

Name: \_\_\_\_\_ Date: \_\_\_\_\_

\*\*\* FINAL ASSESSMENT \*\*\*

**Lab #31: Investigating Relationships Among Species: Simulating The Search for A Drug**

**Pre-Lab Activities**

1. Survey the headings and subheadings to familiarize your self with this lab.
2. Read the **Introduction** completing the given tasks along the way. Now go back and re-read it, this time highlighting main ideas.
3. **Interactive Lecture:** What is phylogeny?
4. Read the **Materials and Methods**, completing the given tasks along the way. Now go back and re-read it, this time highlighting main ideas.
5. Read through the **Procedure** and circle any new or unfamiliar materials.
6. Set up your lab notebook for Lab #31, being sure to include a **PURPOSE** for this lab. Be sure to transfer any important safety information and/or safety symbols into the "Safety Precautions" section.
7. Differentiate between **Procedure** tests study the plants on the molecular level, and which study the gross morphology of the plants. Note which is which below:

	Molecular Level	Gross Morphology Level
Procedure Test (i.e. Structural Characteristics of Plants)		

8. Read through the **Procedure** for the first three tests, Steps 2-10. In the following table, write the objective of each of the tests.

Test	Objective
1. Structural Characteristics of Plants	
2. Structural Characteristics of Seeds	
3. Microscopic Internal Structure of Stems	

9. Review the **Procedure** for the remaining four tests, Steps 14-49. For each test, write its objective, using the information in the introduction to make it specific to the overall purpose of the lab.

Test	Objective
<i>Paper Chromatography to Separate Plant Pigments</i>	
<i>Indicator Tests for Enzyme M</i>	
<i>Simulated Gel Electrophoresis</i>	
<i>Translating the DNA To Make A Protein</i>	

10. Reread the entire lab sheet. As you read, circle concepts and/or activities that relate directly to Lab Rubric Items. This will help you “Achieve” or “Exceed Standard”.

11. As a pair, construct data tables for the data you will need to gather for this lab. **BOTH PARTNERS SHOULD PERFORM ALL TESTS TOGETHER.**

## Introduction

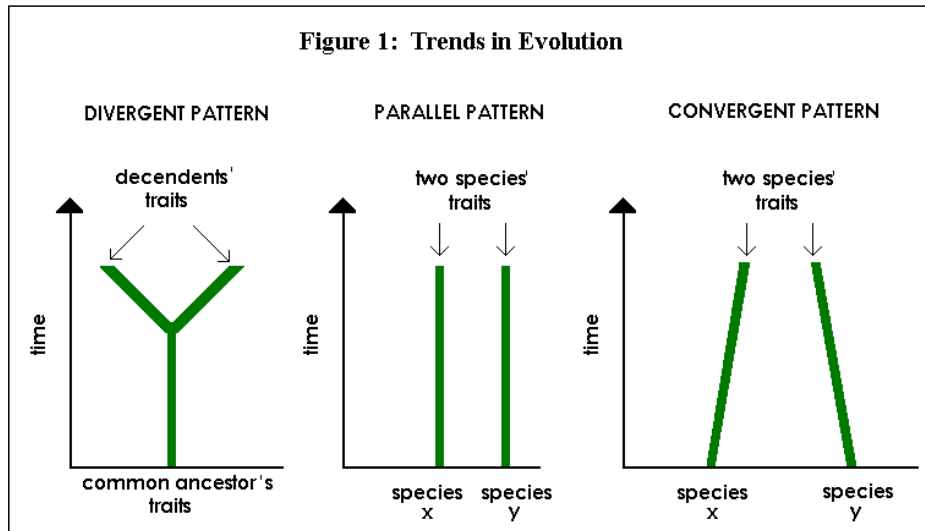
### *Taxonomy*

Humans have been naming and describing organisms since the beginnings of language. The Greek philosopher Aristotle (384-333 B.C.) was grouping plants and animals based on observable similarities over 2,000 years ago. Later, Greeks and Romans created sub-classifications of plants and animals, into groups such as oaks, horses or dogs. This unit of classification became known as a *genus*, the Latin word for group. For example, all cats belong to the genus *Felis*. Hence the field of *taxonomy*, the grouping and naming of living things, was born. Further levels of classification were created by the addition of descriptive terms to the genus, thus forming polynomials. This became quite unwieldy, though. For example, the honeybee had a 12-part name.

In the 1750's a Swedish biologist named Carl Linnaeus (1707-1778) published several books that utilized the polynomial system. As a sort of shorthand, Linnaeus also included a two-word Latin name for each species; for the Florida panther this is *Felis concolor*. Linnaeus' system became known as *binomial nomenclature* and is the system that is currently used in by most taxonomists.

Traditionally, an organism's structure was the basis for taxonomic organization. We classified organisms according to the observable degrees of difference between them. For example, tigers look more like dogs than they do snakes, therefore they must be more closely related to dogs. However, making connections only on the basis of gross morphology can be misleading. Bats and butterflies both have wings, but closer study of the wings' structure reveals that they are entirely different. Bats have an endoskeleton supporting their wings whereas butterflies do not. In this case, we are observing *analogous structures*, or structures that result from *convergent evolution* (See **Figure 1**). These wings are analogous structures, because though they evolved to perform the same function, flight, the structures themselves are very different. Convergent evolution means that two species

evolved similar traits (wings) in response to similar selective pressures (the advantage of flight). However, it does not mean that these organisms are closely related from a genetic standpoint. Think of (or research) another example of analogous structures, considering how the given environment would have selected for those structures. Write the example below.

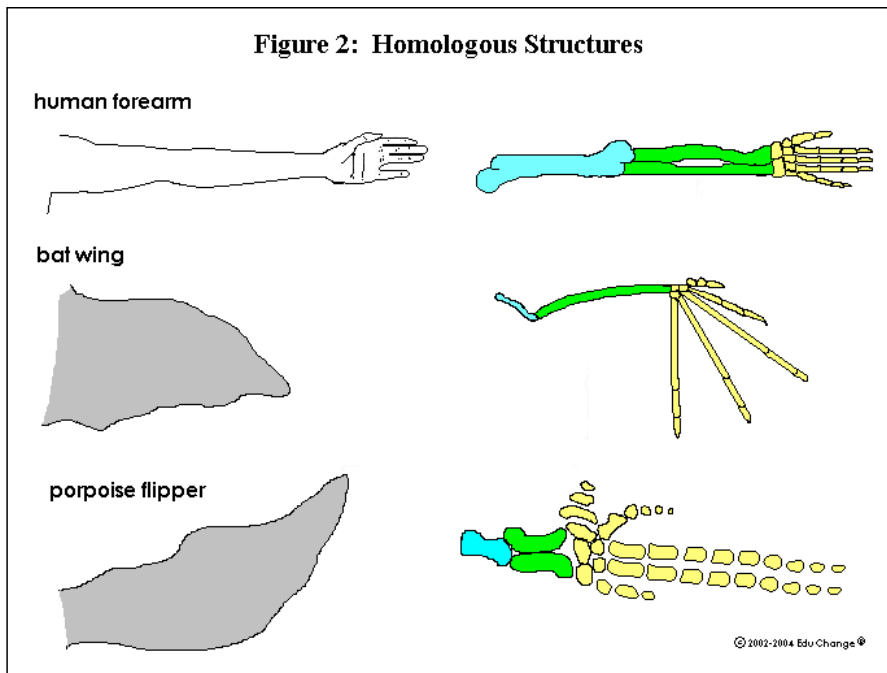


From the occurrence of analogous structures, it can be inferred that the classification of organisms based solely on morphology may not accurately reflect evolutionary relationships. If we consider bat wings and human arms, at first glance the wing and the arm appear to be structured very differently to suit the differing needs of the organism. However, closer inspection indicates that the internal skeleton of these two structures is very similar (See **Figure 2**). This similarity in internal structure could be attributed to a common ancestor who had an original structure that was then modified differently in response to different selective pressures along divergent lines of descendents. Anatomical structures of similar organization and developmental patterns are known as *homologous structures*. Often they are the result of *divergent evolution* (See **Figure 1**). To *diverge* means to separate. Think of Robert Frost's 1916 poem *The Road Not Taken*:

*Two roads diverged in a yellow wood  
And sorry I could not travel both...*

Divergent evolution relates to organisms that shared a common ancestor but who, through exposure to different environments or selecting agents, have become different over time. Bats and humans likely evolved from a common ancestor, but due to temporal and environmental conditions, they differentiated. How is divergent evolution like cell differentiation? Explain in the space provided.

**Figure 2: Homologous Structures**

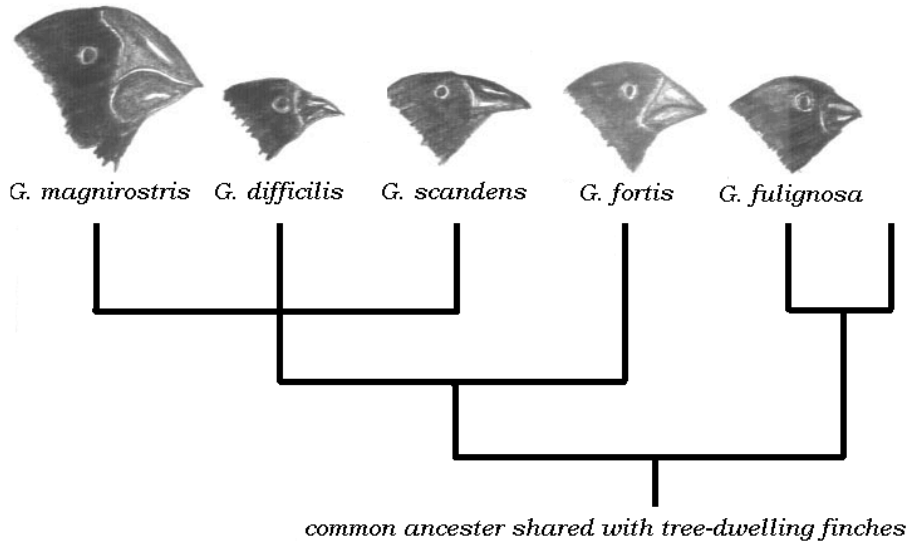


*Phylogeny*

Taxonomists must be very cognizant of homologous and analogous traits when classifying organisms and establishing an organism's *phylogeny* (evolutionary history).. How do taxonomists establish an organism's phylogeny? Traditionally, they have relied on overall similarities between the anatomical and physiological characteristics of different organisms. This is partially how the "Tree of Life" was constructed. This representation had all organisms starting from a common ancestor and then branches based on observable differences formed. **Figure 3** illustrates proposed relationships among Darwin's ground-dwelling Galapagos finches, the *Geospiza* group.

Darwin's finches were originally based upon their beak shape. However, the approach of relying on gross morphology, or structures able to be viewed by the naked eye even with the help of a microscope was limiting when it came down to establishing relationships between species. It is easy to see the difference between dogs and cats, but within the genus *Felis* it is more difficult to establish the phylogenic trees. For example, the beak shapes of the *G. magnirostris* and *G. fuliginosa* in Figure 3 are more similar to each other, though they are not as closely related to each other as the *G. magnirostris* and the *G. difficilis* are. This is where the advances in DNA science have been immensely helpful. Studies of the molecular differences between organisms have served to truly establish the amount of "relatedness" between species or even within a species and have therefore changed how taxonomists construct the phylogeny of an organism. Comment on why this might be true, using your work from Lab #30 to assist you.

**Figure 3: Phylogenetic Tree for Darwin's Ground-Dwelling Finches**



This phylogenetic tree is based upon the paper, Sato et al (1999). Phylogeny of Darwin's finches as revealed by mtDNA sequences, *Evolution*: vol. 6(9).  
The artwork is original.

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*Phylogeny and Drug Development: Finding a New Source of Curof*

Understanding the relationships between plants is becoming more and more important to industries such as pharmaceuticals. Many compounds in nature contain compounds that can slow the progression of or even cure diseases. Meanwhile, there is a growing concern that humans are too quickly depleting the planet's biodiversity and by doing so, eliminating potential cures for diseases such as cancer. The film *The Medicine Man* focuses on this idea as researchers race against road construction to find a compound. Why might a loss of biodiversity be problematic for drug researchers? Record your response in the space provided.

However, if a potential drug is found, researchers are often confronted with other issues: availability, the source's growth rate and growing conditions, to name a few. If these issues prove insurmountable then it may not be practical to manufacture the drug even though its therapeutic potential is real. This obstacle has not prevented scientists from pursuing these therapies; instead they seek to isolate usable compounds in plants that are closely related to the source plants but are more available or are easier to grow and maintain. The logic is that relatives might contain similar compounds and be easier to use commercially.

In our simulation, one such plant is *Botana curus*, which produces Curol, a compound used for treating certain kinds of cancer. Curol cannot be synthesized in the laboratory; it can only be extracted from *Botana curus*. However, *Botana curus* grows very slowly and is on the endangered species list, so the ability to provide Curol in large quantities is limited. How might its rate of growth contribute to its status as an endangered species? How might its status as an endangered species impact its use for drug production? Record both answers in the space provided:

Researchers believe that species more closely related to *Botana curus* are more likely to produce the important substance Curol. Why is this a reasonable assumption? Record your response in the space provided.

Researchers think that three similar plant species (X, Y, and Z) might be related to *Botana curus*. All three of these species exhibit a more rapid rate of growth than *Botana curus* and are more plentiful as well, and therefore not on the endangered species list. What types of observations do you think researchers relied on to inform their initial perceptions on relatedness? Record your thoughts in the space below.

In Lab #31 we are going to use both morphological and molecular data to compare these three plant species to *Botana curus*. Upon completion of the study, we will need to construct a phylogenetic tree for these four organisms based on all available data. Then we will state which of the plant species is most closely related to *Botana curus* and is therefore most likely to contain a Curol-like compound. We will recommend this species for further scientific study.

## Materials and Methods

In Lab #31 we will be relying on a variety of techniques that we have gained familiarity with through the first thirty lab experiences in *Investigations in Biology and Chemistry*. To conduct our initial hypothesis-forming tests we will rely on visible and microscopic structure to inform our ideas. Once we have established what we believe to be the closest relative of *Botanus curol*, we will use additional tests to confirm or refute our hypothesis. These additional tests include:

- paper chromatography (Lab #20)
- enzyme test (Lab #11)
- gel electrophoresis (Lab #30)
- and protein analysis (Modeling DNA Replication to Understand Mutation Types).

None of these techniques are new to us, nor are we making any major changes to the normal procedures one follows for these techniques. To better prepare yourself, take out the labs mentioned above and review the **Materials and Methods** and **Procedure** sections of these labs.

## Materials

4 plant samples  
4 seed samples  
Slides of stems of each plant sample  
Light Microscope  
Chromatography paper  
4 capillary tubes  
1 100 mL beaker  
Pencil  
Ruler  
1 spot plate  
Dropper bottles containing each extracts of each of the four plants  
Indicator powder  
Scoopula or small spatula  
Nuclei containing DNA for each of the four plants  
4 pairs of scissors

## Procedure

### *Structural Characteristics of Plants*

1. Record the names of the four organisms
2. Study structures of plants
3. Record similarities and differences

### *Structural Characteristics of Seeds*

4. Record the names of the four organisms
5. Study structures of seeds
6. Record similarities and differences

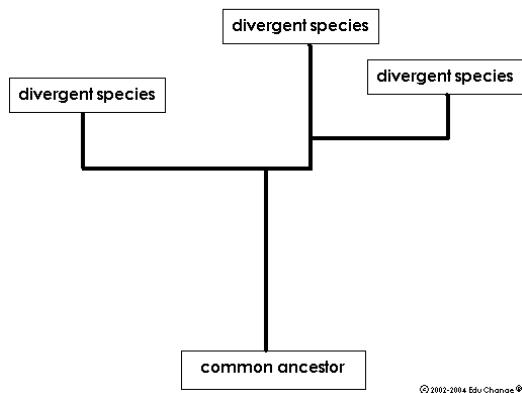
### *Microscopic Structures of Stem*

7. Record the names of the four plants
8. Use the lowest magnification of examine slides of the cross section of the stems of the four organisms
9. Record similarities and differences

### Construction of Hypothesis

10. As a pair review your data from the **Procedure** Steps 2-10.
11. Construct a hypothesis based on your data that indicates which species—X, Y or Z—you believe to be most closely related to *Botanus curos*.
12. Record the hypothesis in your lab notebook in the section titled “Hypothesis”.
13. Create an initial phylogenetic tree for these four plants based on data from first three tests. Do this by starting with a common ancestor as your tree trunk. As you extend your tree trunk, create a branch as an organism appears to differentiate from the others (See Fig. 4)

**Figure 4: Model Phylogenetic Tree**



### Paper Chromatography

14. Record the names of the four plants
15. You must wear safety goggles for this test
16. Draw a pencil line 2 cm from the bottom of the chromatography paper and place four dots along the line.
17. Use the pencil to label the top of the chromatography paper with four species you will be testing.
18. Use a clean capillary tube to transfer samples of each of the extract to the chromatography paper. Be sure to allow spots to dry between applications.
19. Add enough water to cover the bottom of the beaker about 1 cm deep.
20. Fold the chromatography paper in the middle and place upright in the beaker.
21. Remove the chromatogram from the solvent before the solvent front reaches the top of the paper.
22. Once the chromatogram is done, record your observations of the colors and relative amounts of pigments for each species.
23. Record similarities and differences between species.
24. Clean up and return materials

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### | *Indicator Test for Enzyme M*

25. You must wear goggles for the next few steps
26. Obtain spot-plate, 4 plant extracts, indicator powder w/scoop and eyedropper.
27. We are looking for the presence of Enzyme M. If Enzyme M is present, the indicator powder will react with it, producing a gas.
28. Place a small amount of indicator powder in four wells
29. Add a few drops of the first plant extract to the first well.
30. Record your observations
31. Repeat with remaining plant extracts.
32. Clean up and return materials

### *Simulation of Gel Electrophoresis*

33. Obtain materials—plastic bags containing “DNA molecules”; 2 pairs of scissors
34. The strips of paper represent DNA strands found in the nuclei of the four plants. Take a moment and record the names of the four plants, and which color represents which plant.
35. In today’s simulation you will be using Restriction Enzyme T which has the recognition sequence of CCGG. It cuts evenly between the C and the G.
36. Starting at the left end of the sequence, act as RE T to scan and then cut the DNA strand in the appropriate location.
37. Model gel electrophoresis by “Loading” the cut DNA into the “wells” of a gel. The gel is a piece of paper.
38. Remember the smaller fragments will move the farthest.
39. Partners should now act as the electrical current and help the DNA fragments migrate.
40. Upon completion of the “Run” tape down the fragments and record the banding pattern.

### *Protein Synthesis*

41. Obtain the sequence of DNA for each of the four plants
42. Transcribe and translate your DNA sequence into the primary structure of the expressed protein.
43. Record the sequence of amino acids for each plant.

### | *Data Sharing*

44. Share data for the four tests. Each person should record all the data in their notebook.
45. Determine if data supports your initial hypothesis and phylogenetic tree. See **Figure 4** for a model. If not, modify your phylogenetic tree based on molecular data and revise your recommendations to the researchers.

### **Post-Lab Activities.**

1. Construct a final copy of your phylogenetic tree on poster paper. Hang up and complete a Gallery Walk of all group phylogenetic trees. How did your diagram compare to other groups?
2. Explain why all four plant species had some of the same morphological characteristics.
3. Did the addition of molecular data change your initial hypothesis tree? Why or why not?
4. How might the increased accuracy and accessibility of molecular data change traditional phylogenies and therefore taxonomic classifications of organisms?