Biodiversity - Phylogeny and Biogeography Laboratory Investigation Lab

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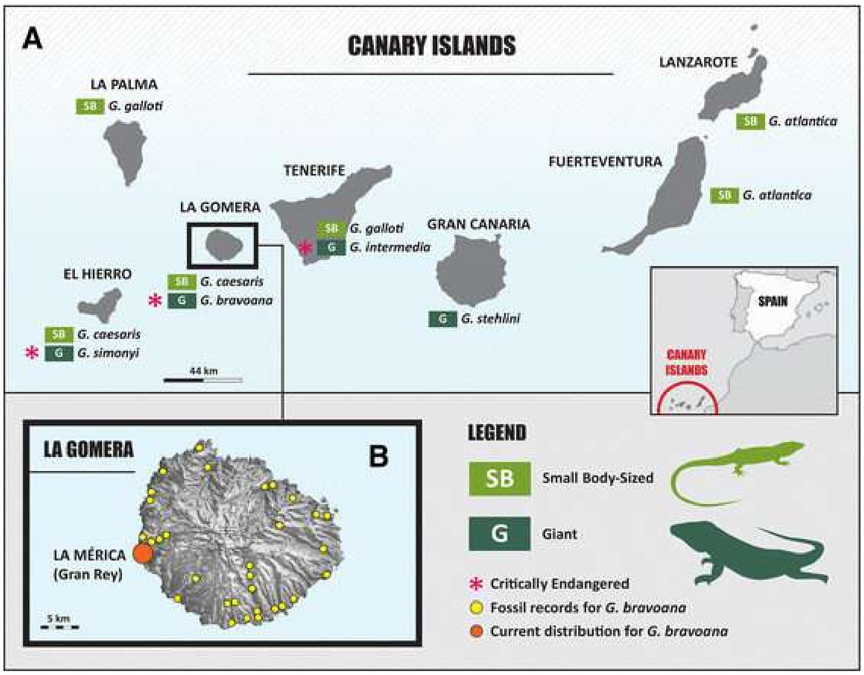
INTRODUCTION

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. 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.

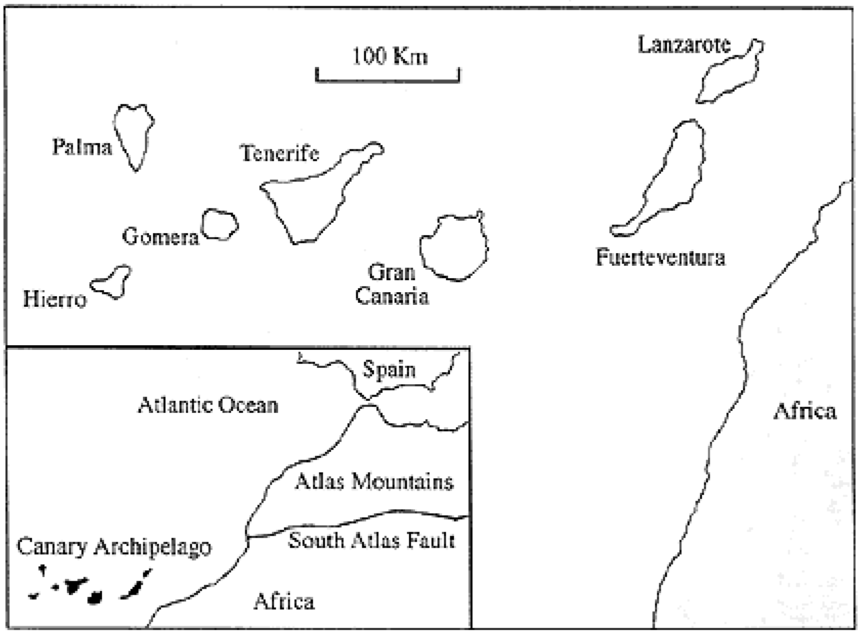
BACKGROUND

Figure 1:
https://live.staticflickr.com/7345/14181783013_4b107e2b32_b.jpg

Figure 1 (left) illustrates one of the many populations of lizards living on the Canary Islands. The Canary Islands form an archipelago of seven volcanic islands just west of the African continent (Map 1). 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, while the smaller 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.



Map 1. The Canary Islands Archipelago. (redrawn from Anguita et al., 1986)



The development of ecosystems on volcanic islands is somewhat unpredictable. However, ecological succession does occur first with pioneer organisms that gradually alter the environment until a stable climax community is established. What is unpredictable is what 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. This investigation deals with three species of lizards of the genus *Gallotia*, and within one of these species, *Gallotia galloti*, and four separate island populations. The arrival of the *Gallotia* lizards was probably by rafting (**See Map 1**). Rafts of natural vegetation are often washed out to sea when high river levels cause river banks to collapse, carrying away both plants and clinging animals. Oceanic currents in this region vary with the seasons. Colonization by airborne organisms, such as insects and birds, usually occurs during storms. In any case, there are some general principles of island colonization:

1) The closer the island to another land mass, the higher the probability of colonization.

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 — Evolution 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 population's of*G. galloti* on the four westernmost islands (**Map 2**) imply continuing evolution? In this investigation, you will use data from geography, geological history, morphology (body size), and molecular genetics to develop answers to these questions.

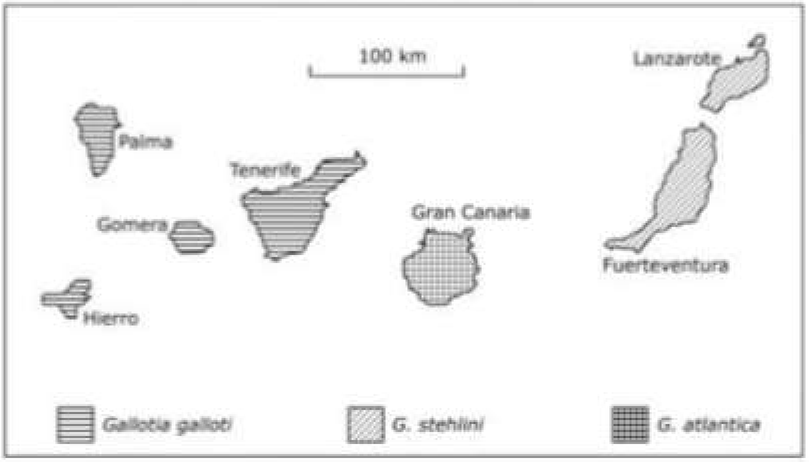
PART I: PHYLOGENY BASED ON GEOGRAPHIC DISTANCE

PROCEDURE

Using **Map 1**, measure the distance in kilometers of each island to the mainland (Africa). List these distances on a separate page. Include the following islands: *Lanzarote, Fuerteventura, Gran Canaria, Tenerife, Gomera, Palma,* and *Hierro*.

1) Which island is most likely to have been colonized first and which last? Tell why you think so.
2) Using **Map 2** and your geographic reasoning, draw on a separate page 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:

atlantica	***stehlini***	***galloti*** **Tenerife**	***galloti*** **Palma**	***galloti*** **Gomera**	***galloti*** **Hierro**



Map 2: Gallotia *atlantica* distribution

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.

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

Lanzarote & Fuerteventura	Gran Canaria	Tenerife	Gomera	Palma	Hierro
24.0	**17.1**	**15.1**	**5.3**	**2.0**	**0.8**

1) Explain how the data in **Table 1** (above) support your phylogeny diagram? Or what changes should you make and why?

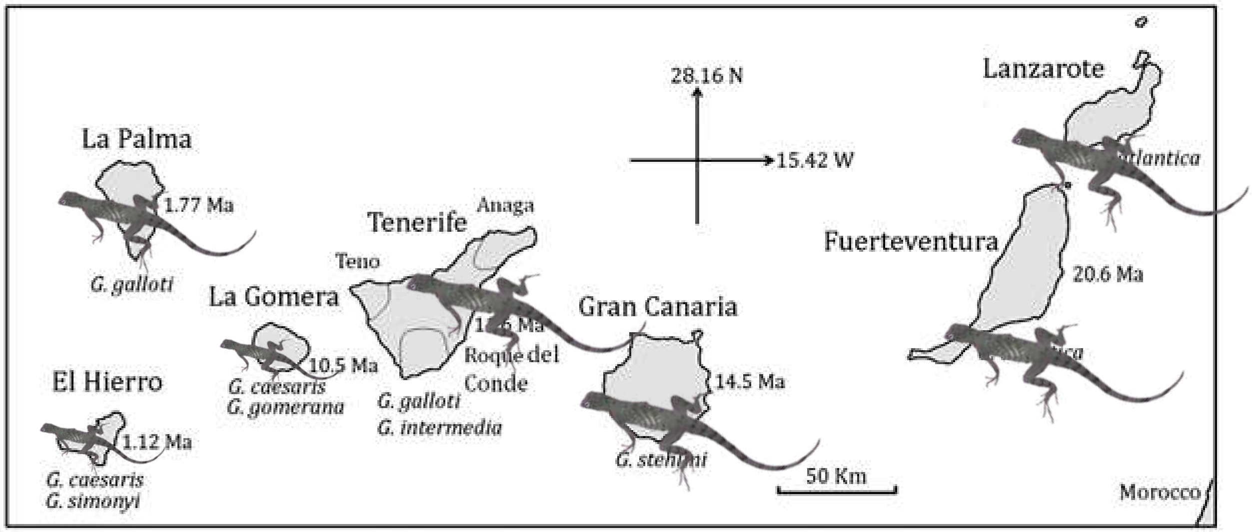
Double Click to Draw

PART III: PHYLOGENY BASED ON MORPHOLOGY

Study the drawings from each lizard population in Figure 2 below. Compare and contrast their body size with the distribution on **Map 2**. To be sure differences were genetic, not environmental, researchers collected individuals from all island populations and bred and raised them in captivity. Their offspring still displayed differences according to their parental characteristics. Draw a new phylogeny chart based on morphological similarities and differences.

Double Click to Draw

2) Compare your two phylogeny charts. Describe how they are different.



PART IV: PHYLOGENY BASED ON MOLECULAR GENETICS

Recent studies by R.S. Thorpe (1993, 1994) have attempted to support 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 its nucleotide 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 more time, the more changes. When two populations are isolated and gene flow between them is restricted, the mutational differences accumulate over time. The longer the isolation the greater the difference.

Thorpe and his colleagues used restriction enzymes to cut the DNA, and gel electrophoresis to separate the fragments. Radioisotope tagging eventually led to the sequencing of the samples of DNA for each of the seven populations. Thorpe tested 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.

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 on **Table 3** for five of the 21 pairs (see chart below). The pairings are listed on **Table 2**. Note that the first pairing has been counted for you. Record your results in **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.

There are 21 possible pairings, each team member selects five pairings other than 1/2.

Student #1	Student #2	Student #3	Student #4
1/3	1/4	1/5	1/6
1/7	2/3	2/4	2/5
2/6	2/7	3/4	3/5
3/6	3/7	4/5	4/6
4/7	5/6	5/7	6/7

	G. stelini	**G. atlantica**	**G. galloti / palma**	**G. galloti / N. Tenerife**	**G. galloti / S. Tenerife**	**G. galloti / Gomera**	**G. galloti / Hierro**
G. stelini							
G. atlantica	**36**						
G. galloti / palma							
G. galloti / N. Tenerife							
G. galloti / S. Tenerife							
G. galloti / Gomera							
G. galloti / Hierro							

INTERPRETATIONS AND CONCLUSIONS

Use the data from **Table 2** to guide you in redrawing your phylogenetic tree of the *Gallotia* lizards of the Canary Islands using both geographic and genetic information. Consider the two populations on Tenerife as a single population so that the phylogenetic tree contains six populations.

Low numbers 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 the higher branches. Branches that are far apart imply greater genetic distance.

1) In **Table 2**, large numbers imply that pairs of populations are less related. Why is this?

2) 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.

3) Which two populations are most closely related? Justify your answer.

4) Why should you expect the populations S. Tenerife (ST) and N. Tenerife (NT) to have fewer differences than other pairings?

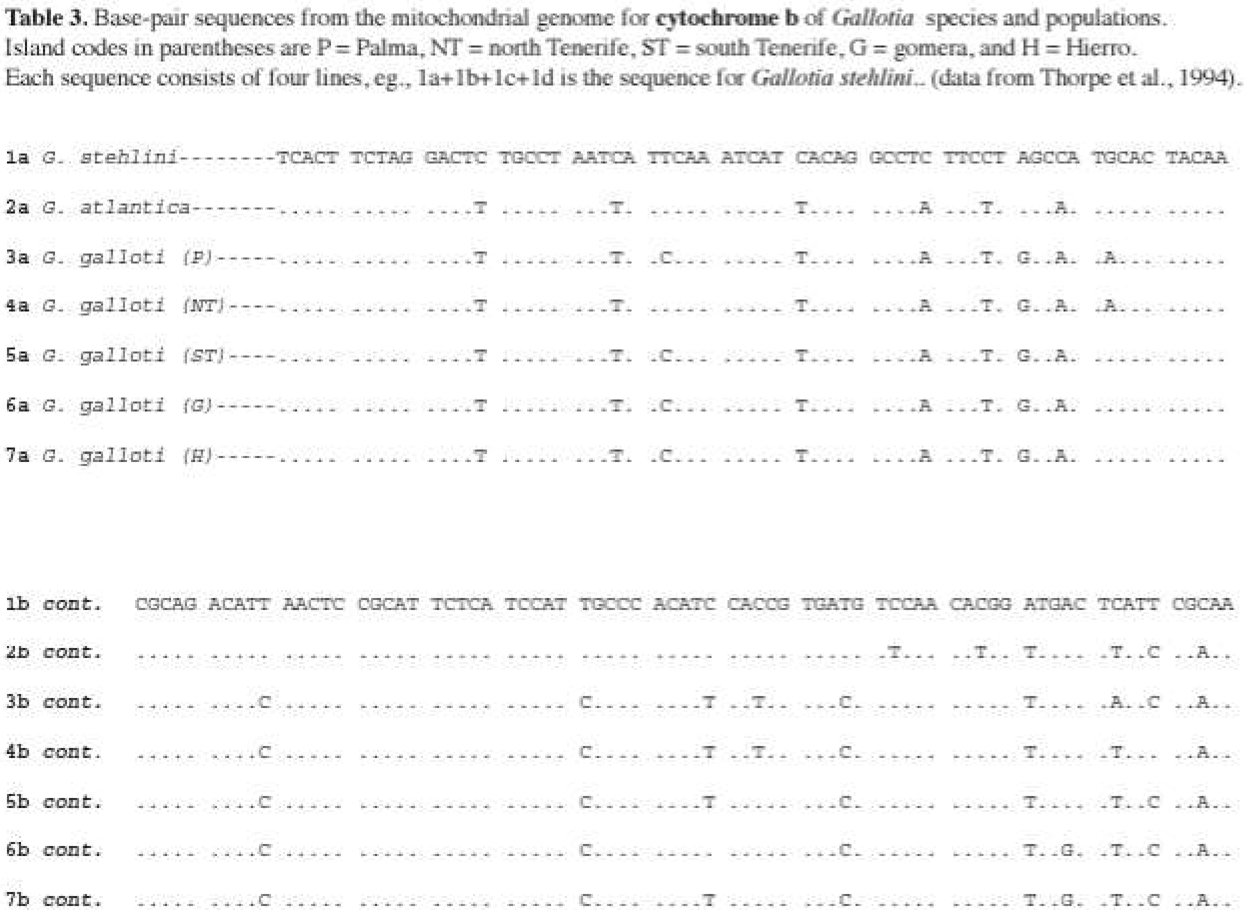
5) Which population is least related to the rest? Why do you say so? Refer to your last phylogeny chart using genetic similarities and differences found in Table 2. Compare it to the phylogeny chart you drew based on the geographic distances and geologic age of the islands.

6) What difference is there between the two phylogenies?

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

8) Predict what is likely to happen to the four populations of *G. galloti* on the four westernmost islands. State what conditions will support this prediction.

Table 3. Base-pair sequences from the mitochondrial genome for cytochrome b of *Gallotia* species and populations. Island codes in parentheses are P = Palma, NT = north Tenerife, ST = south Tenerife, G = gomera, and H = Hierro. Each sequence consists of four lines, e.g., 1a+1b+1c+1d is the sequence for *Gallotia stehlini*. (Data from Thorpe et al., 1994).



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```
1a G. stehlini--------TCACT TCTAG GACTC TGCCT AATCA TTCAA ATCAT CACAG GCCTC TTCCT AGCCA TGCAC TACAA

2a G. atlantica-------..... ..... ....T ..... ...T. ..... ..... T.... ....A ...T. ...A. ..... .....

3a G. galloti (P)-----..... ..... ....T ..... ...T. .C... ..... T.... ....A ...T. G..A. .A... .....

4a G. galloti (NT)----..... ..... ....T ..... ...T. ..... ..... T.... ....A ...T. G..A. .A... .....

5a G. galloti (ST)----..... ..... ....T ..... ...T. .C... ..... T.... ....A ...T. G..A. ..... .....

6a G. galloti (G)-----..... ..... ....T ..... ...T. .C... ..... T.... ....A ...T. G..A. ..... .....

7a G. galloti (H)-----..... ..... ....T ..... ...T. .C... ..... T.... ....A ...T. G..A. ..... .....



1b cont.  CGCAG ACATT AACTC CGCAT TCTCA TCCAT TGCCC ACATC CACCG TGATG TCCAA CACGG ATGAC TCATT CGCAA

2b cont.  ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .T... ..T.. T.... .T..C ..A..

3b cont.  ..... ....C ..... ..... ..... ..... C.... ....T ..T.. ...C. ..... ..... T.... .A..C ..A..

4b cont.  ..... ....C ..... ..... ..... ..... C.... ....T ..T.. ...C. ..... ..... T.... .T... ..A..

5b cont.  ..... ....C ..... ..... ..... ..... C.... ....T ..... ...C. ..... ..... T.... .T..C ..A..

6b cont.  ..... ....C ..... ..... ..... ..... C.... ..... ..... ...C. ..... ..... T..G. .T..C ..A..

7b cont.  ..... ....C ..... ..... ..... ..... C.... ....T ..... ...C. ..... ..... T..G. .T..C ..A..
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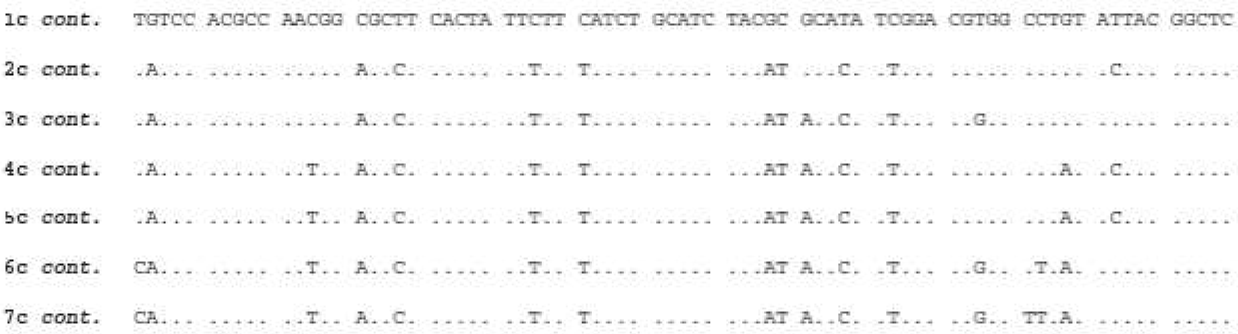

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1c cont.   TGTCC ACGCC AACGG CGCTT CACTA TTCTT CATCT GCATC TACGC GCATA TCGGA CGTGG CCTGT ATTAC GGCTC
2c cont.   .A... ..... ..... A..C. ..... ..T.. T.... ..... ...AT ...C. .T... ..... ..... .C... .....
3c cont.   .A... ..... ..... A..C. ..... ..T.. T.... ..... ...AT A..C. .T... ..G.. ..... ..... .....
4c cont.   .A... ..... ..T.. A..C. ..... ..T.. T.... ..... ...AT A..C. .T... ..... ...A. .C... .....
5c cont.   .A... ..... ..T.. A..C. ..... ..T.. T.... ..... ...AT A..C. .T... ..... ...A. .C... .....
6c cont.   CA... ..... ..T.. A..C. ..... ..T.. T.... ..... ...AT A..C. .T... ..G.. .T.A. ..... .....
7c cont.   CA... ..... ..T.. A..C. ..... ..T.. T.... ..... ...AT A..C. .T... ..G.. TT.A. ..... .....
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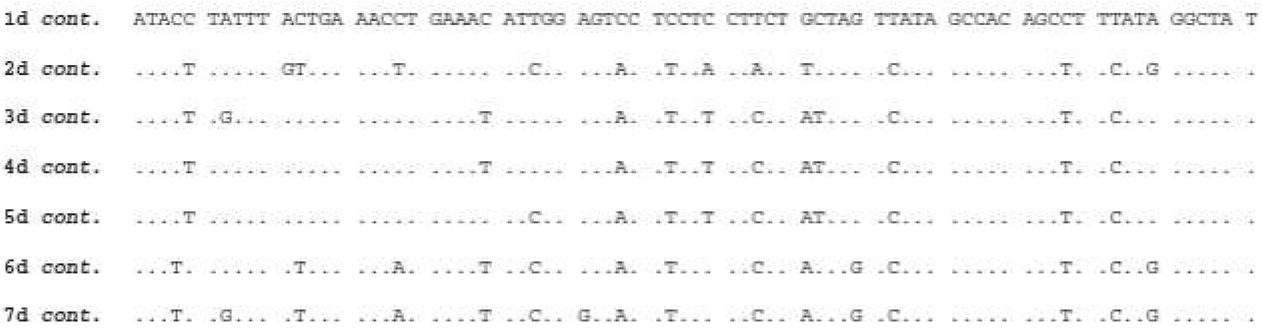

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1d cont.   ATACC TATTT ACTGA AACCT GAAAC ATTGG AGTCC TCCTC CTTCT GCTAG TTATA GCCAC AGCCT TTATA GGCTA T
2d cont.   ....T ..... GT... ...T. ..... ..C.. ...A. .T..A ..A.. T.... .C... ..... ...T. .C..G ..... .
3d cont.   ....T .G... ..... ..... ....T ..... ...A. .T..T ..C.. AT... .C... ..... ...T. .C... ..... .
4d cont.   ....T ..... ..... ..... ....T ..... ...A. .T..T ..C.. AT... .C... ..... ...T. .C... ..... .
5d cont.   ....T ..... ..... ..... ..... ..C.. ...A. .T..T ..C.. AT... .C... ..... ...T. .C... ..... .
6d cont.   ...T. ..... .T... ...A. ....T ..C.. ...A. .T... ..C.. A...G .C... ..... ...T. .C..G ..... .
7d cont.   ...T. .G... .T... ...A. ....T ..C.. G..A. .T... ..C.. A...G .C... ..... ...T. .C..G ..... .
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PART V - Biogeography of the Penelope (Poison Dart Frog)

The poison dart frog (family Dendrobatidae) is a group of brightly colored frogs native to Central and South America. They are renowned for their toxic skin secretions, which are used by indigenous peoples to coat the tips of blowdarts for hunting.

There are over 300 species of poison dart frogs, which are divided into three subfamilies: Dendrobatinae, Colostethinae, and Aromobatinae. They exhibit a wide range of colors, from vibrant reds and yellows to more muted greens and browns.

Image	Common name	Binomial name and authority[7]	Distribution
	Green and black poison dart frog	*Dendrobates auratus* (Girard, 1855)	southeastern Nicaragua on the Atlantic slope and southeastern Costa Rica on the Pacific coast through Panama to northwestern Colombia (Chocó Department)
	Yellow-banded poison dart frog	*Dendrobates leucomelas* Steindachner, 1864	Guyana, Brazil, Venezuela and the extreme easternmost part of Colombia
	Rockstone poison dart frog	*Dendrobates nubeculosus* Jungfer and Böhme, 2004	Guyana
	Dyeing poison dart frog	*Dendrobates tinctorius* (Cuvier, 1797)	Guiana Shield and Venezuela, including parts of Guyana, Suriname, Brazil, and nearly all of French Guiana.
	Yellow-striped poison dart frog	*Dendrobates truncatus* (Cope, 1861)	Colombia

The biogeographic distribution of poison dart frogs is primarily centered on Central and South America, with the highest diversity occurring in the Amazon basin. They can also be found in parts of the Caribbean, including Cuba and Hispaniola. Some species have even been introduced to Hawaii and Guam.

The evolutionary history of poison dart frogs is thought to have begun in South America, around 47 million years ago. The ancestral dendrobatid was likely a small, brown, arboreal frog that fed on insects. Over time, this group diversified into the colorful, toxic species we see today.

One of the most remarkable features of poison dart frogs is their ability to sequester toxic alkaloids from their diet, which they use for defense against predators. Different species of poison dart frog have evolved to produce different types and quantities of alkaloids, which may be used for a variety of purposes, including as a deterrent against predators or to attract mates.

Distribution of Poison Dart Frogs

Analysis:

1. How has divergent evolution contributed to the remarkable diversity of poison dart frogs across their range, and what are some examples of divergent traits among different species?

2. How have historical biogeographic events, such as the formation of the Andes and the separation of Central and South America, influenced the geographic distribution and genetic diversity of poison dart frogs?

3. How have different lineages of poison dart frogs evolved unique adaptations to their specific habitats, such as arboreal versus terrestrial lifestyles or different types of vegetation?

4. What evidence supports the hypothesis that the ancestral dendrobatid frog was a small, brown, arboreal species, and how have modern poison dart frogs diverged from this ancestral form?

5. How do the different types and quantities of alkaloids produced by poison dart frogs reflect their evolutionary history and biogeographic distribution, and what ecological and evolutionary factors have shaped the evolution of these toxic compounds?