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A Model of the Use of Evolutionary Trees (MUET) to Inform K-14 Biology Education

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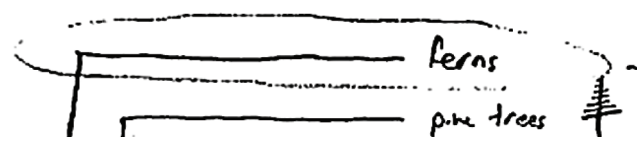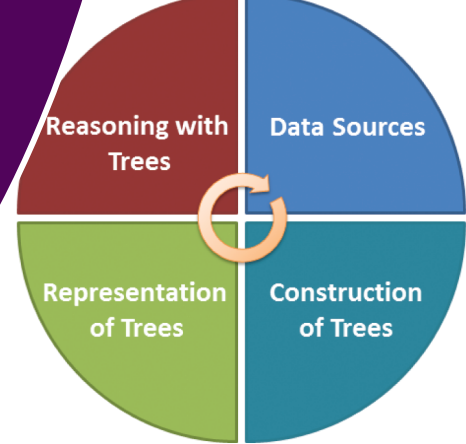
ABSTRACT

Evolutionary trees are powerful tools used in modern biological research, and commonly used in textbooks and classroom instruction. Studies have shown that K-14 students have difficulties interpreting evolutionary trees. To improve student learning about this topic, it is essential to teach them how to understand and use trees like professional biologists. Unfortunately, few currently used teaching frameworks for evolution instruction are designed for this purpose. In this study we developed the Model of the Use of Evolutionary Trees (MUET), a conceptual model that characterizes how evolutionary trees were used by professional biologists as represented in their research publications. The development of the MUET was guided by the Concept-Reasoning Mode of representation (CRM) model as well as a "model of modeling" framework. The MUET was then used to review instructional and assessment material for K-14 classrooms. Future studies with the MUET may inform the development of teaching materials for K-14 classrooms aimed at improving students' understanding of and learning about evolutionary trees.

Key Words: *evolutionary tree; model; biology research; biology; evolution.*

Introduction

In a world where biologists are increasingly called on to address global challenges, evolutionary biology must often be applied to solving food, health, and environment concerns (Carroll et al., 2014). In addition to a need for shared vocabulary and research methods, collaborations that span such disciplinary boundaries must also share an understanding of the role that representations play as important components of scientific research and knowledge creation. Larkin (1983), Dunbar (1995), Kozma et al. (2000), Novick and Catley (2014), and others have investigated how everyone from students to scientists in the fields of physics, biology, and chemistry use symbolic forms to represent or explain phenomena in a problem situation or investigation. Scientists commonly use graphs or equations to express how they perceive a phenomenon or visualize some aspect of their research. In addition, a diagram that represents objects and spatiotemporal changes is often used as a meaningful product of research. Thus a knowledge-building scientific community is structured around the common use of visual representations and tools to investigate and explain scientific concepts and principles.

In the case of evolutionary biology, tree-shaped diagrams are often generated to express understanding of evolutionary relationships among populations or species (Halverson et al., 2011; Meir et al., 2007; Novick & Catley, 2007). Evolutionary trees, also called phylogenetic trees, are used to answer different types of questions. For example, ecologists partly base conclusions about diversity on information embedded in a phylogeny. Medical researchers use evolutionary trees to investigate the origin of an emerging disease and to develop appropriate treatments for it. On the other hand, an evolutionary biologist might compare trees developed with different methods to observe similarities in the outcome, and possibly generate a third tree to reconcile any differences. Evolutionary trees chronicle the sequence of events when new heritable traits emerge (Brooks & McLennan, 1991; Kong et al., 2016; O'Hara 1988). According to O'Hara (1988), evolution explanations depend on this chronicle. Thus evolutionary trees are important representations that have wide-ranging uses and whose interpretations have far-reaching implications, allowing researchers to draw a diversity of inferences about changes over time of relevance to a particular biology subdiscipline.

Since evolutionary trees are powerful tools used in modern biological research, they are commonly presented in textbooks (Campbell & Reece, 2005; Raven et al., 2014) and used in classroom instruction. However, research has shown that

> *Evolutionary trees, also called phylogenetic trees, are used to answer different types of questions.*

students have difficulty understanding evolutionary trees, and many common misconceptions of reading evolutionary trees have been reported (e.g., Baum et al., 2005; Catley et al., 2010; Crisp & Cook, 2005; Gregory, 2008; Halverson et al., 2011; Meir et al., 2007; Omland et al., 2008). According to these reports, examples of such misconceptions are "node counting" and "incorrect mapping of time." Students with "node counting" difficulties assume that the number of nodes on the trees could be counted to indicate how close the relationship is among species on a tree. Students with difficulties to do with "incorrect mapping of time" attribute a sequence in time to the wrong direction of a tree. For example, given a tree that reads from the root at the bottom to the tips at the top, some students incorrectly assume that the oldest species are at the tips on the left and the youngest species are at the tips on the right of the tree. These and other related misconceptions are not unusual to find among students at K-14 levels (e.g., Halverson et al., 2011; Meir et al., 2007), suggesting that evolutionary trees are hard for students to understand and that improved teaching approaches are urgently required to address such problems.

Perhaps it is unrealistic to expect K-14 students to correctly read and understand evolutionary trees if they are not taught how biologists use evolutionary trees. As mentioned above, many current textbooks for K-14 classrooms introduce evolutionary trees. However, unlike textbooks for advanced undergraduate students, teachers, and scientists in the life science disciplines (e.g., Baum & Smith, 2012), few general biology textbooks for K-14 classrooms attempt to explain the structure of evolutionary trees with theoretical underpinnings (Catley & Novick, 2008). Although peer-reviewed journals have published classroom activities and laboratory exercises that are ready for K-14 teachers to use in the classroom (e.g., Baldauf, 2003; Davenport et al., 2015; Eddy et al., 2013), there is a need to establish whether these sources are sufficient to help students form an understanding of evolutionary trees. Thus, here we introduce a conceptual map on the use of evolutionary trees by professional biologists, and we show how to use this as a framework to guide decisions about instructional materials for teaching and learning of evolutionary trees in the K-14 classroom. The Model of the Use of Evolutionary Trees (MUET) characterizes professional biologists' use of evolutionary trees in scientific research.

Evolutionary trees represent the tree of life, which is often used as a metaphor, research tool, or model to explore the evolution and genealogical relationships of life (Mindell, 2013). As shown in this definition, evolutionary trees can be considered as a kind of model. Evolutionary trees and the MUET are two different kinds of models, as the term "model" means different things in different contexts (Baker, 2015). An evolutionary tree is a diagram generated to depict a hypothesis of evolutionary relationships among any level of taxonomic group: individuals, families, populations, species, genera, and higher-order taxa (Halverson et al., 2011; Meir et al., 2007; Novick & Catley, 2007; Raven et al., 2014). It provides all the information relevant to determining the degree of evolutionary relatedness among groups (Baum & Smith, 2012). MUET is a conceptual model for characterizing how professional biologists have used evolutionary trees, designed to inform K-14 teaching and learning. The development or testing of teaching materials can be informed by MUET to guide instruction on the use and interpretation of evolutionary trees. By applying MUET, teachers will be equipped to handle decisions in selecting teaching materials relevant to evolutionary trees.

The Research Questions

The guiding research question is: How can we develop the MUET to characterize components that general biologists included when they applied evolutionary trees in scientific research reported in the journal *Science* during the period of investigation, 2012 through 2013? The follow-up question is: How can the MUET be used to target science learning to instructional materials with evolutionary trees for effective teaching and learning?

Theoretical Framework: The Concept-Reasoning Mode of Representation (CRM) Model

The development of MUET was informed by the Concept-Reasoning Mode of representation (CRM) model of Schönborn and Anderson (2009). According to this model, the soundness of a person's prior knowledge of the scientific concepts (C) depicted by any given mode of representation (M) influences the soundness of their reasoning abilities (R). In addition to the soundness of their reasoning with the concepts (R-C), the nature and quality of the mode of representation (M) and reasoning with the representation (R-M) all influence how well the representation is understood and interpreted. The concepts and reasoning behind evolutionary trees as a visual representation can be approached from the framework of the CRM model to examine how biologists use tree thinking. Guided by the CRM model, the MUET should cover four key areas: (1) it should depict the basic components of the visual representation of evolutionary trees, which corresponds to the relevant representational modes (M) of the CRM model; (2) it should also cover the relevant concepts represented by the evolutionary trees, which corresponds to concepts (C) in the CRM model; (3) it should cover reasoning (R) with the concepts (R-C) and representations (R-M) to do with the application of tree thinking for the purposes of a research study; and (4) it should integrate all the factors represented in the CRM model to construct an understanding of the use of evolutionary trees for communicating scientific research.

○ Methods

The "model of modeling" framework (Justi & Gilbert, 2002) was chosen to guide the development of the MUET. This framework has four stages: (1) decide on a purpose and select appropriate research journals for the development of an initial MUET; (2) produce a mental model and create a diagram of the MUET; (3) conduct thought experiments and design empirical tests for the MUET; and (4) modify the MUET based on empirical test results to fulfill the initial purpose (Justi & Gilbert, 2002; Mendonça & Justi, 2013). Guided by this framework, four investigators participated in the data collection and data analysis. The investigators included: a doctoral candidate in biology education with a background in molecular biology research experience applied to the construction of evolutionary trees representing genetic diversity of *Pelteobagrus fulvidraco* populations in the Yangtze River (Kong et al., 2009); a doctoral candidate with research experience in regenerative medicine and in molecular biology, biotechnology, and biophysics (Nasir et al., 2010); a doctoral student in biology whose

undergraduate focus was in evolution, ecology, and conservation; and a biology professor who teaches evolutionary biology. Three of the investigators have interpreted tree results as part of their biological research. Furthermore, all of the investigators have been reading and interpreting evolutionary trees to aid in scientific understanding with students at various educational levels: two taught courses where preservice elementary school teachers learned to construct trees from taxon-character data; two worked on evolutionary tree thinking with teachers at the middle school and high school levels; three taught students to construct, use, and read evolutionary trees for scientific content understanding at the undergraduate level; one taught a graduate student research seminar course where articles with evolutionary trees were critiqued. However, because only one of the investigators had experience with using tree results for their own biological research application, four external experts were consulted, as detailed below in the "Data Analysis" section.

Data Collection

Since a full spectrum of biology subfields can be viewed through the lens of evolution, we considered it important to base the development of the MUET on research reports by biologists in all biology disciplines, not just from evolutionary biologists. This would give us a broader understanding of reasons for using evolutionary trees and render the MUET more representative of the full range of phylogenetic tree usage by biologists. In an attempt to identify the most appropriate source of such reports, we conducted a pilot study with various "high-impact journals" (those considered to be highly influential with biologists), and found that the journal *Science* has many more figures with evolutionary trees than other high-impact journals. Thus the journal *Science* was selected for this study.

Data was collected from issues published in 2012 and 2013 by identifying articles with evolutionary tree figures in two rounds. In the first round, two investigators worked independently to collect articles with tree-shaped diagrams published in issues of the journal *Science* between January 6, 2012, and August 9, 2013. The investigators then worked independently to identify evolutionary trees from these tree-shaped diagrams. In so doing, as per the criteria recommended by Kong et al. (2016), they excluded any tree-shaped diagrams that did not depict evolutionary history. Finally, the two investigators were joined by a third investigator, and together they reached 100 percent agreement about the selection of evolutionary tree articles for our study. In the second round, articles published in the journal *Science* in the rest of 2013 were collected by one investigator based on the exclusion criterion. Overall, articles collected from these two rounds are data for this study (Table 1).

Data Analysis

Guided by the "model of modeling" framework (Justi & Gilbert, 2002), the MUET was developed and revised several times until all investigators unanimously agreed that it was applicable to all the articles collected in the first round. Thereafter, a content analysis (Cole, 1988; Krippendorff, 1980) was performed to obtain the frequency of each component of the MUET. During the development of the MUET, the process was enhanced by accessing relevant information from a wide range of literature resources and through consulting experts in the field. For example, and more specifically, we developed a Glossary (see Supplemental Material), and adopted the Felsenstein (2004) introduction to tree formats, and Hershkovitz and Leipe's (1998) and Delsuc et al.'s (2005) introductions to the methods of constructing trees. We also consulted with two evolutionary biology experts in systematics who are full professors and two graduate students who were doing evolutionary biology research. Based on all this feedback, we revised the MUET and then applied it to descriptions of trees that had been found in the first and second rounds of the data collection. To test the Inter-Rater Reliability (IRR) (Tinsley & Weiss, 2000) of one investigator's coding result, another investigator randomly selected and coded ten articles using the MUET. Thereafter these two investigators' coding results were used to calculate the Cohen's Kappa, an IRR statistic index, by applying SPSS Version 22 (IBM, 2013). As reported in Table 2, the IRR statistic index of each component of the MUET reached 80 percent, which means that the investigators' coding results were reliable.

Validation of the MUET

The MUET was validated in three ways. First, although the components of the MUET were developed based on already published literature and by consulting experts in phylogenetics as described above, to further test its validity, we examined each component of the MUET for consistency with the tenets of the widely recognized textbook *Tree Thinking: An Introduction to Phylogenetic Biology* (Baum & Smith, 2012). Second, since the MUET was developed using the trees that were found in the first round of data collection, to further

Table 2. Inter-Rater Reliability (IRR) for each component of the MUET.

The MUET Component	Inter-Rater Reliability (IRR)
Representation of trees	100.0%
Data sources	88.6%
Construction of trees	100.0%
Reasoning represented by trees	82.0%

Table 1. Number of articles and tree figures collected from the journal *Science*.

Round	Period	Issues	Issues with Tree Articles	Articles with Trees	Tree Figures
First	01/06/2012–08/09/2013	83	47	72	107
Second	08/16/2013–12/20/2013	19	6	11	22
Total		102	53	83	129

Table 3. Description of trees in the journal _Science_ using the MUET.

| MUET | Subcategory | Tree Figures | | Total |
| | | 1/2012–8/2013 | 8/2013–12/2013 | 2012–2013 |
		n = 107	n = 22	N = 129
Representation of trees	Rooted tree	50	17	67
	Unrooted tree	57	5	62
Data sources	Molecules	91	15	106
	Morphological traits	11	3	14
	Developmental patterns	4	0	4
	Behavior	1	0	1
Construction of trees	Distance-based methods	23	1	24
	Maximum parsimony	14	1	15
	Maximum likelihood	37	13	50
Reasoning with trees	Cladistics	62	18	80
	Homology	107	22	129
	Homoplasy	35	3	38
	Chronology	50	17	67

validate the model, we applied it to the second round of data collected. As shown in Table 3, the MUET was successfully applied to the rest of the trees, which suggested that the data behind MUET was saturated in that MUET shows how all of the evolutionary trees were represented and used by biologists, who reported research with trees in the journal _Science_ from the period of investigation, 2012 through 2013. Third, an evolutionary tree selected from an issue of the journal _Science_ was used to describe how the MUET is applied. As shown below in the section "Validation of the MUET . . . ," the MUET successfully described an evolutionary tree that was published in a 2013 issue of the journal _Science_.

○ Findings

MUET Components from Evolutionary Trees in Research Reported from 2012 to 2013 in the Journal _Science_

As shown in Figure 1, the MUET is a conceptual model that depicts the basic components included by professional biologists when they used evolutionary trees to report their research in a _Science_ journal publication. The MUET consists of four categories and includes arrows to represent how the four components are used in sequence when biologists deploy evolutionary trees in their research. In the MUET, "Data Sources" refer to various types of traits (e.g., morphological traits) collected from organisms that were used to construct trees. Following the direction of the arrow, "Construction of Trees" refers to various methods of analysis (e.g., maximum parsimony) used by biologists to construct trees. "Representation of Trees" refers to the basic components of evolutionary trees (e.g., branches) as

depicted in research articles. "Reasoning with Trees" refers to four basic types of reasons (e.g., homology) for including tree figures as revealed or expressed by professional biologists' use of evolutionary trees. The four types of reasoning are defined and explained in Table 4. Logical connections between the concepts of data collection (C of CRM), the analysis and interpretation of data (R or reasoning with data), and the tree mode of representing the information (M of CRM) are related to the ultimate interpretation of the diagram (RM of CRM, meaning how and why scientists reason about a particular research problem with an evolutionary tree as their mode of representation). This process tends to be iterative, with inferences based on tree diagrams feeding back into gathering additional data to address further biological research questions. In practice this can be a fully circular, continuous process.

Validation of the MUET with an Evolutionary Tree from a 2013 Research Article

To illustrate the MUET, we apply the MUET components to the example shown in Figure 2. Guided by the MUET, Figure 2 can be understood by the following four aspects:

Data Sources. The tree is constructed based on various 16S rRNA gene sequences. These data sources were compared for the construction of the tree-shaped part of this diagram. These 16S rRNA gene sequences were obtained from the bacterial strain taxa labeled in the tree.

Construction of Trees. Since the authors of Figure 2 did not detail any methods used to construct their tree, the corresponding author of the article was contacted. The response stated that a neighbor-joining (NJ) method as a distance-based method was applied to the data to construct this tree.
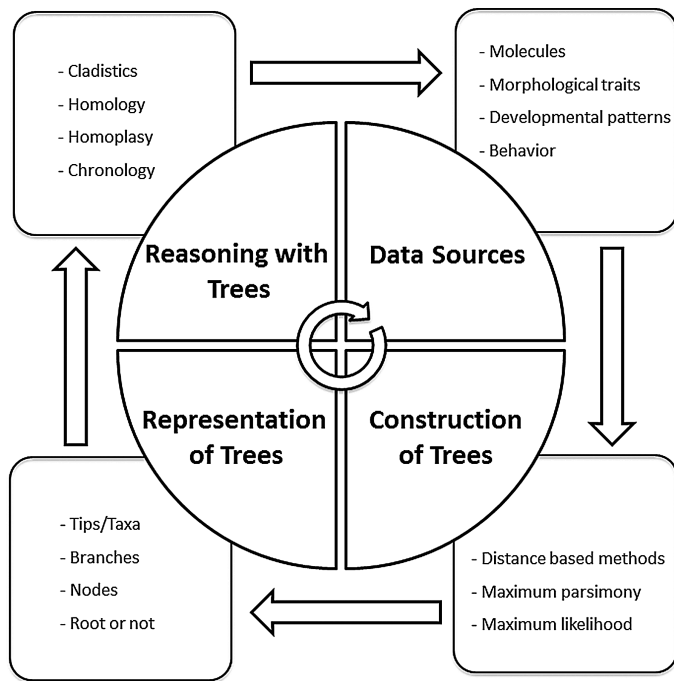
Figure 1. A Model of the Use of Evolutionary Trees (MUET). The MUET consists of four components. Arrows on the model highlight the fully cyclical nature of the research process by representing how the four components are related to each other for the biologist who reports research with an evolutionary tree figure. "Reasoning with trees" refers to the reason why an evolutionary tree was used to report research findings. Such reasoning often raises questions that could be answered with additional data. "Data Sources" refers to data that were selected to construct the tree. "Construction of Trees" refers to the methods used for constructing the tree. "Representation of Trees" refers to the depiction of components and modes of representing evolutionary tree figures in research articles.

Representation of Trees. As mentioned above and in the Figure 2 caption, the entities portrayed at the tips of the tree in Figure 2 are various species of bacteria. This particular example is a rooted bifurcating tree with the names of bacteria species displayed in text at the end of each branch. As detailed in the figure legend, this tree provides additional information with different colored fonts as well as different background colors for groups of organisms on the tree.

Reasoning with Trees. Reasons for including the tree depicted in Figure 2 include cladistics, homology, homoplasy, and chronology. Cladistics is shown in that this tree details taxonomic diversity and evolutionary relatedness between different species. Each branch is a clade. Homology resulting from common ancestry is shown because the sharing of 16S rRNA gene sequences was the basis for clustering the species on branches of the tree. Homoplasy is shown because species share similar features that are inconsistent with the branching pattern of the tree. As described by the authors, the outer ring, the middle ring, and the inner ring display different features that in many cases are shared among organisms that are not on the same branch of the tree, thus indicating homoplasy. Figure 2 is a rooted tree with the root indicated at the middle and bottom of the tree. The direction from the root to the tips of the tree shows the chronology of evolutionary processes that produced diversification, resulting in many more new species of bacteria from the earliest ancestor compared to now.

MUET as a Framework to Improve Teaching about Evolutionary Trees

The *Framework for K-12 Science Education* (NRC, 2012) suggests that K-12 life science students cannot fully understand scientific ideas without engaging in the scientific practices by which such ideas are developed and refined (Bybee, 2013). According to recommendations, such practices must be demonstrated in the context of specific content (NRC, 2012). The MUET is tightly aligned with the dimension of science practices for K-12 science classrooms. For example, by "Data Sources," we mean the character traits listed in Figure 1. Students must decide which traits they will investigate when defining their research question or when planning and obtaining their data

Table 4. Definition and explanation of each type of reasoning with trees listed in Figure 1.

Reasoning	Definition	Explanation
Cladistics	Cladistics is an approach commonly used in biology to make assumptions about the evolutionary history of organisms. In detail, cladistics uses patterns of synapomorphies (i.e., traits of a common ancestor that were inherited and are shared by a group of taxa) (Novick et al., 2010) to infer the order of lineage divergence of organisms in evolutionary history. The principle of parsimony is typically adopted when biologists use cladistics (Goldsmith, 2003; Henning, 1966). For teaching in K-14 classroom, cladistics, as a type of reasoning with trees, is simply defined as an approach used to classify organisms based on whether the organisms share common characteristics inherited from their most recent common ancestor.	If an evolutionary tree is constructed by using synapomorphies to show the lineage divergences of organisms, it means cladistics was used to construct that evolutionary tree. An evolutionary tree which was constructed without using synapomorphies to show the lineage divergences of its entities on the tree is recognized as not based on cladistic reasons.

Table 4. Continued

| Reasoning	Definition	Explanation
Homology	In biology, homology refers to any of a group of organisms' traits that were derived from a similar trait in an ancestor they shared (Hall, 2012). These traits are called synapomorphies, which can be morphological, molecular, or behavioral characteristics shared by a group of taxa as a result of their inheritance from a common ancestor (Novick et al., 2010). Homology involves reasoning that is focused on the historical nature of homologs to understand biological phenomena (Ereshefsky, 2012).	All the trees showed homology because the organisms that shared similar traits derived from a common ancestor were clustered together on a branch of the tree.
Homoplasy	In biology, homoplasy refers to the presence of any similar traits that are shared by different organisms but that were not derived from the similar trait in a common ancestor.	When two entities on an evolutionary tree show similar traits that came from different lineages, the tree reveals homoplasy. Convergent evolution explains why homoplasy occurs.
Chronology	Chronology refers to the science of ordering events by occurrence in time.	Rooted trees show chronology because the roots represent the most distant ancestor in time. Unrooted trees do not show complete chronology, or the chronology is unclear because the most distant ancestor in time has not been indicated (Kong et al., 2016).
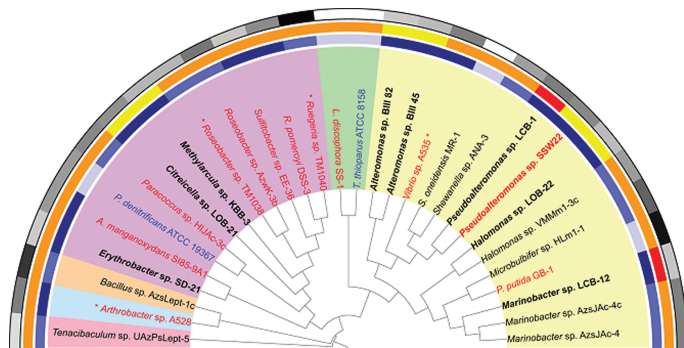
Figure 2. Example to illustrate how MUET was tested. This figure shows superoxide production across a broad phylogenetic and ecological diversity of bacteria. The background colors of the entities represent that these entities belong to the *Bacteroidetes* (pink), *Actinobacteria* (blue), *Firmicutes* (orange), *Alphaproteobacteria* (purple), *Betaproteobacteria* (green), and *Gammaproteobacteria* (yellow). The font colors of the entities represent that the organisms were isolated from benthic (black), planktonic (red), and soil (blue) habitats. Moreover, this tree provides additional information displayed as rings around the tree. As described by the authors of this tree, the outer ring represents the value of relative superoxide production, where white represents values that were below detection, and black represents values that were the observed highest rate. The middle ring shows the organisms that are psychrophiles (yellow), mesophiles (orange), and thermophiles (red). The inner ring shows the habitats of the entities that are freshwater (light blue), estuarine (medium blue), and marine (dark blue). Source: Diaz et al. (2013), used with permission.

(Practices 1, 3, and 8 of the *Framework*). By "Representation of Trees," we refer to the symbolic features listed in Figure 1. The nodes and branches and the arrangement of tips in a tree must be understood when "developing and using" a tree model, "analyzing and interpreting data," or "evaluating and communicating information" with a tree (Practices 2, 3, and 8 of the *Framework*). Furthermore, by "Reasoning with Trees," we refer to how and why one should reason about research with an evolutionary tree. Nearly all "Practices of Science and Engineering" are involved when considering how and why to reason about research data with an evolutionary tree. In fact, some might argue that "Reasoning with Trees" would even correspond to Practice 1 if the teacher prompts students to be "asking questions" that could be answered with additional data (as suggested by the circular arrow in Figure 1). Clearly, the MUET components might help a teacher and students understand how the research process relates to the evolution concepts and evolutionary tree representations in their lessons.

When preparing instructional materials for teaching about evolutionary trees, using the MUET as a lens might help teachers more easily clarify the learning goals of their classrooms. For example, data sources provide a foundation for construction of trees. Representation of trees is the result of tree construction, and both of these should be understood for students to more fully understand the reasoning behind professional biologists' use of evolutionary trees. Thus, the MUET also provides criteria to check whether necessary components are present in instructional resources and assessments. For example, in *College Board AP ® Biology Lab Manual*, "Investigation 3: Comparing DNA Sequences to Understand Evolutionary Relationships with BLAST" (College Board, 2012) is aligned with the MUET. This activity has students using DNA sequences as data sources to construct trees by using a Basic Local Alignment Search Tool (BLAST). The students construct a tree by

Table 5. Evaluation of instructional materials and assessments according to the Model for the Use of Evolutionary Trees (MUET).

Evolution Education Material	Data Sources	Construction of Trees	Representation of Trees	Reasoning with Trees
Instructional Materials Tested with Undergraduate College Students				
Phylogenetics Laboratory: Reconstructing Evolutionary History (Catley & Novick, 2012)	Data sources mentioned in Parts I and II include molecular data (HOX gene sequences) and morphological traits (developmental patterns and body structures such as type of skeleton).	Students are taught to use cluster analysis of molecular or morphological data to construct a tree.	Students are instructed with an example to draw rooted bifurcated trees with nodes and the origin of traits indicated along the branches of the tree. No examples of unrooted trees are provided.	Parts IV–VI have students comparing trees based on clustering of molecular or morphological data, to find evidence for homology and homoplasy and to trace the origin of traits on the tree.
Phylogeny Assessment Tool (PhAT) (Smith et al., 2013)	In the PhAT, data sources are morphological traits of organisms such as large canine teeth.	In Part B of the PhAT, students were expected to use the parsimony principle to decide which tree is a better hypothesis for related organisms in the PhAT.	The PhAT shows two rooted bifurcated trees with traits marked along the branches of the trees. No examples of unrooted trees are provided.	Based on morphological data, the PhAT assesses students' ability to consider both gain and loss of traits in chronology for reasoning about homology and homoplasy.
The Great Clade Race (Goldsmith, 2003)	Traits are represented using various shapes (e.g., circles, squares, diamonds) without specifying if these are molecular, cell, tissue, or morphological traits of organisms.	Cladistics is introduced as a method to examine patterns of shared derived characters to infer a sequence of divergence within groups of organisms. Maximum parsimony is introduced to guide cladistic analysis.	Two different correct answers with the same branching order are given as rooted bifurcated trees with traits marked along branches of the tree. No unrooted tree is given as an answer for this exercise.	Cladistics, homoplasy, and homology are introduced in this paper.
Instructional Materials Tested with K-12 Level Students				
The Lizard Evolution Biointeractive Virtual Lab (HHMI, 2015)	Data sources include morphological data, which are the lengths of the lizards' bodies, hindlimbs, and tails (Module 1), and molecular data, which are mitochondrial NADH dehydrogenase subunit 2 (ND2) DNA gene sequences and five transfer RNA (tRNA) sequences from different populations (Module 2).	Module 1 introduces cluster analysis based on animal traits such as appearance and morphology. In Module 2, cluster analysis of eight anole species is done by DNA comparisons using MABL (Dereeper et al., 2008).	An unrooted bifurcated evolutionary tree was constructed to depict eight anole species. A distantly related species was introduced to "root" the tree with nodes, branches, and the living species at the tip. Students are able to omit the outgroup to re-root the tree with the midpoint or examine an unrooted tree for the eight anole species.	To compare the rooted or unrooted evolutionary trees generated by students with published rooted and unrooted trees provided in worksheets, the students must reason about chronology, trace the origin of traits, cladistics, homology, and homoplasy (convergent evolution).

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following simple step-by-step guidelines. However, to fully understand evolutionary trees, students need to interpret what the tree means after the tree is constructed, as well as understanding the basic principles embedded in the use of BLAST. For example, in a tree constructed by students, what do the tips and nodes represent? Does the tree show chronology? The example procedure also requires students to consider what their findings mean in terms of the evolution of a gene they might find in several different kinds of organisms they would test. According to MUET, a teacher might also prompt students to explain the chronology for changes in a gene by ordering events in the history of life on Earth, or for a particular trait, the teacher might prompt students to explain what additional data might help distinguish homology (traits derived from the similar trait in a common ancestor) from homoplasy (similar traits of organisms that are not derived from a common ancestor, see Table 4).

Thus, by systematically checking each component of the MUET, teachers can guide and improve the implementation of instructional activities in their classroom. Besides the AP biology lab manual activity mentioned above, Table 5 lists additional examples of instructional materials selected for high school and the undergraduate college levels. According to this analysis, an undergraduate instructor who implements *The Great Clade Race* (Goldsmith, 2003) may opt to assign an activity from *The Lizard Evolution Biointeractive Virtual Lab* (HHMI, 2015) to give students experience with various data sources and with interpreting trees that are rooted or not. In our view, using the MUET and its components to analyze these or other instructional materials related to evolutionary trees (e.g., Collins et al., 2006; Knuffke & Mainhart, 2013; Young et al., 2013) will better equip instructors to make more informed decisions about how to advance their students' understanding of evolutionary trees and their various applications in biology.

Implications for Teaching

In summary, by developing the MUET to characterize biologists' use of evolutionary trees in their scientific publications, we provide K-14 teachers and students with a useful model and a sound basis for understanding how evolutionary trees are used to advance scientific research. The MUET is closely aligned with scientific practices in the *K-12 Science Education Framework* (NRC, 2012) as well as with resources for teaching tree thinking. Therefore K-14 educators and students are encouraged to use MUET as a simple guide to promote expert-level tree thinking abilities in the classroom. Furthermore, our review of instructional standards and educational resources provides benchmarks for understanding the gaps and positive effects of each source. Where there are gaps, the referenced materials could be modified or additional resources could be adapted as supplements. Identifying limitations in the coverage of instruction is vital to the success of evolution education, to ensure that each student has the chance to receive a more complete education in the vital area of biological evolution.

Limitations of this Study and Directions for Future Studies

The MUET shows basic components used by some general biologists when reporting research with evolutionary tree figures. But since we only examined the journal *Science*, the MUET might not accurately

represent how more specialized evolutionary biologists use trees. It is likely that the concepts, data, and tree thinking visualizations are reported differently in journals like *Trends in Ecology & Evolution, Molecular Biology and Evolution*, and *Methods in Ecology and Evolution*, which target evolutionary biology experts. Thus, future research studies are needed to explore potential differences in research reports with evolutionary tree figures published by evolutionary biologists, compared with the figures designed to communicate with a general science audience. Also, the components of MUET correspond to tree figures published in 2012–2013. A future study might track how components in evolutionary tree figures have changed from earlier to more recent times. Thus our research methods hold promise for advancing biology education to keep pace with rapid changes in biology as a research science.

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