Name ___________________________ Date _______ Instructor ____________________

# ISLAND BIOGEOGRAPHY AND EVOLUTION: SOLVING A PHYLOGENETIC PUZZLE WITH MOLECULAR GENETICS

**PURPOSE: To use evidence from biogeography, geology, morphology, and genetics and biochemistry to analyze the evolution of *Gallotia* lizards of the Canary Islands**

**INTRODUCTION**

Ever since Charles Darwin formulated his hypothesis on how the finches of the Galapagos Islands evolved into 13 species, islands have been a prime target for the study of evolution. By their very nature, islands are isolated and are essentially a living laboratory of evolution. In this investigation, you should be familiar with the terms speciation, geographic isolation, gene flow, gene pool, and reproductive isolation. You will work with real data from real populations of lizards from the Canary Islands. The data will include observations of lizard morphology (body form), geological age estimates of various islands in the Canary Island Archipelago, geographic distances, and genetic distances based on nucleotide base differences in DNA between different populations of lizards.

**Background** —The Canary Islands form an archipelago of seven volcanic islands just west of the African continent (Figure 2). The island chain starts about 85 km (50 miles) west of the continent, following a fault line of the Atlas Mountains in northern Africa. Geologists theorize that a geologic hot spot of upwelling magma has been drifting westward for the past 20 million years, gradually forming the islands as it moves. Thus the most eastern island, Lanzarote, is oldest (about 24 million years old), while the most western island, Hierro, is the youngest (about 0.8 million years old). Volcanic islands are particularly good laboratories for evolutionary science because they can be dated accurately using radioactive isotope decay and because they start out as lifeless masses of rock emerging from the sea.

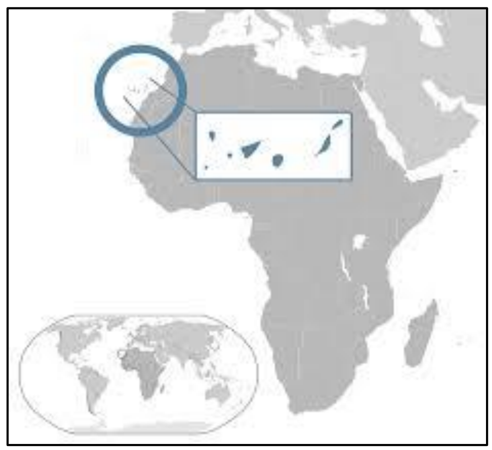

**Figure 1.** The Canary Islands lie 85-400 km off the northwest coast of Africa.

The development of ecosystems on volcanic islands is somewhat unpredictable. It is unclear which plant and animal species will colonize these new environments. Much of this is left to climate, proximity to other land masses, and of course, chance. Figure 2 illustrates three of the many species of lizards living on the Canary Islands. This investigation deals with three species of lizards of the genus *Gallotia*, and within one of these species (*Gallotia galloti)*, four separate island populations. The arrival of the *Gallotia* lizards was probably by rafting, when rafts of natural vegetation carrying clinging animals washed out to sea and then landed on new islands.


**Figure 2.** Three related species of *Gallotia* lizards on the Canary Islands. A) *Gallotia galloti*. Four populations exist on the four islands of Hierro, Palma, Gomera, and Tenerife. B) *Gallotia stehlini*. C) *Gallotia atlantica*.

There are some general principles of island colonization:

1) The closer the island to another land mass, the more likely it will be colonized.
2) The older the island, the more likely it will be colonized.
3) The larger the island, the more species are likely to be established.
4) Geographic isolation reduces gene flow between populations.
5) Over time, colonial populations become genetically divergent from their parent population due to natural selection, mutation, and/or genetic drift.

**Problem** — Evolutionary biologists have been faced with an interesting problem. What is the phylogenetic history of the three species and seven populations of *Gallotia* lizards on the Canary Islands? Does the presence of four morphologically different populations of *G. galloti* on the four westernmost islands imply continuing evolution? In this investigation, you will use data from biogeography, geological history, morphology (body size), and molecular genetics to develop answers to these questions.

# PART I: PHYLOGENY BASED ON GEOGRAPHIC DISTANCE

Using Figure 3, measure the distance in kilometers of each island to the mainland (Africa).

| **Island name** | Lanzarote | Fuerteventura | Gran Canaria | Tenerife | Gomera | Palma | Hierro |
|---|---|---|---|---|---|---|---|
| **Distance to mainland (km)** | | | | | | | |

**Data Table 1.** Distance between individual Canary Islands and the African mainland in km.

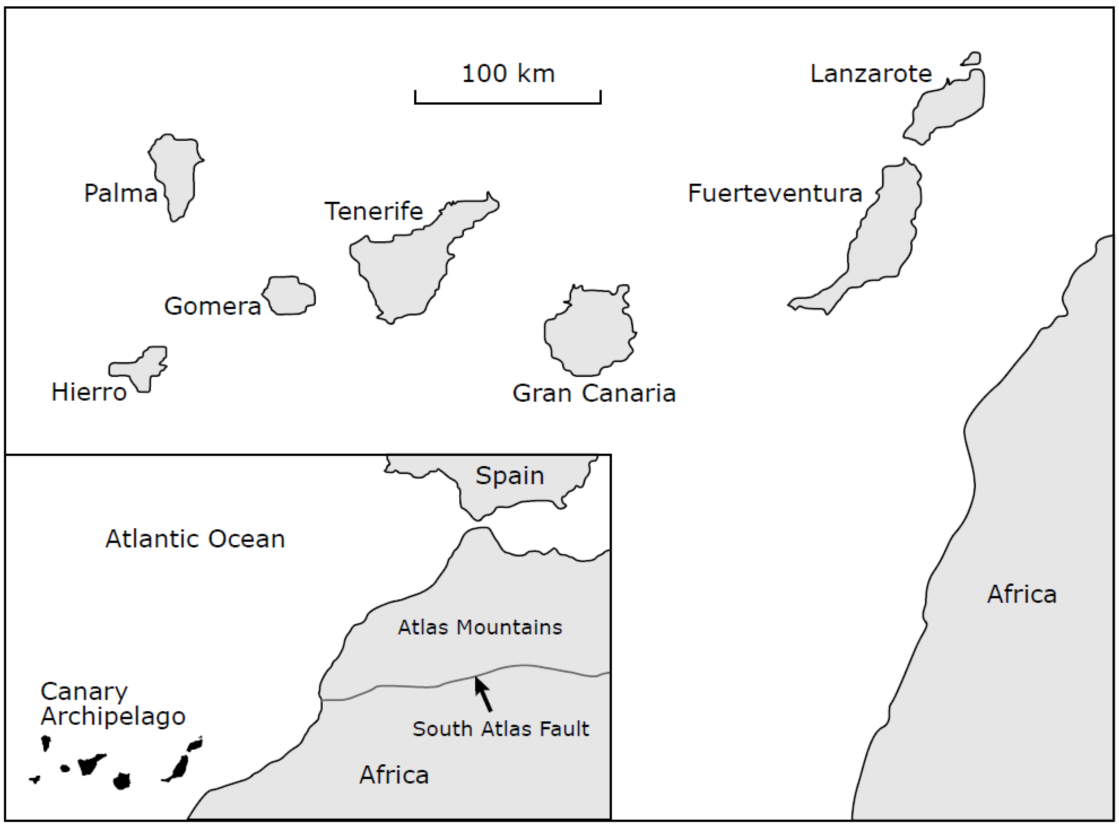


**Figure 3.** Map showing distance from Canary Islands to mainland Africa. Scale shows 100 km.

1) Which island is most likely to have been colonized first and which last? Why do you think so?

2) Using Figure 3 and your geographic reasoning, draw a hypothetical phylogenetic (family) tree of the three species and the three additional populations of *G. galloti*. Your teacher will demonstrate how to draw a phylogenetic tree. Label your end branches with the following population names:

| | | | | | |
|---|---|---|---|---|---|
| *G. atlantica* | *G. galloti* Gomera | *G. galloti* Hierro | *G. galloti* Palma | *G. galloti* Tenerife | *G. stehlini* |

# PART II: PHYLOGENY BASED ON GEOLOGICAL HISTORY

Check your hypothetical phylogenetic tree against the geological data in Table 1. The maximum age of each island was estimated by sampling volcanic rocks found on all islands. The ratio of radioactive potassium to its breakdown product, argon, was used to estimate the age of the rocks.

| **Island name** | Lanzarote and Fuerteventura | Gran Canaria | Tenerife | Gomera | Palma | Hierro |
|---|---|---|---|---|---|---|
| **Maximum age (million years)** | 24.0 | 17.1 | 15.1 | 5.3 | 2.0 | 0.8 |

**Table 1.** Maximum age of the Canary Islands in millions of years. (Anguita et al., 1986)

3) Explain how the data in Table 1 (above) support your phylogeny diagram, or what changes you should make and why.

# PART III: PHYLOGENY BASED ON MORPHOLOGY

The most notable external difference between lizard populations is coloration, which differs significantly between species but is similar within species.

4) Refer back to Figure 1. What differences in coloration do you note between the three species? What similarities do you note?

5) Based on coloration alone, what two species do you hypothesize are most closely related? Why?

Canary Island lizard populations also differ in size. Study the drawings from each lizard population in figure 4A below to compare their relative sizes. (Note: to ensure the observed differences were genetic and not environmental, these individuals were bred in captivity.)

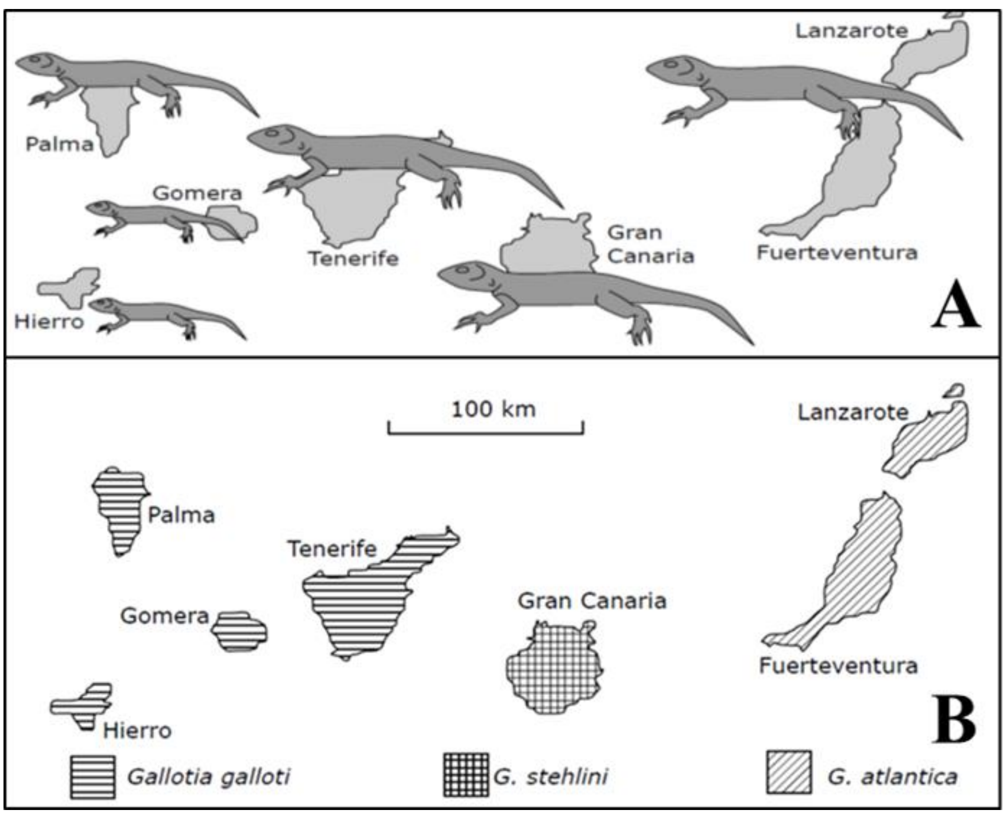


**Figure 4.** A) Relative sizes of typical lizards from each population. B) Species distribution of Canary Island lizards.

6) Compare figures 4A and 4B to analyze size variation between lizard populations. Does the largest variation in size occurs between species or between populations?

7) Based on size, how do you think the four G. galloti populations are related to each other? Why? Draw a phylogenetic tree for these populations.

8) Combine information about coloration and size to draw a complete phylogenetic tree based on morphology. Include all of the following species and subspecies.

| | | | | | |
|---|---|---|---|---|---|
| *G. atlantica* | *G. galloti* Gomera | *G. galloti* Hierro | *G. galloti* Palma | *G. galloti* Tenerife | *G. stehlini* |

9) Compare your two complete phylogeny charts. How are they different?

# PART IV: PHYLOGENY BASED ON MOLECULAR GENETICS

Studies by R.S. Thorpe (1993, 1994) tested various phylogenetic hypotheses by comparing genetic differences among the populations of the *Gallotia* lizards on the Canary Islands. The gene for cytochrome b, which is coded by DNA found in every cell's mitochondria, was used in this study along with DNA from other genes. Cytochrome b is an important substance for cell metabolism and has probably been around since the first prokaryotes. Changes in base sequence (A, T, C, and G) that do not disrupt the gene's function provide us with a kind of evolutionary clock. The rate of mutational changes due to pairing errors is relatively constant. The chances for such mutations are the same for any of these bases. This means that the number of new mutations increases over time. When two populations are isolated and gene flow between them is restricted, the mutational differences accumulate. The longer the isolation the greater the difference.

Thorpe and his colleagues sequenced cytochrome b DNA from all seven populations. Thorpe tested also two populations on Tenerife to see if ecological differences were part of the story. He felt that because Tenerife is moist and lush in the north while arid and barren in the south, populations on that island might have some genetic differences. Also, he wondered if Tenerife was supplying colonizing lizards from two different directions. The results for Thorpe's tests appear on the last two pages of this investigation.

Your task is to count the differences between all pairings of the seven populations and use that data to construct a final phylogenetic tree based on genetic similarities and differences. Low numbers of differences express more genetic similarity and imply more recent common ancestry. Pairs with high numbers are said to have greater genetic distance between them. In other words, large numbers imply they are less genetically alike, have more distant ancestry, and have been separated longer. On a phylogenetic tree, early ancestry is expressed by low branches while more recently evolved are on higher branches. Branches that are far apart imply greater genetic distance.

**Procedure** — There are 21 different pair combinations possible using seven populations. You should work in a team of four. Each person will be responsible for counting all of the base differences for five of the 21 pairs. The first pairing has been counted for you. Record your results in Data Table 2. When all teams are done, the data will be checked for agreement. The easiest way to make accurate counts is to cut the paper into four strips and tape them end to end in the correct order, A to D. You will then compare pairs of strips side by side to count the differences.

| Student 1 = | Student 2 = | Student 3 = | Student 4 = |
|---|---|---|---|
| □ 1/3 | □ 2/3 | □ 3/4 | □ 4/6 |
| □ 1/4 | □ 2/4 | □ 3/5 | □ 4/7 |
| □ 1/5 | □ 2/5 | □ 3/6 | □ 5/6 |
| □ 1/6 | □ 2/6 | □ 3/7 | □ 5/7 |
| □ 1/7 | □ 2/7 | □ 4/5 | □ 6/7 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1** *G. stehlini* | **1** *G. stehlini* | | | | | | |
| **2** *G. atlantica* | 36 | **2** *G. atlantica* | | | | | |
| **3** *G. galloti* Palma | | | **3** *G. galloti* Palma | | | | |
| **4** *G. galloti* N. Tenerife | | | | **4** *G. galloti* N. Tenerife | | | |
| **5** *G. galloti* S. Tenerife | | | | | **5** *G. galloti* S. Tenerife | | |
| **6** *G. galloti* Gomera | | | | | | **6** *G. galloti* Gomera | |
| **7** *G. galloti* Hierro | | | | | | | **7** *G. galloti* Hierro |

**Data Table 2**. Number of DNA base differences in cytochrome b sequences between *Gallotia* populations.

10) In Data Table 2, large numbers imply that pairs of populations are less related. Why?

11) Among the six populations, there are three species. How many base pair differences is the minimum to separate any two species of these lizards? (Remember, don't confuse populations with species.) Give an example to support your answer.

12) Which two populations are most closely related? Justify your answer. Why should you expect these two populations to have the fewest differences?

13) Which population is least related to the rest? Why do you say so?

14) Use the data from Data Table 2 to guide you in redrawing your phylogenetic tree of the *Gallotia* lizards of the Canary Islands using biochemical evidence from cytochrome b sequences. Consider the two populations on Tenerife as a single population so that the phylogenetic tree contains the six populations listed below.

| | | | | | |
|---|---|---|---|---|---|
| *G. atlantica* | *G. galloti* Gomera | *G. galloti* Hierro | *G. galloti* Palma | *G. galloti* Tenerife | *G. stehlini* |

15) Refer to your last phylogeny chart using genetic similarities and differences found in Table 2. Compare it to the phylogeny chart you drew based on the geographic distances and geologic age of the islands. What are the similarities and differences between the two phylogenies?

16) Which species, *G. stehlini* or *G. atlantica*, is the ancestor of the other? Explain your reasoning.

17) Predict what is likely to happen to the four populations of *G. galloti* on the four westernmost islands. Why?