

Jacob B. Landis ^{a,b,} and Julie R. Bokor ^{c,d}

^a Department of Biology, ^b Florida Museum of Natural History, ^c College of Education, ^d Center for Precollegiate Education and Training
University of Florida

Basic phylogenetics and associated "tree thinking" are often minimized or excluded in formal school curricula. Informal settings provide an opportunity to extend the K-12 school curriculum, introducing learners to new ideas, piquing interest in science, and fostering scientific literacy. Similarly, university researchers participating in science, technology, engineering, and mathematics (STEM) outreach activities, increase awareness of college and career options as well as highlight interdisciplinary fields of science research and augment the science curriculum. To aid in this effort, we designed a six-hour module in which students utilized 12 flowering plant species to generate morphological and molecular phylogenies using biological techniques and bioinformatics tools. The phylogenetics module was implemented with high school students during week-long University STEM immersion programs and aimed to increase student understanding of phylogenetics and co-evolution of plants and pollinators. Student response reflected positive engagement and learning gains as evidenced through content assessments, program evaluation surveys, and program artifacts. We present the results of the first year of implementation and discuss modifications for future use in our immersion programs as well as use in multiple course settings at the high school and undergraduate levels.

Background/Students

A plant phylogenetic and plant/pollinator interactions module was conducted with high school students over two consecutive summers. The goal was to increase interest in botany which is often only covered at a cursory level in Florida high schools, as well as to give students practice in actual laboratory techniques used in the research lab. Over the two summers this module was conducted, 247 high school students and 10 teachers participated, as well as 10 high school teachers. The breakdown of participation is below.

Overall number of program participants			2013 Program participants demographics		
Year	2013	2014*	Grade	Participants	Total
Male	62	74	11 th	21	45
Female	50	71	12 th	24	45
Total	112	145	Female	21	38
			Total	42	83

* 10 high school teachers (3M/7F) also participated in the module; 48 students (24M/24F) participated in the forensic module.

Flowering Species Used

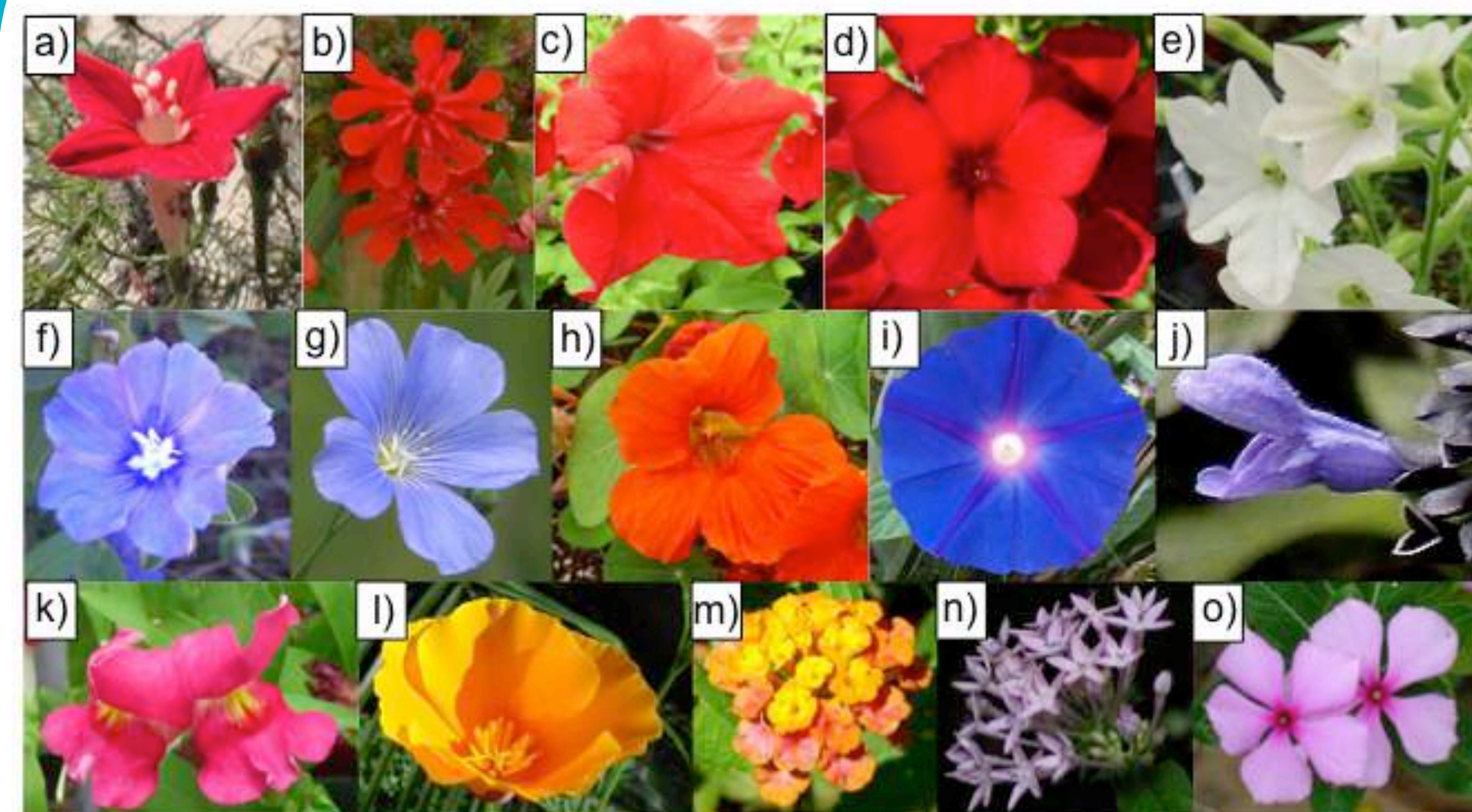
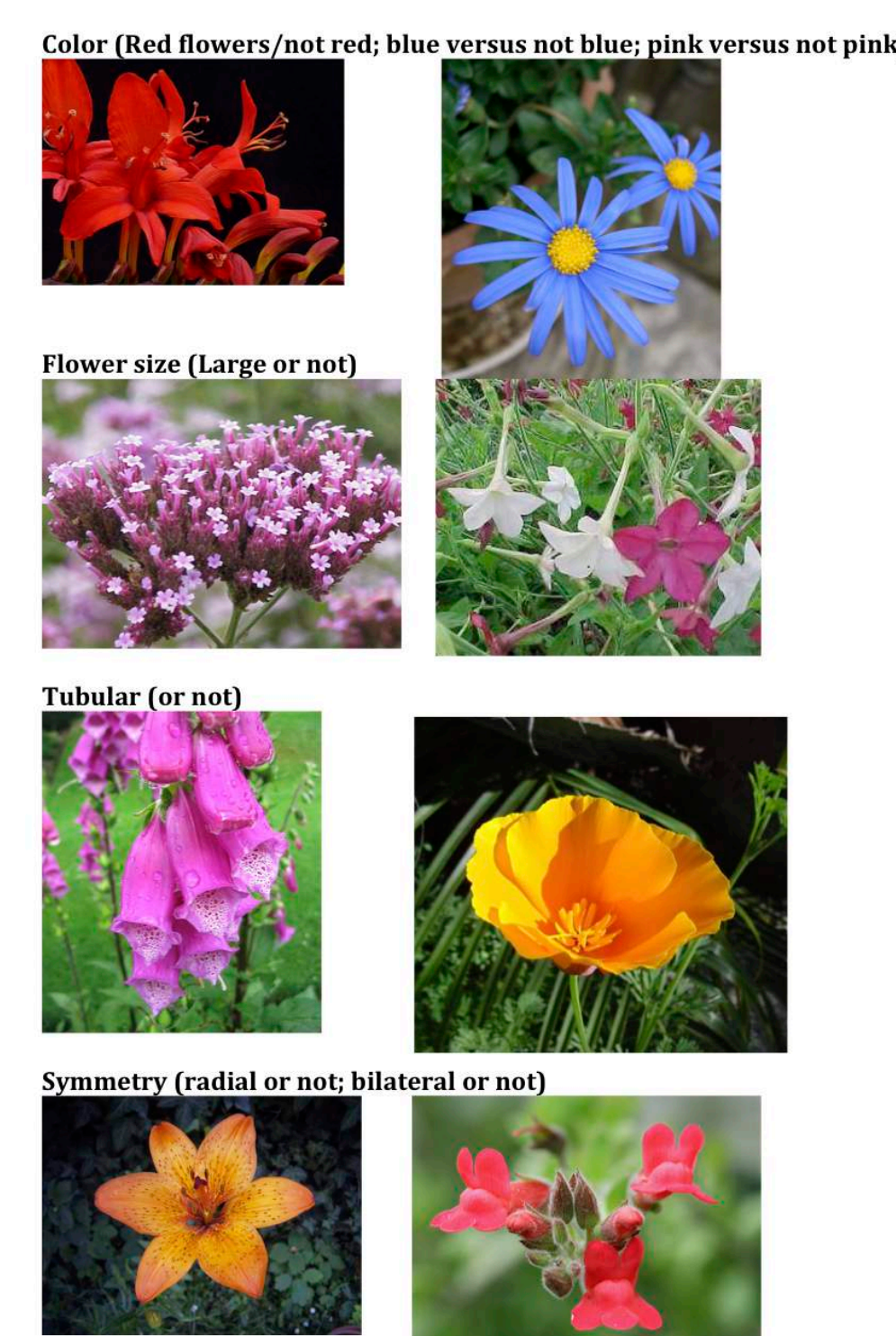


Figure 1. Plate showing the diversity of flowering plant species used in the module, a) cardinal climber (*Ipomoea quamoclit*), b) maltese cross (*Lychnis chalcidonia*), c) supercascade red *Petunia* (*Petunia hybrida*), d) red Phlox (*Phlox drummondii*), e) heavenly scent *Nicotiana* (*Nicotiana glauca*), f) blue daze (*Evolvulus glomeratus*), g) blue flax (*Linum usitatissimum*), h) empress of India (*Tropaeolum majus*), i) morning glory (*Ipomoea violacea*), j) *Salvia* (*Salvia farinacea*), k) snapdragon (*Antirrhinum majus*), l) California poppy (*Eschscholzia californica*), m) *Lantana* (*Lantana camara*), n) *Pentas* (*Pentas hybrida*), o) vinca (*Catharanthus roseus*).

List of plants used during the module including common name, scientific name, known pollinator, Genbank accession numbers for both nuclear and chloroplast genes, as well as source of plant material.

Common name	Scientific name	Pollinator	Nuclear	Chloroplast	Material
Cardinal climber	<i>Ipomoea quamoclit</i>	Hummingbird	AY538323	AY101065	Eden Brothers
Maltese cross	<i>Lychnis chalcidonia</i>	Hummingbird	EF602379	FJ404990	Eden Brothers
<i>Petunia</i>	<i>Petunia hybrida</i>	Hummingbird	DQ208093	AY098702	Burpee
Red Phlox	<i>Phlox drummondii</i>	Hummingbird	JN115041	EF433261	Eden Brothers
Heavenly scent	<i>Nicotiana glauca</i>	Hummingbird	AJ492424	AY098701	Burpee
Blue daze	<i>Evolvulus glomeratus</i>	Bee	EF567109	AY101121	Lowe's
Blue flax	<i>Linum usitatissimum</i>	Bee	JN115032	FJ160887	Eden Brothers
Empress of India	<i>Tropaeolum majus</i>	Bee	AF254020	AB043665	Eden Brothers
Morning glory	<i>Ipomoea violacea</i>	Bee	AY538329	AY101071	Burpee
<i>Salvia</i>	<i>Salvia farinacea</i>	Bee	EU169483	AY570479	Burpee
Snapdragon	<i>Antirrhinum majus</i>	Bee	F1648325	AY591322	Burpee
California poppy	<i>Eschscholzia californica</i>	Bee	DQ912883	JN051803	Burpee
<i>Lantana</i>	<i>Lantana camara</i>	Butterfly	AF437858	HM216633	Lowe's
<i>Pentas</i>	<i>Pentas hybrida</i>	Butterfly	AM267047	AM266961	Lowe's
Vinca	<i>Catharanthus roseus</i>	Butterfly	AF136743	JN574648	Lowe's

Morphological Phylogeny



Characteristics Chart: Top possible characters for each species used for the morphological analysis.

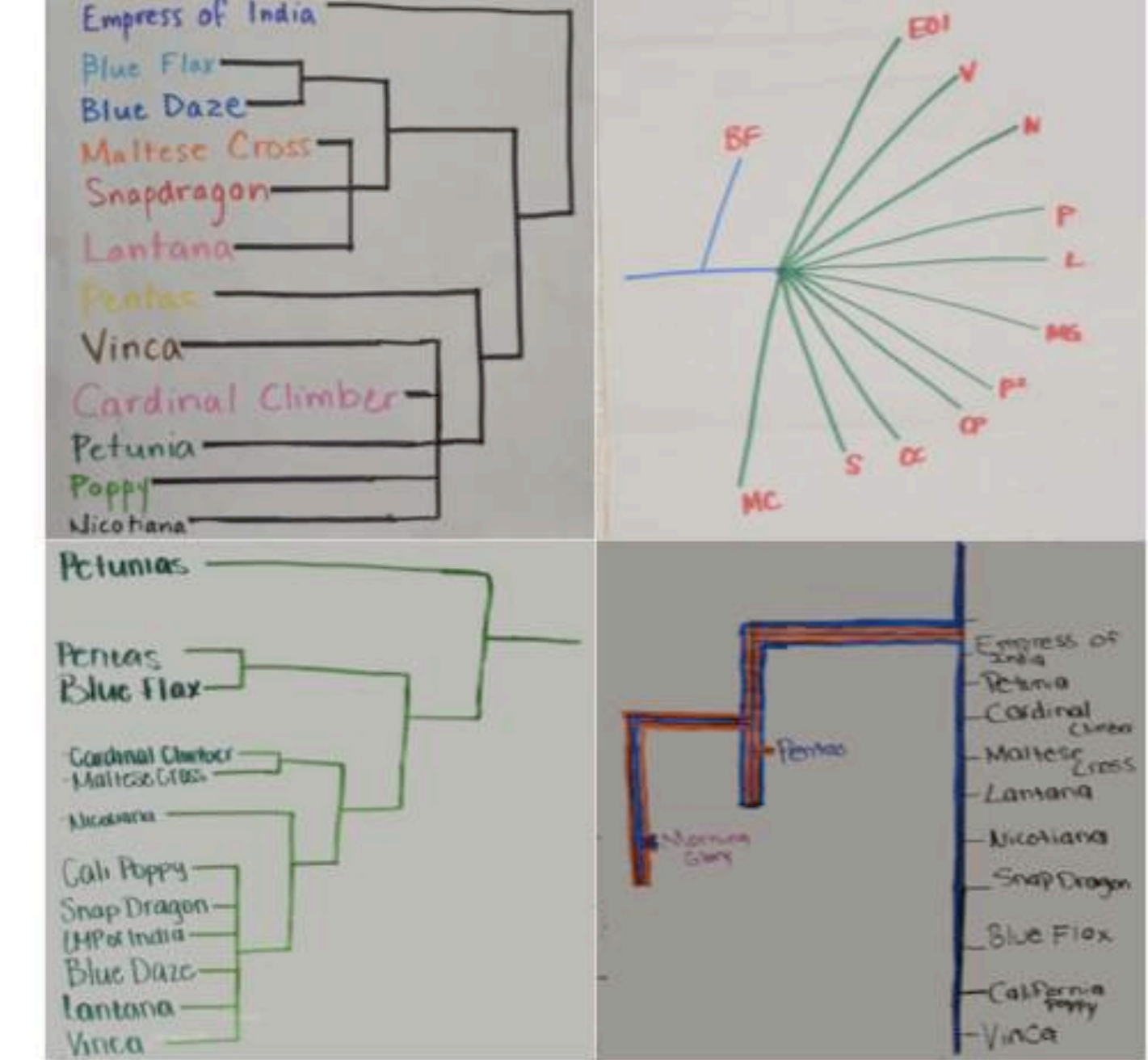
Species	Color (Red flowers/not red; blue versus not blue; pink versus not pink)	Flower size (Large or not)	Tubular (or not)	Symmetry (radial or not; bilateral or not)
Ipomoea				
Lychnis				
Petunia				
Phlox				
Nicotiana				
Evolvulus				
Linum				
Tropaeolum				
Ipomoea				
Salvia				
Antirrhinum				
Eschscholzia				
Lantana				
Pentas				
Catharanthus				

Distance Matrix:

Species	Ipomoea	Lychnis	Petunia	Phlox	Nicotiana	Evolvulus	Linum	Tropaeolum	Ipomoea	Salvia	Antirrhinum	Eschscholzia	Lantana	Pentas	Catharanthus
Ipomoea															
Lychnis	x														
Petunia		x													
Phlox			x												
Nicotiana				x											
Evolvulus					x										
Linum						x									
Tropaeolum							x								
Ipomoea								x							
Salvia									x						
Antirrhinum										x					
Eschscholzia											x				
Lantana												x			
Pentas													x		
Catharanthus														x	



Complete/Developing Understanding Lack of Understanding

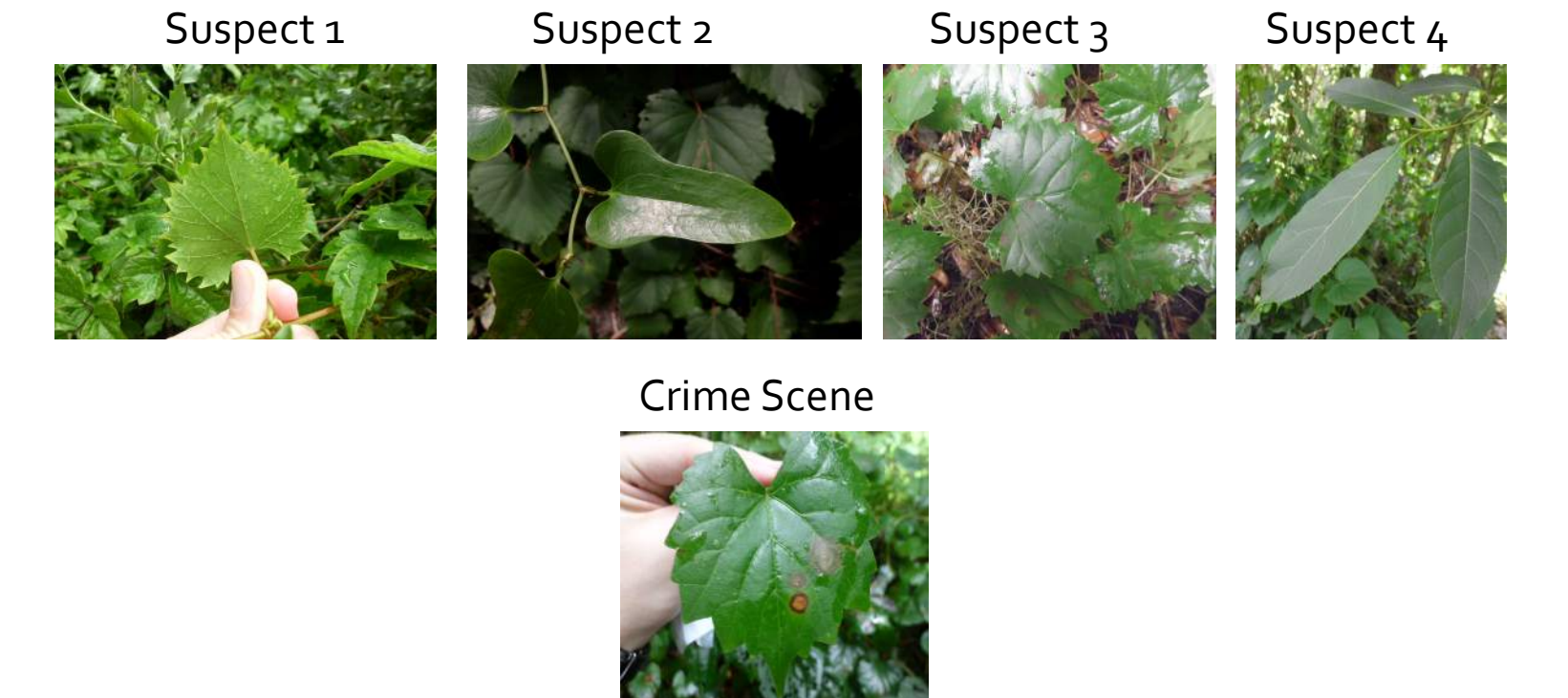
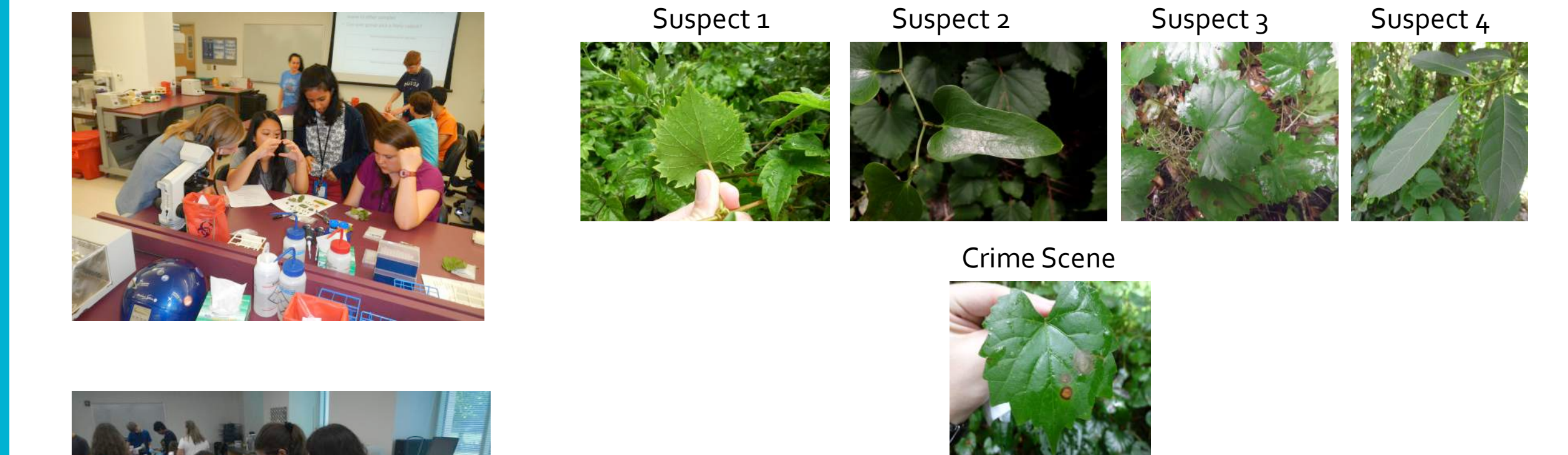


Left: Plants were arranged around the room for student inspection. For the morphological analyses, students were to pick three flower characters useful in distinguishing plants. Examples of possible characters were given, as well as suggestions for scoring. Students scored all 12 species for their selected three characters and completed a differences matrix to use for phylogenetic construction.

Above: Example student phylogenies representing groups that understood the task, and those that lack an element of understanding based on the four criteria rubric modified from Young et al. (2013). Understanding did not represent an accurate topology, since only a small subset of the characters scored were used to create phylogenies.

Additional Activity – Forensic Case

High school students were presented with a story of a murder involving four suspects. They were given evidence to process and determine the culprit. Evidence consisted of leaf samples from suspects and crime scene. With this material, they compared leaf morphology, as well as extracting DNA from all samples. Four combinations of microsatellite markers from *Vitis rotundifolia* were used for PCR and results visualized using gel electrophoresis. Each group of four students had to determine who they thought committed the crime based on the evidence. Overall there were 48 participants over two weeks, all rising sophomores from Florida high schools.



Based solely on leaf morphology, who is the likely criminal?

Based solely on DNA, who is the likely criminal?

Based solely on the fiber analysis, who is the likely criminal?

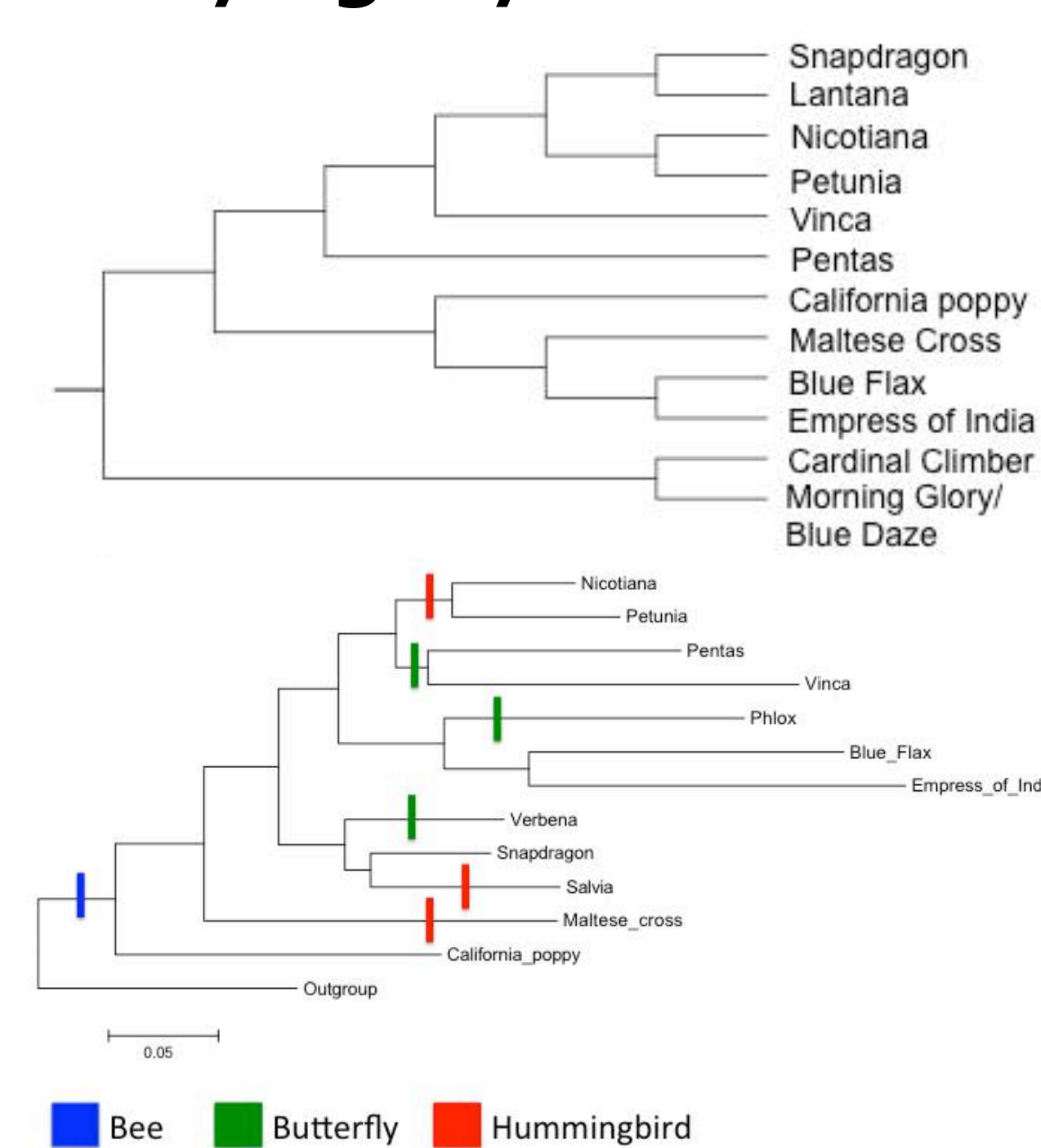
Do all three pieces of evidence tell the same story? If not, how do they differ?

Based on all of the data combined, which suspect (if any) would your group charge with the crime?

Molecular Phylogeny



Students extracted DNA from four plant species using the Extract and Amp Kit (Sigma). They then amplified ITS for each plant species and visualized their PCR products using E-gels (Life Technologies).



Sequences of ITS downloaded from Genbank for all 12 species were aligned in Mega5 using the default parameters for Muscle. A parsimony tree was reconstructed to compare to morphology trees students generated. A maximum-likelihood tree was then generated for students to trace on their three chosen flower characters illustrating how they were evolving in relation to pollinators as shown here.

Recent Iterations Summer 2014

- More reliable assessment instrument. Students completed pre/post assessments to measure conceptual understanding of the plant phylogenetics module. Assessment items were validated prior to module.
- Incorporation of The Great Clade Race from Goldsmith (2003). This exercise was done prior to completing the large morphological phylogeny to give students a deeper understanding of the procedure.
- Increased effort of drawing the big picture and why studying both plants and phylogenetics is important. An example is showing pictures of cats and then asking where did house cats come from?
- Module available online: <http://www.cpet.ufl.edu/resources/plant-phylogenetics/>

Acknowledgments and References

This project was supported from funds from NSF grant IOS-0922742, NSF grant DEB-1406650, The Amborella Genome: A Reference for Plant Biology grant awarded to Pamela Soltis (JBL advisor) and by the FloridaLearns STEM Scholars Project, funded through the Florida Department of Education's Race to the Top Award #670-RA311-4C001; Authority: 84.395A Race to the Top Fund. We would like to thank Michael Chester and Margarita Hernandez for scoring student phylogenies, help implementing the modules and helpful comments throughout. We would also like to thank Sarah Allen, Andy Crowl, Blake Geraci, Grant Godden, Richie Hodel, Barry Kaminsky, Blaine Marchant, Luis Mourino, Kim Segovia, Douglas Soltis, Pamela Soltis, Milda Stanislauskas, Kayla Ventura, and Clayton Visger for volunteering to help with the modules.

Goldsmith, D.W. 2003. The great clade race. *The American Biology Teacher* 65:679-682
 Tamura K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28: 2731-2739
 Young, A.K., B.T. White and T. Skurtu. 2013. Teaching undergraduate students to draw phylogenetic trees: performance measures and partial successes. *Evolution: Education and Outreach* 6:16