can't find anyone, wait out this turn until the second round. During this time, your instructor will introduce one mutation at the blue locus and one mutation at the green locus (one mutation in each of two arbitrarily selected fishes).

- Determine the blue locus genotype of the first offspring.
- Write down the genotype of your parental fish before you begin.
- Put the alleles from the blue locus of your fish in your fists as before, and determine the genotype of the first offspring.
- If your offspring has one of the new mutant alleles, it survives.
- If your offspring lacks one of the new mutant alleles (mutant B1), it may not survive. Flip a coin to determine whether it lives. (heads = lives; tails = dies). This coin flip corresponds to a probability of mortality in non-mutant genotypes that the mutant genotype does not suffer. The non-mutant may, for example, be more susceptible to a predator than the mutant.
- If it dies, return the alleles to your fists, and determine a new genotype of the first offspring. (Repeat the coin flip if it still does not have the mutant B1 allele.)
- If your offspring lives, or if it has one of the mutant alleles, proceed.
- On your gene exchange worksheet, write the new blue locus genotype of the first offspring.
- Replace the alleles from your fists into your fish's genotype card to restore it to its original parental state.
- Determine the green locus genotype of the first offspring.
- Put the alleles from the green locus of your fish in each of your fists, and have the person carrying your fish's mate select one fist. Now you select an allele from the fists of this person.
- No matter what genotype the offspring gets at the green locus, it lives, since the mutant G1 does not alter the fitness of the fish.
- On your gene exchange worksheet, write in the new green locus genotype of the offspring.
- Replace the alleles from your fists into your fish's genotype card to restore it to its original state.
- Determine the mating type of the first offspring as you did in activity 3.
- Determine the genotype and mating type of the second offspring. Use the same method as in 1-3.
- Allow your fish to die. (In this fish population, as before, fish die after they mate.) Follow the instructions in activity 3 for this task.
- Allow your offspring to be born by taking on their new identities. Follow the instructions in activity 3 for this task.

When the whole class has reached the new generation, return to the spawning grounds with your fish, and find it a new mate. Your instructor will not introduce further mutations during this activity. As before, you may not choose a fish carried by someone with whom you have exchanged genes in the most recent generation. Repeat the cycle through the second and third generations.

The difference between this activity and the last is that the number of alleles in the population has probably changed. Though each individual still only carries two alleles at each locus, there may be three different alleles if you examine the whole population at a particular locus. At the blue locus, the new allele B1 improves the probability of reproductive success of your fish (so the old alleles, by comparison, reduce reproductive success.) At the green locus, the new allele G1 has no effect on reproductive success. It is neutral.

Class calculation

Q: What are the new frequencies of each allele? (Complete Table 1, columns labeled 4 only.)

Class discussion

Q: Have the allele frequencies changed at the blue locus?__________________________________
Q: Why?____
____

Q: Have the allele frequencies changed at the green locus?_________________________________
____
Q: Why?____
____
Q: What happened to the new B1 allele?____
Q: What happened to the new G1 allele?____
Q: What does a fitness advantage do for a mutation?____________________________________
____

Part II – using phylogenies to answer evolutionary questions

We're now ready to transition from adaptation and evolution of allele frequencies to speciation. Open the HHMI lizard virtual lab, and carry out the second module, building a phylogeny of the Caribbean anoles.

Open the link Lizard evolution virtual lab (https://media.hhmi.org/biointeractive/vlabs/lizard2/) . If that doesn't work, go first to HHMI lizard evolution launchpad (https://www.biointeractive.org/classroom-resources/lizard-evolution-virtual-lab) .

Then, work in pairs to answer the following worksheet;

Table 4: Gene Exchange Worksheet

Activity____________ Generation______________

Parent 1 genotype: ________________________

Parent 2 genotype: ________________________

		first offspring	second offspring
blue locus:	1st allele		
	2nd allele		
green locus:	1st allele		
	2nd allele		
mating type (circle one):		XX / XY	XX / XY

Activity____________ Generation______________

Parent 1 genotype: ________________________

Parent 2 genotype: ________________________

		first offspring	second offspring
blue locus:	1st allele		
	2nd allele		
green locus:	1st allele		
	2nd allele		
mating type (circle one):		XX / XY	XX / XY

Activity____________ Generation______________

Parent 1 genotype: ________________________

Parent 2 genotype: ________________________

		first offspring	second offspring
blue locus:	1st allele		
	2nd allele		
green locus:	1st allele		
	2nd allele		
mating type (circle one):		XX / XY	XX / XY

Activity____________ Generation______________

Parent 1 genotype: ________________________

Parent 2 genotype: ________________________

		first offspring	second offspring
blue locus:	1st allele		
	2nd allele		
green locus:	1st allele		
	2nd allele		
mating type (circle one):		XX / XY	XX / XY

Activity____________ Generation______________

Parent 1 genotype: ________________________

Parent 2 genotype: ________________________

		first offspring	second offspring
blue locus:	1st allele		
	2nd allele		
green locus:	1st allele		
	2nd allele		
mating type (circle one):		XX / XY	XX / XY

Activity____________ Generation______________

Parent 1 genotype: ________________________

Parent 2 genotype: ________________________

		first offspring	second offspring
blue locus:	1st allele		
	2nd allele		
green locus:	1st allele		
	2nd allele		
mating type (circle one):		XX / XY	XX / XY

Module 2: Phylogeny

1. In module 1, you identified which species of lizards were most similar to one another based on relative limb length and toe pad size. In this module, you determined which lizards are more similar to one another based on what type of information?

2. Are the species of lizard that are more similar to one another according to body type also more closely related based on the results obtained in this module? Explain your answer.

3. The figures below show two phylogenetic trees similar to the one you constructed in the virtual lab but with more lizards. The trees below show the evolutionary relationships among species from four ecomorphs from the four largest Caribbean islands.

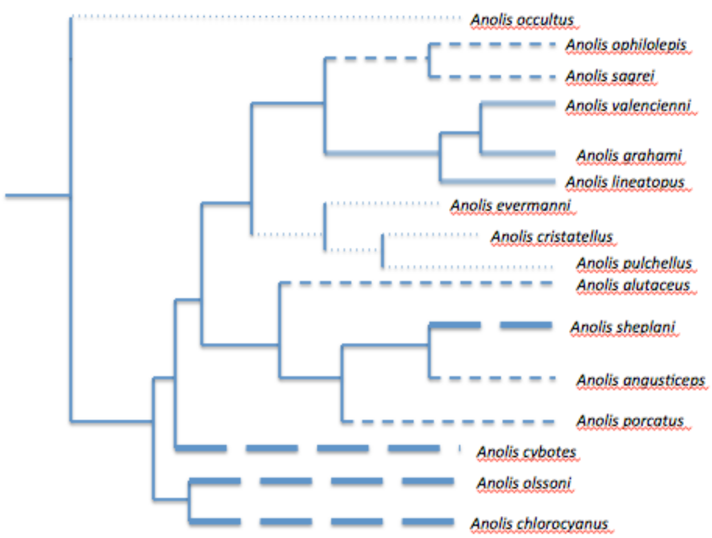


Figure 1. Phylogeny of anole lizards on four of the major Caribbean islands color-coded according to geographical distribution. Light dotted line, Puerto Rico; small dashed line, Cuba; large dashed line, Hispaniola; and solid line, Jamaica.

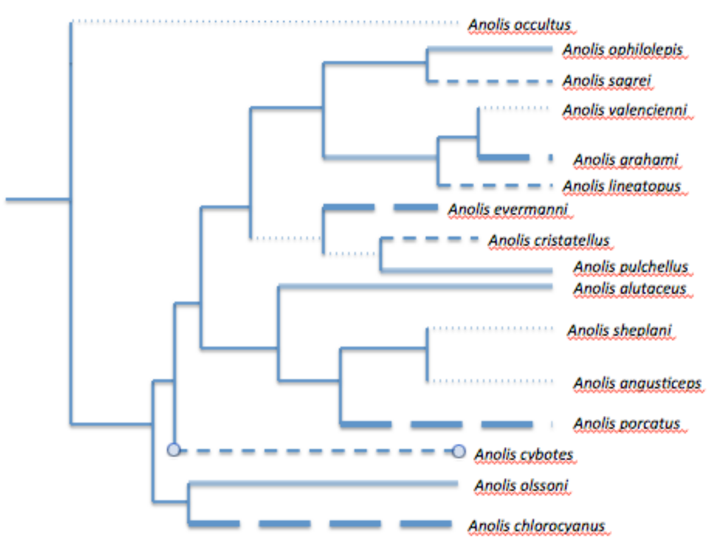


Figure 2. Phylogeny of anole lizards in the four major Caribbean islands colored in according to ecomorph. Light dotted line, twig; small dashed line, trunk-ground; large dashed line, trunk-crown; solid line, grass-bus.

What conclusion can you draw about the evolution of the *Anolis* lizards based on these figures?

4. What is convergent evolution? Use evidence from the trees to explain how the *Anolis* lizards are an example of this concept.

If there is time, we can watch the second part of the video on J Losos work on anole evolution, and complete the following handout

The Origin of Species: Lizards in an Evolutionary Tree

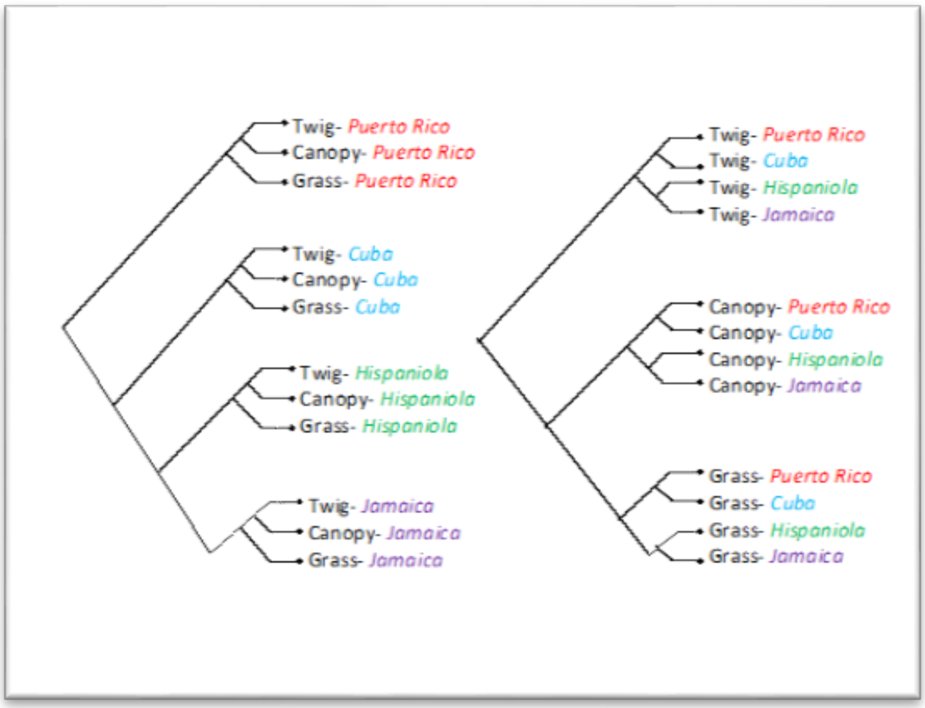
hhmi BioInteractive

Film Guide
Student Handout

NAME_______________________ **DATE**_________________

This handout supplements the short film *The Origin of Species: Lizards in an Evolutionary Tree*.

1. Puerto Rico, Cuba, Jamaica, and Hispaniola have species of anole lizards with distinct body types, including the grass lizards, which have long tails; the canopy lizards, which have large toe pads; and the twig lizards, which have short legs. Anole species with each of these three body types exist on each of the four islands. The phylogenetic trees in the figure below illustrate two hypotheses for how these types of lizards may have evolved.



   a. Select the pair of statements in the table below that accurately describe the phylogenetic trees in the figure above: __________

	Tree on the Left Side of the Figure	**Tree on the Right Side of the Figure**
A	The twig lizard on Puerto Rico evolved first and is the ancestor of all the other lizards.	The twig lizard evolved first on all of the islands, and then the canopy and grass lizards evolved from the twig lizard.
B	Body types evolved repeatedly and independently on each island.	Different body types evolved once, and then populations of individuals with those body types ended up on different islands.
C	Different body types evolved only once, and then populations of individuals with those body types ended up on different islands.	There are two ancestors to all the lizards, the twig lizard and the canopy lizard.
D	Puerto Rico is the origin of all three lizard body types.	Each body type evolved repeatedly and independently on each island.

b. Select which tree in the figure illustrates the most likely hypothesis for how the different species of anole lizards evolved on the Caribbean islands according to the film:
_____ the tree on the left _____ the tree on the right

c. Using evidence presented in the film, explain the reasoning behind your answer in the question above (Part b).

2. Over many generations, natural selection favors those traits that enable populations to live successfully in a particular habitat. A scientist discovered two species of anole lizards that live in different habitats and display the characteristics listed in the table below. (The scientists based these observations on a sample of 20 lizards from each species.)

Observations of Two Species of Anoles		
Species	**A**	**B**
Habitat	**High trunks and branches**	**Lower trunk and ground**
Body length	130-191 mm	55-79 mm
Limb length	Short	Long
Toe-pad size	Large	Intermediate
Color	Green	Brown
Tail length	Long	Long

a. Describe two differences between the two species of anoles.

b. Formulate two hypotheses to explain why each of these differences may have evolved.

c. Describe an experiment that would test one of your hypotheses stated above.