MORPHOLOGICAL AND MOLECULAR ANALYSIS OF PLANT PHYLOGENY AND DIVERSITY

LEARNING OBJECTIVES

Upon completing this exercise, students will be able to:

- Apply knowledge of plant anatomy and morphology to the study of plant diversity.
- Name the major taxonomic groupings of plants and describe their features.
- Describe major innovations and patterns of trait evolution in plants.
- Understand phylogenetic relationships among different plant taxa.
- Identify important characters, assign character states, and code characters for phylogenetic analysis.
- Construct, compare, and interpret phylogenetic trees using morphological, anatomical, and molecular data.
- Have a deeper connection to the natural world around them, and the habitat attributes offered by local parks and ecosystems.
- Apply knowledge of stele and vascular bundle anatomy to evolution and phylogenetic inquiry of plants.
- Use sectioning and staining skills to obtain stelar anatomy data.
- Obtain practice using anatomical terms to describe and analyze plant vascular traits.

Develop a consensus phylogeny based on different data.

SUMMARY

Evolutionary biologists use morphological, anatomical, ecological, genetic, and other forms of data in phylogenetic analyses to test hypotheses that investigate different biological questions. Because each type of data has its own, assumptions, strengths, and limitations, it is important to recognize these features of the data and take them into consideration when interpreting results.

This laboratory module explores plant diversity and evolution through phylogenetic analyses using 1) plant morphological and anatomical data, 2) microscopic stelar architecture and 3) molecular data. The combination of analyses using different forms of data will promote deeper understanding of important evolutionary innovations, patterns of diversity, and major trends in plant evolution. In this exercise, you will collect morphological and anatomical data to construct an initial phylogeny of the study organisms that describes broad patterns of trait evolution among taxa. Next, you will compare your data with researched molecular phylogenies using all three genomes (nuclear, chloroplast, and mitochondrial) for a more fine-grained analysis of relationships among taxa. You will then use the data from the different data sets to develop a consensus phylogeny to describe relationships among taxa and patterns of

trait evolution. Comparisons of phylogenies also provides an opportunity for critical analysis of phylogenetic data interpretation

PROCEDURE

1. Prior to lab, review the characteristics of different plant groups (bryophytes, seedless vascular plants, gymnosperms, and angiosperms) described in your lab manual and textbook.
2. Research habitat requirements of these groups and study local maps to find an ecotype that may house the plant group you have been assigned.
3. Explore the local ecotype you researched, and collect samples of this type of plant. If you find more than one type (such as a fern and a horsetail and/or "ground pine") collect some of each. Take only parts of plants, and never take the entire plant if it is the only member of its species in the vicinity. Make sure that you collect part of the rhizome of ferns and lycopodium. Store your samples in a glass of water or a moist ziploc bag with a damp paper towel wrapped around the cut part.
4. When you return to the lab, prepare slides of roots, rhizomes or stems, and leaves at your station. Stain with Toluidine blue. Maintain some intact parts of your plants for morphological analysis.
5. Your professor may supplement your station with prepared slides and preserved specimens.
6. Working in small groups, rotate among the stations on the different tables and observe the fresh material, living plants, preserved specimens, and microscope slides. Using the list of characters and character states provided in Table 1, identify the characters and score the character states for what best represents the specimens at each station.
7. Make note of the traits that are 1) similar among the plant specimens at the different stations and 2) different among the plant specimens at the different stations.
8. Record the character states in Table 2. Make note of some of the genera and species names on the specimens at the different stations.
9. After recording the character states for the different stations, make a prediction about what the phylogentic relationships will be among the taxa at the different stations. Draw your predicted phylogeny in the space below for Figure 1.
10. Next, use your data matrix to generate a phylogenetic tree using Mesquite that has each station as a separate taxon in your analysis. Indicate the character state changes on your phylogeny. This is your ***morphological tree.*** After looking at your data matrix, can you identify the plant groups represented at the different stations?
11. Use the annotations panel to place images of representative plants from each broad group at the tips of your phylogenetic tree. Include images from your microscopy.

12. Use literature to compare your morphological tree to molecular trees constructed using all three genomes (For example: Bowman, 2013 https://doi.org/10.1016/j.pbi.2012.10.001)

8. Indicate which species on the molecular trees are from the different plant taxa represented at the stations (i.e., bryophytes, seedless vascular plants, gymnosperms, monocots, and dicots). You may need to look this up online or in your text.
9. Next, map your morphological trait character changes would occur on the appropriate clades. (This can be done by hand or on a computer.)
10. How do the morphological and molecular trees compare? Do they show the same patterns of relationships among taxa? Do they show the same patterns of character evolution? Explain. Do the molecular trees all show the same patterns of relationships among taxa? What is similar? Different? What do you think this means?
11. How should you resolve the differences among phylogenies? How should you come to consensus?
12. Are the different plant groups (bryophytes, seedless vascular plants, gymnosperms, angiosperms) monophyletic? How can you tell? What does this indicate?

Table 1. Characters and character states for plant phylogenetic analysis.

Character	Character states (Coding)
Body	0: unicellular and multicellular 1: multicellular adults only
Leaves with veins	0: absent 1: present
Pollen production	0: absent 1: present
Woody tissues	0: absent 1: present
Xylem present in stem	0: no 1: yes
Xylem present in root	0: no 1: yes
Seed production	0: no 1: yes
Fruit production	0: no 1: yes
Flower	0: no flowers 1: yes, flower parts in multiples of 4 or 5 2: yes, flower parts in multiples of 3
Woody female cones produced	0: no 1: yes
Stem	0: small or non existent in some species 1: present, obvious, may or may not branch
Protostele	0: no 1: yes
Siphonostele	0: no 1: yes
Eustele	0: no 1: yes
Actinostele	0: no 1: yes

Table 2. Data matrix of character states for outgroup and five ingroup taxa.

Character	Chara (outgroup)	Station A	Station B	Station C	Station D	Station E
Body						
Leaves with veins						
Pollen production						
Woody tissues						
Xylem present in stem						
Xylem present in root						
Seed production						
Fruit production						
Flower						
Woody female cones produced						
Stem						
Protostele						
Siphonostele/ Dictyostele						
Eustele						
Actinostele						

Figure 1. Predicted phylogeny of plant groups at different stations based on morphological traits.
(Draw your tree in the space below.)

Stelar Phylogenetics and Anatomy

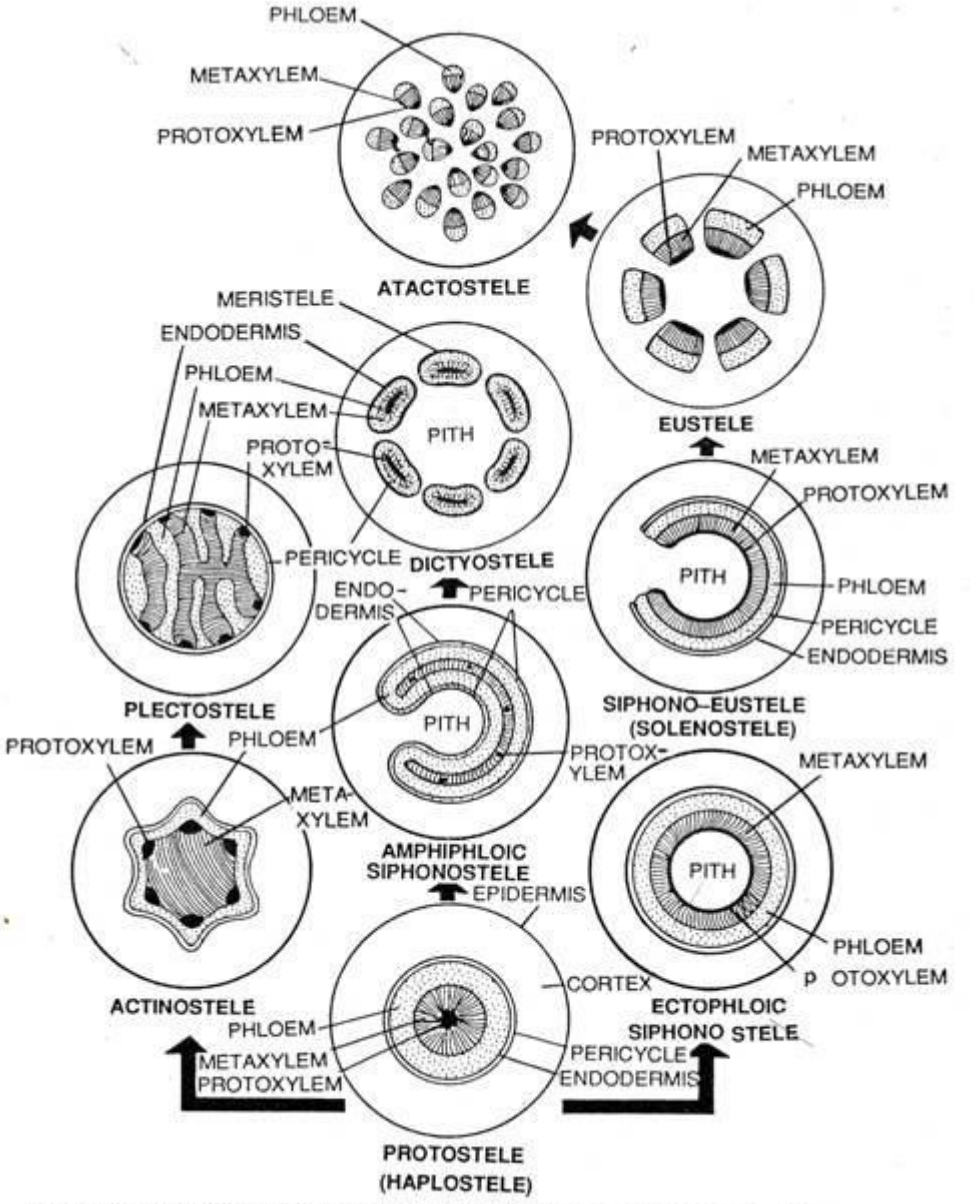

Fig. 37.43. The stelar system. Different types of steles arranged in evoluntionary sequence.

Jeffrey, 1910