

Article

High School Students' Learning and Perceptions of Phylogenetics of Flowering Plants

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Submitted April 25, 2014; Revised July 23, 2014; Accepted July 24, 2014
Monitoring Editor: Kenneth Miller

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 and highlight interdisciplinary fields of science research and augment the science curriculum. To aid in this effort, we designed a 6-h 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 83 high school students during a weeklong university STEM immersion program and aimed to increase student understanding of phylogenetics and coevolution 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 in multiple course settings at the high school and undergraduate levels.

Just as beginning students in geography need to be taught how to read maps, so beginning students in biology should be taught how to read trees and to understand what trees communicate. O'Hara (1997, p. 327)

INTRODUCTION

Basic phylogenetics and associated “tree thinking” are often minimized or excluded in formal school curricula, even though they can help form the basis for much of the study

and understanding of biology, particularly the “ability to analyze the evolutionary ‘why’ questions” (O'Hara, 1988, p. 151; see also O'Hara, 1997; Gregory, 2008). Accordingly, some advanced high school courses have placed greater importance on phylogenetics as a tool to help students understand evolution as the unifying theme in biology. For example, the Advanced Placement Biology curriculum places heavy emphasis on the study of evolution and relatedness of species and includes tree thinking and the use of cladistics in an evolution-centered laboratory activity (College Board, 2012) and specific learning objectives in the curriculum framework (LO1.17, 1.18, 1.19; College Board, 2013). However, the same cannot be said for the K–12 science curriculum. For example, phylogenetics is not included in the recently released *Next Generation Science Standards* (NGSS; Achieve, 2013). NGSS promotes deep, foundational knowledge of evolutionary theory; however, it falls short in recommending explicit instruction in tree thinking and use of phylogenetics. Recognizing this void, scientists engaged in discipline-based education research are developing lessons on phylogenetics (National Research Council [NRC], 2012a) for inclusion in secondary schools (Rau, 2012; Kovarik *et al.*,

DOI: 10.1187/cbe.14-04-0074

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2013) and undergraduate-level courses (Meir *et al.*, 2007; Lents *et al.*, 2010; Smith *et al.*, 2013; Young *et al.*, 2013) to facilitate student comprehension of evolutionary concepts. Use of diagrammatic depictions such as phylogenetic trees provide a model that increases student understanding of science topics (Schwarz *et al.*, 2009) and can facilitate understanding of evolution by providing a visual representation of relatedness among species (O'Hara, 1997; Gregory, 2008; Catley *et al.*, 2012).

Complementary to the formal school setting, informal science experiences offer the opportunity to stimulate science learning in low-stakes environments that facilitate learner choice in engaging in participatory learning activities and can augment and extend the formal school curriculum (Bell *et al.*, 2009). Informal settings are traditionally associated with museums, zoos, and planetariums, but postsecondary institutions provide another venue for engaging youth in a variety of academic and career options situated in authentic research environments by opening their laboratories for classroom visits, community events, and apprenticeship programs. University scientists are playing an increasingly larger role in broadening public awareness of science research, as federal grant applications are judged not only on intellectual merit but also on their broader impact to the public (National Science Foundation [NSF], 2012). Engaging learners across the K–20 spectrum has benefits for all stakeholders (Andrews *et al.*, 2005). For current graduate students, the next generation of researchers, developing methods for communicating their scientific research to a broader audience with the support and guidance of faculty advisors and educational outreach professionals is becoming an important skill (Dolan *et al.*, 2004; Laursen *et al.*, 2007).

Researchers' participation in immersion programs serves multiple functions, not the least of which is to communicate current research and scientific practices as emphasized in *A Framework for K–12 Science Education* (NRC, 2012b) and *NGSS* (Achieve, 2013), to introduce potential members of their community of practice (Wenger, 1998) to authentic scientific inquiry, and to stimulate interest in the diversity of science careers. Botany, however, is not traditionally viewed with great interest by secondary school students in the United States or internationally (Schussler and Olzak, 2008; Bybee and McCrae, 2011), and this lack of interest is reflected in the school curriculum. Botany is often given cursory attention by teachers and students in secondary school levels in deference to animal systems, an emphasis that continues into the postsecondary setting (Uno, 1994; Hershey, 1996). In our experience, we have noted a similar sentiment among beginning undergraduate students in their approach to the botany section of the introductory biology course. However, this situation does not imply a void of educational research related to the learning of phylogenetics.

Previous work has shown that students often hold several common misconceptions regarding reading and interpreting phylogenetic trees. For example, Catley and Novick (2008) demonstrated that undergraduate students have difficulties inferring which taxon should or should not be included in a clade, even when the definition of a clade is provided. Additional studies have shown that students have difficulty understanding most recent common ancestry and the implication of taxa sharing a most recent common ancestor (Catley and Novick, 2008; Catley *et al.*, 2012; Gregory, 2008). Baum

and colleagues (2005) illustrated that, when presented a phylogenetic tree, students tend to infer time by reading the tips, which actually does not convey any timescale. Owing to a lack of instruction in tree thinking, these are just some of the common misconceptions that exist as students approach phylogenetic analysis.

To stimulate interest in botany and the diversity of applications within the field, as well as to foster understanding of phylogenetics in secondary students, we developed a 6-h module for high school students engaged in a weeklong science, technology, engineering, and mathematics (STEM) immersion program at a major southeastern university. In keeping with the overall goal of the immersion program, the module was designed to engage students in active learning and to facilitate their understanding of the wide breadth of academic disciplines and potential careers. The aim of this study was to better understand how this plant phylogenetics module impacted the engagement and learning of high school students. With our design-based approach (McKenney and Reeves, 2012), we intended to add to our understanding of how students learn phylogenetics, while also informing our next design iteration of the curriculum module. The results presented here represent year 1 of our study and provide a benchmark to inform our future design and implementation.

METHODS

Program Description

The plant phylogenetics module was embedded within a weeklong residential STEM immersion program composed of multiple science and engineering modules developed and led by researchers across the university campus. Students explored such diverse topics as molecular biology, materials engineering, and wetlands ecology, complete with indoor laboratory and outdoor field experiences. Students resided in on-campus housing and participated in both social- and STEM-enrichment evening activities under the guidance of undergraduate and postbaccalaureate science majors. Additionally, students worked in pairs to document their experiences using tablet computers, generated daily blog posts, and constructed STEM career presentations that were posted on each week's social learning platform. Pre/post content assessments were administered using a Web-based survey instrument that students accessed through individual accounts on the social learning platform.

Participants

The students who attended the program included males and females entering their junior or senior years of high school (Table 1). During their first day on campus, the 83 students provided consent to serve as research participants. The participants were selected to attend the program by mentors in their individual school districts, which were located in rural areas of the state. These sparsely populated and under-resourced districts struggle with providing rigorous STEM course work and career opportunities for students. Consequently, students from rural districts are less likely to take advanced courses, due to low enrollment and a lack of resources (Lee and Luykx, 2007).

Table 1. Program participants demographics

	Participants		Total
	11th	12th	
Grade ^a	11th	12th	
Male	21	24	45
Female	21	17	38
Total	42	41	83 ^b

^aGrade listed is the grade the student entered after completion of the summer immersion program.

^bOne student did not complete the program evaluation; therefore the evaluation $n = 82$. Two students did not complete the pretest, and one student did not complete the posttest; therefore the assessment $n = 80$.

Module

The plant phylogenetics module engaged students in an experimental sequence including observation of morphological characteristics, DNA extraction, amplification, and verification of polymerase chain reaction (PCR) product, immersing them in an authentic experience consistent with current laboratory research practices. The context of the module is the coevolution of plants and their pollinator species and how morphological and molecular phylogenetic trees can help researchers understand the relationship between plants and pollinators through investigations of systematics. The module was sequenced to scaffold student understanding of phylogenetics using visible traits to first

construct a morphological tree, which facilitates construction and understanding of complex evolutionary relationships based on genetic data made visible in computer-generated molecular trees (Wood *et al.*, 1976; Linn, 2000; Belland, 2014). Before implementation of the formal research study, the module was pilot tested for functionality and feasibility with a similar sample of high school students.

Description of the Module Activities

The phylogenetics module consisted of four main activities: 1) background information on phylogenetic analyses and importance of botanical knowledge, 2) collection of floral morphological features, 3) common laboratory techniques for generating molecular data, and 4) phylogenetic analyses involving morphological and molecular data. All students, working in groups of three or four with assistance from undergraduate, graduate, and postdoctoral researchers, were given ample opportunity to participate and contribute during each activity.

Activity 1. Plants and Pollinators. Before hands-on activities began, a short presentation covering topics such as the importance of plants, phylogenetics, types of pollinators, and pollinator attractions were covered. Phylogenetic theory and pollination biology were briefly explained (Judd *et al.*, 2007). Each student group was given plants from four of the 12 species being utilized in that particular week, with a total of 15 species used throughout this project and each group receiving at least one representative flowering plant for each pollinator: hummingbird, bee, and butterfly (see Figure 1 and

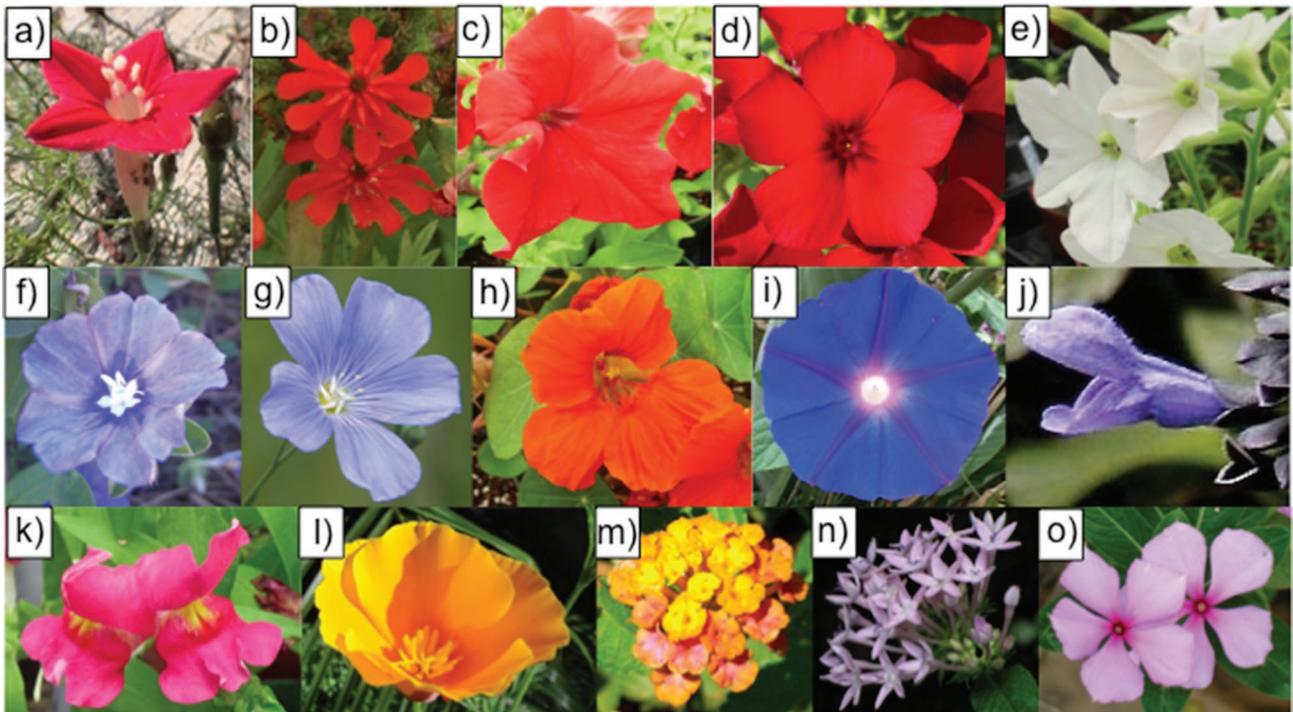


Figure 1. Plate showing the diversity of flowering plant species used in the module. (a) Cardinal climber (*Ipomea quamoclit*); (b) Maltese cross (*Lychnis chalconia*); (c) supercascade red petunia (*Petunia hybrida*); (d) red phlox (*Phlox drummondii*); (e) heavenly scent nicotiana (*Nicotiana glauca*); (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*); and (o) vinca (*Catharanthus roseus*). Flowers of a–e are hummingbird pollinated, flowers of f–i are bee pollinated, and flowers of m–o are butterfly pollinated.

Table 2. List of plants used during the module, including common name, scientific name, known pollinator, GenBank accession numbers for both nuclear and chloroplast genes, and source of plant material

Common name	Scientific name	Pollinator	Nuclear	Chloroplast	Material
Cardinal climber	<i>Ipomea quamoclit</i>	Hummingbird	AY538323	AY101065	Eden Brothers
Maltese cross	<i>Lychnis chalcedonia</i>	Hummingbird	EF602379	FJ404990	Eden Brothers
Petunia	<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
Salvia	<i>Salvia farinacea</i>	Bee	EU169483	AY570479	Burpee
Snaptadragon	<i>Antirrhinum majus</i>	Bee	FJ648325	AY591322	Burpee
California poppy	<i>Eschscholzia californica</i>	Bee	DQ912883	JN051803	Burpee
Lantana	<i>Lantana camara</i>	Butterfly	AF437858	HM216633	Lowe's
Pentas	<i>Pentas hybrida</i>	Butterfly	AM267047	AM266961	Lowe's
Vinca	<i>Catharanthus roseus</i>	Butterfly	AF136743	JN574648	Lowe's

Table 2 for a complete list of flowering plants). Students were asked to infer the pollinators of each of their flowers after learning about common pollination syndromes and associated floral morphologies (Judd *et al.*, 2007; see also Fenster *et al.*, 2004).

Activity 2. Molecular Biology. Students began the molecular biology portion of the module by extracting DNA from the leaves of each of their plants as described in the REDExtract-n-Amp Plant PCR Kit (Sigma-Aldrich, St. Louis, MO). This was followed by DNA amplification of a nuclear gene (ITS: *Internal Transcribed Spacer*) that is often used in systematic studies of plants using universal primers (White *et al.*, 1990; Sang, 2002; Alvarez and Wendel, 2003). Students confirmed the success of their PCR procedure by gel electrophoresis (E-gel; Invitrogen, Grand Island, NY). Between the DNA extraction, DNA amplification, and gel electrophoresis procedures, short presentations helped familiarize students with the rationale behind each procedure to aid conceptual understanding of what was occurring from plant leaf to DNA band on an agarose gel and the further step of DNA sequencing, which produces the molecular data needed for the construction of molecular phylogenies. Owing to limited time, the student samples were not sequenced; instead, previously published sequence data were used for further analysis. (All protocols and presentations are available at www.cpet.ufl.edu/resources/plant-phylogenetics.)

Activity 3. Morphological Phylogenies. Students recorded 10 floral features in a characteristics chart, scoring traits as binary (e.g., presence or absence of the trait) for selected floral features, such as flower color, size, shape, and orientation (see Supplemental Table S1). Many traits were evaluated subjectively and varied between students and groups. For characteristics such as flower size, each group developed its own criteria of large versus small flower and used these guidelines to score all species. Individuals rotated to other groups to observe, score, and discuss all 12 species of plants.

Using their completed characteristics chart, each group constructed a distance matrix incorporating the number of differences between each species pair for three selected

characters (see Table S2). Using the information for total number of differences between species, groups constructed a morphological phylogenetic tree using a parsimony framework (Baum and Smith, 2013). Undergraduate and graduate students worked with each group to assist in the development of the trees and help students understand the process of selecting traits and determining evolutionary relatedness. Groups drew their phylogenies on chart paper and posted them so all groups could identify similarities and differences (see Figure 2 for examples).

Activity 4. Molecular Phylogenies. DNA sequences for a nuclear marker (ITS) and a chloroplast marker (*trnL-trnF* spacer) were downloaded from GenBank (www.ncbi.nlm.nih.gov/genbank; see Table 2 for accession numbers) for each of the plant species in advance and saved in FASTA format on each student laptop to import into MEGA5 (Tamura *et al.*, 2011). Whole-group instruction was provided to guide student groups through phylogenetic analysis of the nuclear marker (ITS) using a parsimony framework including subtree pruning to identify the best topology. Students were then encouraged to do the same analysis on the chloroplast data (*trnL-trnF*) to see whether the two trees differed (see Hall, 2011, for additional examples and walkthroughs of analyses). Phylogenies constructed with nuclear markers are often incongruent with those that are chloroplast-based, due to hybridization, different modes of inheritance, and different rates of evolution (Sang, 2002; Alvarez and Wendel, 2003). At the conclusion of the module, students compared and contrasted the topology of trees between the molecular and morphological data sets generated by MEGA5 (Figure 3) and discussed the differences and similarities observed and the utility of each type of data in phylogenetic analysis.

Research Methodology

Design-based research was used for this investigation, exploring student content knowledge gains and engagement with and perceptions of the plant phylogenetics module. Design-based research is characterized by investigating our theoretical understanding of learning, as an intervention is developed, tested, and revised in an iterative manner

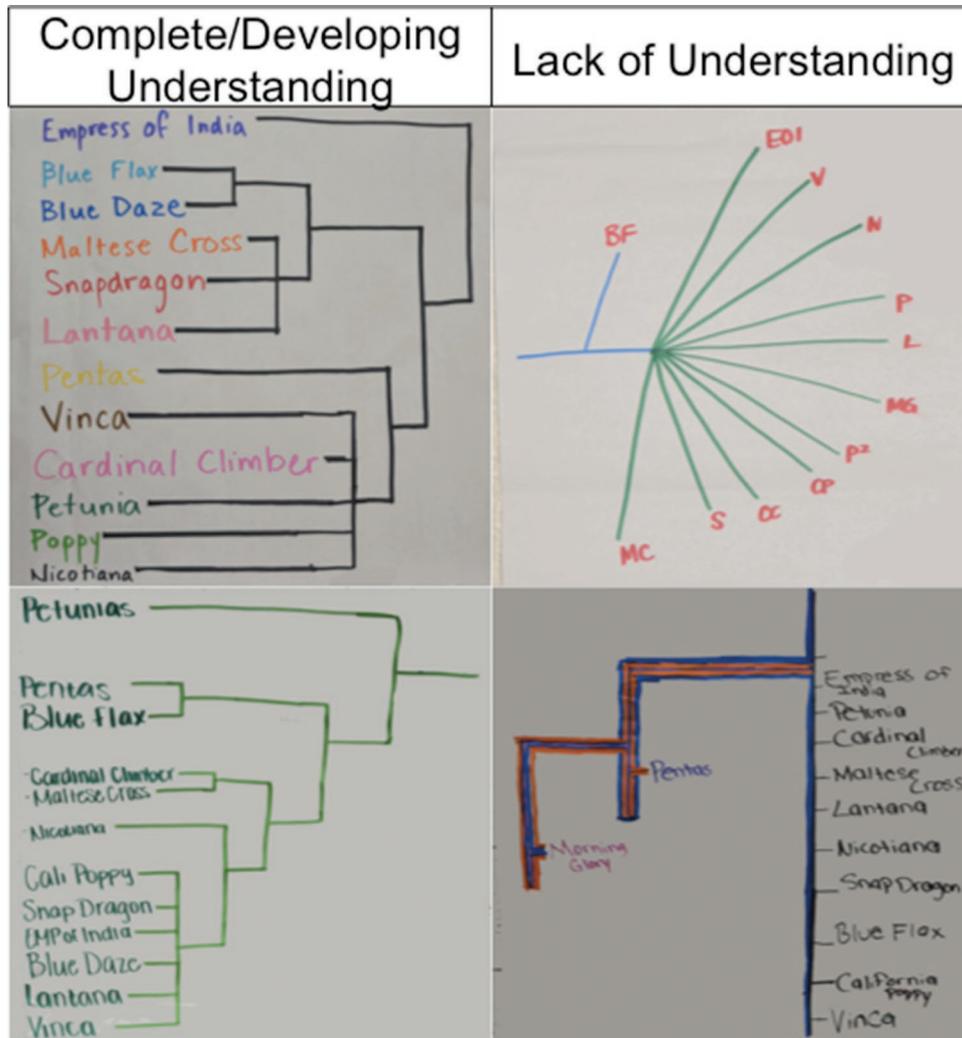


Figure 2. Student phylogenies constructed during modules representing groups who understood that task and those who lack an element of understanding based on the four-criteria rubric designed for this module. Understanding did not represent an accurate topology, because only a small subset of the characters scored were used to create phylogenies.

while situated in a real-world context (Barab and Squire, 2004; Hoadley, 2004). The results of this study represent the first iteration of the design–test–revise steps of the research cycle. While considerable scholarship addresses phylogenetics and tree thinking with undergraduate students, secondary students have only recently become the subject of investigation (Catley *et al.*, 2013). Therefore, this study establishes initial conceptions of our student population and provides a baseline for future investigations. Common with other design-based research studies, a mixed qualitative/quantitative approach was used to better understand how participants “think, know, act, and learn” (Barab and Squire, 2004, p. 5) as they experience a plant phylogenetics module.

Three data sources were utilized in this study: a survey of science content knowledge, the phylogenetic trees that were constructed by student groups, and a follow-up program evaluation survey. The survey instrument for science content knowledge was prepared and used as a pre/post repeated measure (Table S3). Our use of this form of proximal

assessment was based on the specific nature of the content and our long-term strategy for multilevel assessment (Ruiz-Primo *et al.*, 2002). Five survey items were developed by the science researchers (J.B.L.) and reviewed by science education researchers (J.R.B., K.J.C.). The four forced-response items were scored at 1 point each, and the one open-ended response item was scored at 2 points, for a total of 6 points possible.

During the module, participant groups used chart paper to construct morphological phylogenetic trees that were collected and scored. The scores were not shared with participants. These artifacts serve as evidence of the participants’ ability to build and apply phylogenetic trees to answer questions about evolutionary relationships. The following rubric, modified from Young *et al.* (2013) was used to score the artifacts based on four criteria. Each criterion was judged as either successfully fulfilled (1 point) or not fulfilled (0 points) for an overall total of four possible points. Three members of the research team (J.B.L.) independently scored the trees and then discussed their scores to a consensus. The interrater reliability for the independent scoring was 70%.

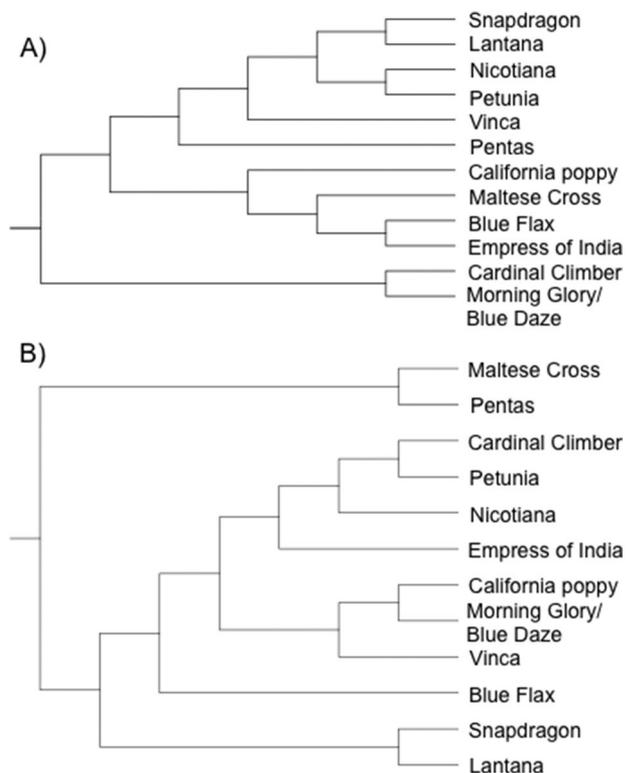


Figure 3. Representative phylogeny from (a) nuclear markers and (b) completed 10-character data matrix for morphological features of flowers. In both trees, morning glory and blue daze occupy the same place in the topology, because they were exchanged in different weeks of the module due to lack of flowering some weeks. Both phylogenies were constructed under parsimony criteria in MEGA5 with default settings.

- Criterion 1: Species present and connected. All 12 species utilized in the module were included in the phylogenetic tree, with connections between all species.
- Criterion 2: Common ancestor. Tree indicates a common ancestor to all species represented by branching from a single root.
- Criterion 3: Branching pattern. Relationships between species were represented using branching morphology, with each node giving rise to either two species, or two clades containing multiple species if a polytomy was present. When a node gave rise to one species, but then was left blank, this was scored as a 0.

- Criterion 4. Choice of characters. Students were to pick three characters they thought would be useful in distinguishing relationships. When relationships could not be distinguished for most species, this represented a poor decision in character choice.

To assess student engagement and perceptions of the module, we prepared a short follow-up survey. Participants were asked to first evaluate the activities by rating them as excellent, good, fair, or poor, and then to include comments to justify their rating. The survey was administered at the conclusion of the weeklong immersion program. Additionally, field notes were used to document the implementation of the module, as were any informal discussions with students and teacher chaperones and among the researchers. These data were used to provide insight into the implementation and to validate the analysis from the other data sources.

RESULTS

Content Knowledge

A paired-sample *t* test was conducted on the participants' scores from the content knowledge survey. A statistically significant increase was found from pretest ($M = 2.70$, $SD = 1.24$) to posttest ($M = 3.73$, $SD = 1.41$), $t = 5.75$, $df = 79$, $p < 0.001$ (Table 3). Cohen's *d* was calculated as 0.643, suggesting a medium effect size for the module as a learning intervention (Cohen, 1988).

Because the module utilized a researcher-designed instrument, we used an analysis of the individual question items to further explore the impact on student content knowledge (Table 4). While responses to question items 2, 3, and 4 showed a significant increase from pre- to posttest, indicating improvement in student understanding of convergent evolution, utility of molecular analysis, and sequence of molecular techniques, responses to items 1 and 5, which probed student understanding of researcher subjectivity and asked students to define a molecular technique, respectively, did not increase. In fact, participants performed worse at posttest on question 5. The largest increase in mean score was for question item 3, with a pretest mean of 0.4375 and a posttest mean of 1.0938. This item contained a short-answer response, and student scores ranged from 0 (incorrect choice and explanation) to 2 (correct choice and explanation) with partial credit awarded. While only 28.8% of the students scored 1 point or higher on the pretest, 60.0% scored 1 point

Table 3. Descriptive statistics for content assessment^a

Question item	Item content	Point value	Pretest		Posttest	
			Mean	SD	Mean	SD
1	Researcher subjectivity	1.0	0.6000	0.49299	0.6000	0.49299
2	Convergent evolution	1.0	0.5250	0.50253	0.6500	0.47998
3	Molecular vs. morphological data	2.0	0.4375	0.72642	1.0938	0.93489
4	Sequence of molecular techniques	1.0	0.6125	0.49025	0.8875	0.31797
5	DNA electrophoresis	1.0	0.5250	0.50253	0.5000	0.50315
Phylogenetics module		6.0	2.7000	1.24168	3.7313*	1.41409

* $p < 0.001$ (2-tailed), $df = 79$.

^a $n = 80$.

Table 4. Content assessment correct responses by question item

Question item	Pre		Post		<i>t</i> test		
	Number correct	% Correct	Number correct	% Correct	<i>t</i>	Significance	Cohen's <i>d</i>
1	48	60.0	48	60.0	0.000	1.000	0.000
2	42	52.5	52	65.0	1.997	0.049*	0.223
3 ^a	8	10.0	36	45.0	5.632	0.000*	0.630
4	49	61.3	71	88.8	4.666	0.000*	0.522
5	42	52.5	40	50.0	-0.363	0.717	-0.041
Module	—	—	—	—	5.752	0.000*	0.643

* $p < 0.05$ (2-tailed), $df = 79$.

^aQuestion 3 was worth 2 points due to the combination of forced response and short answer. Selection of B was valued as 1 point, and the explanation was worth an additional 1 point with 0.5 point given. The value presented here is the number and percentage of students who earned all 2 points.

or higher on the posttest. Many students demonstrated an increased understanding regarding the role of researcher variation in scoring morphological traits and the benefits of molecular analysis. However, other students still chose to create a phylogeny with a team of researchers analyzing 50 morphological traits rather than 1500 base pairs of sequence from each species, illustrating the difficulty of changing prior conceptions (see Box 1).

Morphological Trees

Trees could not be scored as a correct or incorrect topology, because participants were only required to use three of the 10 characters, and multiple most-parsimonious trees with differing topologies could be recovered for all data sets. Trees were therefore scored in a way to represent participants' general understanding of phylogenetics and as a proxy for their understanding of evolutionary theory. We assume this to be participants' first exposure to phylogenetic analysis, and these models therefore represent their understanding of phylogenetics as constructed during the module.

Each participant group was successful in constructing a valid representation of morphological analyses. Overall, the trees could be grouped into three categories (see Figure 2): complete understanding (score 4), developing understanding (score 2–3), or lack of understanding (score 0–1). The trees that were categorized as complete understanding or developing understanding generally included a clear branching pattern of connections between species, the representation of a common ancestor, and a choice of characters that were phylogenetically informative. Trees categorized as lack of understanding typically did not include a clear structure of relationships and often utilized characters that were not informative. For the 24 trees scored, three were deemed to show complete understanding, while 17 showed developing understanding, and four exhibited a lack of overall understanding.

Perceptions of the Module

Eighty percent of the participants responded that the day 1 activities were excellent or good. These activities involved plant biology, including flower structure; pollinator types and syndromes; plant and pollinator interactions; and

biotechnology, including DNA extraction and preparing samples for PCR. The second day of the module included gel electrophoresis; visualizing and discussing gel electrophoresis results; implications about genetic relatedness; scoring morphological characteristics of the 12 plant species in the laboratory; constructing a phylogenetic representation of evolutionary relatedness based on three selected morphological characteristics; using genetic sequence data to generate a molecular phylogenetic tree illustrating genetic relatedness using MEGA5; and discussing the comparative advantages and disadvantages of morphological and molecular phylogenetics. Even though this day was conceptually

Box 1. Example student responses for question item 3

You are part of a four-person research team performing a phylogenetic analysis with 100 species. Which method would you choose and why? Support your decision.

- 50 morphological characters (morphological phylogeny)
- 1 gene consisting of 1500 base pairs of DNA (molecular phylogeny)

Desired response and change in knowledge:

"I would choose answer a because it is less to deal with and if there are that many species to work with it will be less to go through in comparison to 1,500 base pairs of DNA" (Student 78, pretest).

"It would be more accurate and in the long time less time consuming using the answer choice b. With morphological phylogeny it matters a lot on how one sees the trait. For example a flower may score as tubular for one person not the other" (Student 78, posttest).

"Using 50 morphological characters offers a broader spectrum for the analysis" (Student 21, pretest).

"The gene and base pairs because they can be found with a computer, making them easy to compare" (Student 21, posttest).

Enduring misconceptions:

"Morphological characters would be more informative than 1 gene" (Student 1, posttest)

"Well I would use morphological phylogeny, I say this because I feel I would better be able to decode the alike and dislikes [*sic*] traits and construct a graph or chart out of that information" (Student 6, posttest).

Table 5. Student perceptions of plant phylogenetics module

Open codes	Theme	Description	Student quotes
Good activity Enjoyed Critical thinking Interesting	Interesting and engaging	Overall perception of the plant module	“Pretty cool and eye opening.” “Challenging but also really fun.” “Really fun and in-depth lab.” “The experiment was fun and educational, and I learned more about plants.”
Real scientist Tool use Liked instructor	Community of practice	Includes references to tool use, discourse, and working within the community in groups of peers and knowledgeable others	“The instruments were very cool to use and I enjoyed working with them.” “It was nice to use lab equipment I haven’t used before.” “Using a thermocycler was cool.” “[The instructor] seemed passionate about his work.”
Hands-on Labor intensive Hard work Challenging Group work	Active learning	Characteristics of participatory science learning including: hands-on activities as well as collaborative learning with peers	“I liked learning how to extract DNA.” “Liked the hands on! Drawing.” “We were able to do hands on things and work as a team.” “Really fun and in-depth lab, seeing and experiencing lab work is definitely useful.”
Like plants Doesn’t like plants	Views of plants	Positive and negative perceptions of plants in general or the field of study.	“I liked how we learned about plants and why studying botany is important.” “I learned how to examine differences in plants I was not knowledgeable about.” “I have no interest in how plants are related.” “The subject matter was not boring. Botany just isn’t my thing.”
Didn’t work Lack of interest Not novel Confusion	Discontent	Reflects negative perceptions of the module due to disinterest or frustration	“Confusing, didn’t really pop out.” “Hard to keep our attention and maintain enthusiasm.” “We have done this before.” “Not my cup of tea.”

more difficult for the participants, 74% indicated excellent or good on their evaluation, similar to the score for day 1 of the phylogenetics module. Student comments suggested that the decline in favorable perception was attributable to the challenging experience of constructing phylogenetic trees.

The constant comparative method (Erickson, 2012; Creswell, 2014) was used to examine student responses in the module evaluation. The student responses were brief and reflected generally positive, negative, or neutral perspectives of the plant phylogenetics module. Many in vivo codes were used during open coding such as *bored*, *interesting*, and *hard work*. Initial codes were combined into conceptual codes that were then grouped during axial coding to allow themes to emerge from the data. Emerging themes were identified to characterize the students’ engagement and perceptions of the plant phylogenetics module: *interesting and engaging*, *community of practice*, *active learning*, *views of plants*, and *discontent*. (See Table 5 for open codes, themes, descriptions, and example student quotes.) These themes were also identified in the field notes, blog posts, and informal conversations, validating the findings of the qualitative analysis of the program evaluation surveys.

Consistent with the quantitative evaluation results, the participants expressed favorable perceptions of the plant phylogenetics module, considering it *interesting and engaging* and describing it as “Pretty cool and eye opening.” Participant comments about using the tools and techniques of the science researchers (“I know how to do it professionally now!”)

and interacting with more knowledgeable others (“[The instructor] seemed passionate about his work”) suggest that they felt part of a *community of practice*. Additionally, the participants saw value in participating in a research laboratory group (“Seeing and experiencing lab work is definitely useful”). Some participants found certain activities they had already experienced elsewhere to be less engaging, but they indicated that they saw the value of those activities for other students, recognizing that members of a community of practice have different levels of developing knowledge and experience. Opportunities for *active learning* also resonated with the participants, who cited the hands-on nature of the module, drawing components, and working in groups (“We were able to do hands-on things and work as a team” and “This event taught us teamwork, and how to think critically”) as positive attributes, while listening to talks was considered “boring.”

In the evaluation survey, many participants shared their *views on plants*, and some indicated that they enjoyed studying plants (“I liked how we learned about plants and why studying botany is important”), although several others indicated a lack of interest in botany (“Not everyone is in love with plants”). Specific comments related to the module reflect this variation as well. Some participants seemed to disengage with the activity due to their lack of enthusiasm with the organism, while others expressed that plants were not their favorite topic of study but they understood their importance. Some participants’ perceptions

also reflected *discontent* with the module activities, either due to the lack of interest in the experimental procedures (“We have done this before”) or difficulty understanding the module content or procedures (“Confusing, didn’t really pop out”).

Design-Based Research Findings

Because design-based research considers the context of the learning environment to be a crucial component of the enactment of new curricula, we present some of these aspects here to provide insight to the way our participants would “think, know, act, and learn” (Barab and Squire, 2004, p. 5) as they experienced the plant phylogenetics module. To engage participants at the very beginning of the module, we addressed the question “Why study plants?” to the whole group. Participants suggested examples, including the importance of products such as clothing, food, and medicine. Responses were linked to familiar agricultural practices, consistent with the economies of the areas these students represented. These naïve understandings of botany did not include more sophisticated applications such as species conservation, evolutionary theory, or the use of genetic engineering for improved production, indicating lack of student prior exposure to advanced botany research and biology topics.

As participants worked together to score the characteristics, they discovered through firsthand experience that one of the issues with scoring morphological characteristics is human subjectivity. Heated discussions ensued among participant groups regarding scoring, one example being whether a flower should be classified as tubular or clustered. The graduate and undergraduate students were often called to mediate these discussions and to prompt each participant to explain his or her rationale and to encourage each group come to a final consensus. However, as discussed later, our emphasis on a group consensus may have caused confusion for the participants on question item 1 during the posttest.

While the module was specific to molecular phylogenetics, we did point out advantages and disadvantages with both molecular and morphological trees, to help the students understand that multiple techniques are used in science to answer different research questions. With the availability of DNA sequence data, molecular analysis facilitates the investigation of a large number of characters of modern-day plants, whereas morphological analysis with fewer characteristics still dominates studies in paleobotany, an area of study for which molecular data are difficult to impossible to recover. However, there are also cases, such as reconstructing the first flower, in which these two methods are often used in conjunction (Doyle, 2008).

DISCUSSION

The immersion program in which our module was situated was composed of upper-level high school students. In our state, all students take biology by the 10th grade, so we assume each program participant successfully completed general biology and passed the required end-of-course exam. We further assume they will not have any further exposure

to biology curricula in high school. Although these students had previously taken biology in high school, their prior knowledge was low (as evidenced by the pre-assessment), perhaps hindering larger learning gains as they grappled with new concepts. Because prior knowledge is the largest predictor of learning gains (Schraw *et al.*, 2005), we view our finding of a medium effect for a 6-h module as encouraging and worthy of further design and analysis. It demonstrates that, taken out of the K–12 science curriculum sequence, students do increase their understanding of phylogenetics as a result of a very brief intervention. Our study suggests that purposeful integration of tree thinking in the science classroom could foster student understanding of evolutionary theory even further. However, as others have discussed, limited explicit instruction in tree thinking in K–12 classrooms, as well as inaccurate representations of phylogenetics in classroom textbooks, leaves students vulnerable to enduring misconceptions and a limited understanding of evolutionary concepts (Catley and Novick, 2008).

Owing to the short duration and informal nature of immersion programs and science camps, learners do not typically develop deep conceptual understanding, and indeed, it can take weeks or longer for learners to reconcile preconceptions with new information and form new knowledge (Dierking *et al.*, 2003). While the content knowledge gains demonstrated by our plant phylogenetic module are statistically significant, the medium effect size suggests the practical significance of the learning gains must be considered in the context of this study and not generalized, particularly considering the low-stakes setting of an informal environment. However, we are encouraged, as this represents a promising outcome.

Item analysis revealed two question items that did not show a statistically significant increase in scores from pretest to posttest: question item 1 and item 5. Question item 1 was aimed at measuring student understanding that scoring morphological traits is subjective. Student groups were required to come to a consensus on how to score each characteristic and then to use that guideline for all plants. This practice might have transferred to question item 1 during posttest administration, with students mistakenly considering how their group scored as a collective unit rather than how each individual student or scientist scored the traits. Without the ability to probe further, however, we are uncertain what caused confusion and, consequently, no change in student mean scores. Question item 5 probed student understanding of gel electrophoresis, offering four choices, including the intended response of “passing electricity through a gel to separate molecules based on size.” However, on this recall question, more students answered incorrectly after the module. A common posttest response was b) “Identifying pieces of DNA by sequence,” suggesting a misconception was either introduced or reinforced. It may be conceptually difficult for students to understand the band they see on the agarose gel consists of a fragment of DNA several hundred bases long and that identification of sequence data are not possible at this level. However, students were presented with a sequencing technique, which does use electrophoresis to separate fragments and produce sequence data, albeit in a different manner, but the inclusion of this information may have contributed to the ambiguity in student posttest response. After review of the question and the student responses, both

answers should be considered correct and this question revised in future implementation.

Question item 2 indicated statistical significance, however, the low significance combined with detailed analysis of the student responses indicates room for improvement in the module. This question addresses the misconception students often have that similar appearance, habitat, or locomotion indicate close evolutionary relatedness (Catley *et al.*, 2012; Young *et al.*, 2013). While all students recognized molecular data as evidence of evolutionary relatedness, 45% of the students still believed that morphology and/or geographic area are good indicators of relatedness by answering d) “all of the above.” It is possible that inclusion of morphological phylogenetic trees may have reinforced the misconception of similar characteristics indicating evolutionary relatedness.

When probed during the module wrap-up, students voiced their frustration with phylogeny due to its difficulty and lack of a decisive answer; these same frustrations were also evidenced in their evaluation comments. This activity required students to use critical-thinking skills and negotiate with group members, important 21st-century skills and practices emphasized in science education reform. Additionally, it challenged the tenable nature of science that students are taught in formal schooling. These findings are consistent with Barab and Hay (2001), who reported participants in an apprenticeship program had difficulty grappling with alternative findings, because such findings presented a challenging notion to the students who “all too often view science as getting the correct answer” (p. 96).

Creating morphological trees served multiple purposes in our module. It allowed students to discuss the advantages and disadvantages of morphological and molecular trees by having a visual model to reference (Lehrer and Schauble, 2006; Schwarz *et al.*, 2009). Additionally, morphological trees scaffold (Belland, 2014) student understanding of more complex phylogenetic trees that utilize abstract molecular data. Creating morphological trees is historically rooted, and we see tree sketches utilized by Lamarck and Darwin based on physical characteristics to illustrate their thinking about species relatedness (Gregory, 2008). Stepping students through the historical approach based on morphological data can help them build their knowledge and scaffold their thinking to reason through molecular-based phylogenetic analysis (Lin *et al.*, 2010).

A larger idea that provided the impetus for the module was that different species of the same plant have evolved different pollinator syndromes such that coevolution has occurred between plants and the species that pollinate them. The students were very good at identifying likely pollinators based on plant characteristics and considered the traits of the pollinators that allowed them to be adapted for certain plants. Using applied phylogenetics, students observed the frequency of evolution of traits using the comparative method. This allows for determination of correlations between a particular trait, in this case, any flower characteristic such as color, size, or orientation, and a particular adaptation that would select for such traits, in this case, pollinators (Baum and Smith, 2013).

The majority of the students viewed the module favorably; however, there were some students who did not fully engage in the activities due to lack of interest in either the

study of plants or the techniques used. This is a challenge for a multidiscipline immersion program and was confounded by the diversity of the participants’ interests and previous experiences. Informal science settings aim to provide positive experiences for all learners. Positive science experiences encourage learners and increase their likelihood of engaging in scientific activities and retaining favorable attitudes about STEM issues (Sadler, 2009). The programmatic design of the immersion program that contained our module exposes all participants to a variety of disciplines, not just those they believe they are most interested in (i.e., medical or engineering fields). The perceptions documented in this study represent one temporal glimpse of a diverse group of high school students, and they may be short-lived. A longitudinal study is needed to determine what affective and cognitive impacts can be attributed to the plant phylogenetics module, but even then it is difficult to exclude other factors and isolate the effects of one module (Laursen *et al.*, 2007).

Modifications

This study explored the implementation of a plant phylogenetics module with high school students in an informal, situated-learning environment. We have described conditions that may have constrained larger content knowledge gains, but we feel the module has great utility in a number of settings with secondary and postsecondary learners. In the high school and undergraduate classrooms, this module can be included in the course curriculum to complement instruction in a number of topics, thereby providing more opportunities to elicit prior knowledge and correct misconceptions than we could in our brief opportunity (e.g., botany, evolution, phylogenetics, systematics, DNA techniques). The module could also be used as a summative experience to bring several concepts and techniques together. When used in a formal science classroom, the content assessment should include more questions to assess understanding of a larger range of concepts and applications. Additionally, in a formal setting, students would have more time to process new information, as the module would likely take place over a series of class meetings, allowing time for reflection and assimilation of new knowledge.

Instructors may wish to consider introducing smaller data sets (Lents *et al.*, 2010) or more divergent species (Young *et al.*, 2013) to build student understanding of phylogenetics. O’Hara’s (1997) comparison of phylogenetic trees to map reading highlights that students need to be taught how to read and make sense of the representations. Starting with small data sets, students can practice drawing trees by hand and then use a heuristic computer search to generate the most parsimonious tree with a larger set of data. In our module, flower characters such as flower size, flower shape, and whether flowers were clustered or individual had the most phylogenetic signal for the species utilized. Incorporating these characters into the morphological analyses made reconstructing the tree easier than using other characters that had much less signal. Purposeful instruction in tree thinking can guide students in building their understanding of evolutionary relatedness, and the use of phylogenetic trees as mental and physical models aids their developing knowledge. Starting with clear, well-defined examples can scaffold student learning toward deeper conceptual understanding of evolutionary theory (Lents *et al.*,

2010; Young *et al.*, 2013). Students could also compare the best morphological tree with the molecular tree and discuss similarities and differences. Additionally, students could generate a most parsimonious molecular tree and map characteristics and pollinators onto it, allowing them to see how similar traits have evolved multiple times, resulting in convergent evolution.

Although we were quite restricted in our informal setting, if time and resources allow, students could follow their plant samples from DNA extraction through sequencing and analyze their own plants' molecular data. In a course emphasizing biotechnology and molecular techniques, this is an excellent opportunity to engage students in an authentic experimental sequence that has immediate relevance, as students could develop phylogenetic trees using plants found on campus or in the community.

In an informal setting such as a university, museum, or science center, pollinator and plant species (actual specimens or images) can be displayed for students to predict co-evolved pairs. This would provide another opportunity for active participation and kinesthetic learning in which students can see physical representations of adaptations and observe and explain how species relate. For implementation in shorter time intervals, the module could also be separated into two parts, starting with the morphological characteristics and matching species. Students can then move into the molecular activities and discuss speciation events and how different pollinator syndromes have evolved multiple times.

Looking ahead to our implementation next summer, we will attend more to building student understanding of phylogenetics by scaffolding instruction first through an activity that introduces phylogenetics without any terminology or reference to evolution (Goldsmith, 2003). Other brief activities, such as introduction of organism cards, small DNA sequences, and reflection, should allow students to build their understanding of phylogenetics and its applications and to develop their tree-thinking skills. Additionally, use of previously developed assessments will facilitate assessment of student misconceptions (*i.e.*, Meir *et al.*, 2007; Sandvik, 2008; Catley *et al.*, 2013)

Implications

This study calls attention to the lack of understanding and difficulty high school students have attending to phylogenetics. It reinforces the call from scientists to include explicit instruction in the K–12 curriculum starting in elementary grades and building conceptual understanding as students progress through their formal school career. Limited inclusion of phylogenetics in both our state science standards and the NGSS tasks scientists and education researchers with finding ways and venues to assist our students in developing tree-thinking skills.

The goal of the plant phylogenetics module was to increase student awareness and understanding of botany and the construction and application of phylogenetic trees through the morphological and molecular analysis of plants and their pollinators. Exposing learners to fields underrepresented in their formal schooling environment expands the breadth and depth of their knowledge, and they are able to develop appreciation for more disciplines. Perhaps this exposure will

allow students to consider pursuing plant science in their postsecondary education and career.

Additionally, typical of informal learning environments, the larger goal is to support scientific literacy for application to the real-world context in which the learner resides. At the conclusion of 1 wk, the students were gathering in an area with lantana, one of the flowers included in the plant phylogenetics module. The program coordinator queried the students about the plants and asked them to predict the pollinator. Some students suggested it was bee pollinated while others argued for butterfly pollinators. Both groups were able to call upon knowledge gained during the plant phylogenetics module to justify their responses. Hearing the students engage in discourse common to a particular community of practice that they experienced in a situated-learning environment indicated that learning had occurred during the students' weeklong immersion program and that they were able to apply their new knowledge to their everyday life. We consider that success.

Accessing Materials

All module materials, including presentations and handouts, can be accessed at the following website: www.cpet.ufl.edu/resources/plant-phylogenetics.

ACKNOWLEDGMENTS

This project was supported from funds from NSF grant IOS-0922742, The Amborella Genome: A Reference for Plant Biology grant awarded to Pamela Soltis (advisor for J.B.L.), and 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. The authors thank Michael Chester and Margarita Hernandez for scoring student phylogenies and helping to implement the modules and for helpful comments on the manuscript. We also thank Sarah Allen, Andy Crowl, Blake Geraci, Grant Godden, Luis Mourino, Douglas Soltis, Pamela Soltis, and Milda Stanislauskas for volunteering to help with the modules.

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