“I think”: integrating project-based learning and case study to teach fundamentals of evolutionary tree-thinking

Thomas J. Firneno Jr[1*], Gabrielle T. Welsh[1], Peter J. Nimlos[1], and Erica L. Larson[1*]

[1] *Department of Biological Science, University of Denver, Denver, Colorado, 80208, USA*

* *corresponding authors:* thomas.firneno@du.edu and erica.larson@du.edu

ORCID ID: 0000-0002-4975-2794 (TJF)
ORCID ID: 0000-0002-8492-2509 (GTW)
ORCID ID: 0000-0002-5726-3325 (PJN)
ORCID ID: 0000-0003-3006-645X (ELL)

Abstract

The theory and practice of evolutionary tree-thinking is pervasive through many scientific fields and is a critical component of biological literacy. Many elements of tree-thinking are introduced early in undergraduate biology education. However, basic concepts are often not revisited/reinforced and are assumed to have been fully conceptually grasped in upper-level courses and beyond. Here, we present a project-based activity that we developed to aid upper-level biology students to learn, conceptualize, and practice tree-thinking. This approach allows them to identify the misconceptions that they may have about tree-thinking, while reinforcing the theories and concepts that they may have encountered in introductory courses. It also integrates several pedagogical styles (instructor-led and student-centered), along with an organismal case study to make concepts concrete and realistic to students.

Key words: phylogeny, systematics, active learning, field crickets

Introduction

Background

The central concept of evolutionary biology is that the diversity of organisms on earth is descended from common ancestors, and that we are all connected like tips of branches on a single tree of life (Avise 2006; Baum and Smith 2013; Darwin 1859). Evolutionary relationships, both within or between groups of organisms, are depicted in evolutionary trees (i.e. phylogenetic trees). Being able to build, visualize, and interpret evolutionary trees is crucial for developing an accurate understanding of evolution, organizing biological diversity, and to effectively investigate and communicate evolutionary phenomena. This practice is known as "tree-thinking" and is a critical component of biological literacy (Baum and Offner 2008; Baum and Smith 2013; Gregory 2008; Halverson 2011; Novick and Catley 2016; Novick and Catley 2013; Sandvik 2008). Tree-thinking is an important component of many undergraduate courses including introductory biology, evolution, and organismal-based courses, as well as extending into other fields such as medicine, forensics, and anthropology (Baum and Offner 2008). Undergraduates are typically introduced to concepts of tree-thinking (e.g., terminology and tree topology, parsimony, trait evolution, relatedness) in introductory courses and it is often assumed in upper-level courses that this level of instruction is sufficient for students to interpret evolutionary trees in texts and primary literature. However, research has shown that even advanced biology undergraduates still struggle with a range of misconceptions about the building and interpretation of evolutionary trees (highlighted in Table 1; (Baum et al. 2005; Baum and Offner 2008; Blacquiere et al. 2020; Dees et al. 2014; Dees and Momsen 2016; Gregory 2008; Halverson 2011; Lents et al. 2010; Meir et al. 2007; Meisel 2010; Novick and Catley 2013; Sandvik 2008).

In our experience, these misunderstandings stem from several factors including: (1) significant lengths of time between being introduced to concepts of tree-thinking and revisiting or applying concepts; (2) commonly misconstrued concepts are not explained thoroughly or clearly when introducing tree-thinking; and/or (3) a lack of any application (e.g. tree building practice/interpretation) to reinforce and allow students to fully grasp abstract concepts of tree-thinking. Misconceptions of tree-thinking can hinder deeper understanding of evolutionary theory and have an effect on students' acceptance of the evidence and scientific validity of evolutionary theory (Gibson and Hoefnagels 2015; Gregory 2008; Meisel 2010). Moreover, misconceptions formed early in students' college careers can impact their success in upper-level courses, often leading students to leave biology majors (Chen X 2013; Cherif A, Adams G, Movahedzadeh F, Martyn MA, Dunning J. 2014; Ingram and Nelson 2006; McKeachie et al. 2002). Therefore, even slight misconceptions in either introductory or upper-level courses could have consequences that drive students to leave the discipline (Heddy and Nadelson 2013; Mead et al. 2015).

Abstract concepts like tree-thinking can be made more intuitive and concrete with the use of hands-on pedagogical techniques (Brewer and Zabinski 1999). There is considerable evidence that hands-on activities are more effective than traditional lectures because (1) students become active participants in their own learning (Alters and Nelson 2002; Freeman et al. 2014; Gardner and Belland

2012; Hake 1998; Nelson 2008; Smith et al. 2005) and (2) hands-on activities tend to engage students from historically excluded groups in STEM (Ballen 2020; Ballen et al. 2017; Estrada et al. 2016; Haak et al. 2011; Theobald et al. 2020). Furthermore, several case studies have shown that tree-building exercises improve students' abilities to build, read, and interpret phylogenies, and more broadly, think about evolution (Eddy et al. 2013). Here, we present a project-based activity for teaching and reinforcing evolutionary tree building and interpretation. Project-based learning often combines various pedagogical strategies that use a project(s) as a central component. These projects extend over a period of time and vary in complexity, actively engaging students in autonomous work and problem solving, and stimulating critical thinking and decision making (Bell 2010; Berchiolli et al. 2018; Blumenfeld et al. 1991; Guo et al. 2020; Larmer et al. 2015). The other major component of project-based learning is instructor feedback at pivotal times during the exercise(s) to aid students in reflection and recalibration, which ultimately facilitates deep-learning and comprehension of the major concepts being covered.

The project-based activity that we present spans six-hours across three class periods and integrates a traditional instructor-lecture to introduce the concepts of tree-thinking and tree building, student-centered instruction to carry out the activities within the exercise, and a case study to bring abstract concepts into a novel, real-world framework. Students are asked to work in small groups to encourage collaboration and discussion throughout the process, which has been shown to be beneficial and effective in making theory more tangible to students (Allen and Tanner 2005; Buckberry and Burke da Silva 2012; Freeman et al. 2014; Prince 2004). Our project-based activity builds on prior work that provides frameworks for teaching tree-thinking (Baum et al. 2005; Baum and Offner 2008; Meisel 2010) and tree building (Burks and Boles 2007; David 2018; McCullough et al. 2020; Sokal 1983a; Sokal 1983b; Sokal 1983c; Sokal 1983d). Additionally, our project fulfills several recommendations for best practices in teaching phylogeny (and evolution, in general) more effectively by making broad use of active learning, directly addressing student misconceptions, incorporating multimodal instruction, and introducing opportunities for communication and collaboration (AAAS 2011; Nelson 2008; NGSS Lead States 2013). We designed this project to lay foundational concepts of tree-thinking, and then reinforce these concepts through hands-on activities and an engaging case study (herein defined as a teaching tool to show the application of theory and concepts to real life, biological examples/situations).

Below, we outline our learning goals and objectives for this exercise to assist instructors in designing comprehensive and meaningful assessment questions. Learning goals are broad and achievable, though not always measurable, statements of what the exercise is intended to accomplish. Whereas, learning objectives describe specific and measurable learning outcomes that can be assessed by the end of the exercise.

Learning Goals

1. Build a foundation in evolutionary tree-thinking including building, interpreting, and evaluating evolutionary trees.
2. Graphically and verbally represent evolutionary tree-thinking.
3. Build quantitative skills used to create and evaluate phylogenies.

Learning Objectives

1. Build morphological character matrixes and corresponding phylogenies.

2. Use a real-world case study to construct morphological and genetic character matrixes, along with corresponding phylogenies.
3. Discuss how taxa differ and are related morphologically and genetically.
4. Build and evaluate phylogenies by implementing basic bioinformatics tools.
5. Apply what they have learned to deeper discussions of tree-thinking, tree building, and evolution broadly.

Significance of Field Crickets as a Case Study

To allow students to connect abstract concepts of tree-thinking and tree building, we integrated a case study using field crickets (Order: Orthoptera; Family Gryllidae, Subfamily Gryllinae) . Field crickets can be found throughout the world and are familiar occupants of backyards in rural, suburban and urban environments (Byerly et al. in press). Their songs, which males use to attract and court females, can be heard during warm seasons and they are ubiquitous, though often unnoticed organisms. This makes field crickets ideal to introduce students to “hidden” biodiversity. Moreover, cricket species are fairly cryptic - for example many field crickets were considered a single species as recently as the 1950s (Alexander 1957) and new species have even been described in recent years (Gray et al. 2020; Weissman and Gray 2019). This makes field crickets an excellent, yet challenging study system for students to explore the scientific literature, identify key traits that can distinguish species, and compare different types of evidence for evolutionary relationships (morphology versus molecular). We introduced field crickets in an introductory presentation (see Instructional Strategy: Day 1 - Additional File 3) by covering some of their general characteristics (e.g. life history, natural history) and the importance of the group taxonomically (e.g. hybridization, poorly understood evolutionary relationships of some species).

Intended Audience

We developed this project in a small (< 20 student), upper-level (2nd-4th year) undergraduate evolution course. However, it is flexible enough to be used in advanced high school biology classes, undergraduate introductory courses for both majors and non-majors, upper-level classes for students majoring in ecology or evolution, and/or undergraduate laboratories. Based on ideal group sizes for project-based learning and manageability of work for instructors, we believe that it can be effectively used in various class sizes ranging from small (15 students) to medium (≤ 50) (Bilgin et al. 2015; Chen and Yang 2019; Kanter and Konstantopoulos 2010; Kooloos et al. 2011; Mahasneh et al. 2018). We have personally observed that this project can successfully engage students and reinforce conceptual knowledge in exploring and implementing evolutionary tree building techniques.

Instructional Strategy

In this paper, we present this project-based activity as we have used it. The activities are intended to span six hours across three class periods, with about two weeks between each class/session (general timeline included in Figure 1). However, we have included extensions and variations for adapting it to specific classes depending on the timeframe, comprehension and educational level, and class size. We have not used all of the variations that we suggest. We simply present what has worked for us and what we believe will be the most effective modifications or extensions based on education literature and our own experiences. We encourage instructors to adjust this project as necessary for their teaching style, size of class, and availability of time and materials. Here, we describe each part of the project-based activity in further detail.

Before Class

Before beginning the project-based activity, the instructor(s) should familiarize themselves with the material, teaching strategy, and learning goals and objectives, making modifications for their class level and size. We also suggest, if time allows, that instructors conduct an evaluation prior to the introductory lecture to determine what topics of tree-thinking students could benefit from focusing on or highlighting (see #1 in Extensions of Activities section). If needed, instructor(s) can modify the provided worksheets (Additional File 1) or the example introductory lecture (Additional File 2) to cover specific parts of phylogeny that students find challenging. Instructor(s) should also tell students in advance to bring laptops to subsequent sessions and to download both MESQUITE (Maddison 2021) and MEGA (Kumar et al. 2008; Tamura et al. 2021) or provide them with the necessary tools for the exercise (e.g. using a computer lab with pre-downloaded software). The students should familiarize themselves with the basic concepts of tree-thinking and tree building via their textbook, instructional videos, or supplemental text.

During Session 1

Prior to the beginning the project-based activity, we presented a lecture (~ 40-50 minutes) that discussed the basic concepts of tree-thinking (e.g., what evolutionary trees are, how to interpret them, character states, homology), while also addressing the misconceptions that students may have coming into the class. This allowed us to mitigate/alleviate these misconceptions prior to the actual activity. We have included an example lecture that we used as Additional File 2. The lecture can be presented in a prior class, or the same day as the following activity - depending on the length of the class.

We then split the class into small groups (3-5 students each), which could be done by the students themselves or randomly by the instructor. We gave students the accompanying In-Class and Out-of-Class Activity 1 (Additional File 1) either in hard copy or as a PDF through Canvas. We presented the first activity ("trait-to-tree") using hypothetical organisms (In-Class Activity 1 – see Additional File 3). Here, we also discussed and walked through how to choose binary character traits and how to build a character matrix from these traits in the context of their hypothetical organisms. We then allowed the student groups 15-20 minutes to choose their traits and create their matrices. After this, we reviewed a brief example of building a phylogeny from a character matrix and then allowed the students another 15-20 minutes to create their phylogenies from their character matrixes. An example of a student's matrix and tree are provided in Figure 2.

After the completion of In-Class Activity 1, we introduced the field cricket case study and Out-of-Class Activity 1 (see Additional File 3). Students were asked to think about a set of synthesis questions from the In-Class Activity 1. These questions were designed to enable students to make connections across concepts that were covered in the pre-activity lecture and the tree-building In-Class Activity 1.

Out-of-Class Activity

The out-of-class activity and case study were designed to reinforce the skills that students learned during the first class. It was intended to be challenging to (1) show students that tree building and tree-thinking is not always easy, (2) allow students to analyze complex tree-thinking problems, (3) engage students in the natural history of a particular group of organisms, and (4) give students the opportunity to practice searching primary literature and online sources. Students were asked once again to decide on characters (this time they did not have to be binary), code the characters into a matrix, and then follow a tutorial (Additional File 4) to use the program MESQUITE to build their phylogenies. We encouraged students to find characters within the primary literature (Gray et al. 2020; Weissman and Gray 2019; specifically, Weissman et al. 1980) and other potential sources (Orthoptera Species File, http://orthoptera.speciesfile.org/; or Singing Insects of North America, https://orthsoc.org/sina/). When necessary, we provided students with a character bank (Additional File 5). Students were also encouraged to work with their groups on this activity outside of class. An example of a student's matrix and tree are included in Figure 3a.

During Session 2

For the first quarter of the second session (for us, this was 25-30 minutes out of a 2-hour session) we discussed the synthesis questions of In-Class Activity 1 and reviewed the student groups' trees from Out-of-Class Activity 1. Again, this was used to answer any questions or issues about the out-of-class activity, reinforce concepts presented in the first session, and mitigate any misconceptions that students had about the material/activities.

Next, we presented the continuation of the field cricket case study that introduced the use of molecular data to create phylogenies (In-Class Activity 2 – see Additional File 1). This portion of the exercise used a mixture of instructor-guided and student-centered independent work to familiarize students with common tools for mining genetic data from repositories and creating and interpreting phylogenies and diversity metrics from genetic data. We presented a very brief introduction (no more than 15-20 minutes) i to familiarize students with the basic concepts covered in this activity (e.g., GenBank, BLAST, DNA barcoding, sequence alignment, pairwise genetic distance, neighbor joining and other tree building methods – see Additional File 6).

First, we gave student groups the GenBank accession number of one of their ingroup field cricket species, which they used to find and download the 16S sequence of that cricket through the GenBank plugin in MEGA. Students then used the BLAST (Altschul et al. 1990) plugin in MEGA to BLAST against their downloaded 16S sequence and see if they were able to find their other ingroup taxa provided on the activity sheet. The resulting sequences of their BLAST queries that match their respective taxa in the activity sheet were then downloaded into their 16S alignment in MEGA. The students ended up with an alignment file in MEGA of eight field cricket taxa . From their initial alignment, students then used the CLUSTALW (Thompson et al. 1994) function to align their actual data. Once their data were aligned, we instructed students on how to export their alignment as a .meg file that could then be used to analyze the alignment. Students used their newly imported alignment data to (1) assess conserved versus variable sites in their alignment, (2) create a pairwise genetic distance matrix and (3) a neighbor joining tree using MEGA. An example of a student's alignment and tree are included in Figure 3b.

After student groups had finished the activity sheet , we asked them to review their data and analyses from both the Out-of-Class Activity 1 and In-Class Activity 2 and compare their results for their character-based and gene-based phylogenies. We also answered questions or concerns that the students had. Finally, we asked students to prepare to discuss their data, analyses, and trees, as well as review and answer the Synthesis and Review questions at the end of the In-Class Activity 2 for the final session.

During Session 3

Our goal in the final session of this project was for students to discuss their data and trees, think more deeply about the concepts that we covered in the first session and apply those concepts to the data, analyses and results they completed throughout the project. First, we asked student groups to briefly (no more than 5 minutes per group) and informally discuss their results for both their character- and gene-based trees. After this, we proceeded to go through the Synthesis and Review questions in a forum style discussion to see how students were thinking about the activities and the data that they collected and analyzed. Through this discussion we tried to discuss common misconceptions with tree-thinking and gauge how the activities mitigated these misconceptions by creating a more concrete foundation to the abstract notion of tree building and tree-thinking.

Implementation Tips for Activity

The exercises within this project-based activity are easy to implement in any style room (e.g. lecture hall, smaller classroom, teaching laboratory). The only required items for the project are student laptops and a projector. We recognize that this project, having been designed and implemented over the course of six hours or class time, may not fit many or most allotted class periods. This is why we have designed this project as three independent in-class activities and one out-of-class activity, which allows instructors to utilize all or just a part of the project. Portions of the project can be made into out-of-class activities. For example, we created a tutorial for MEGA/In-Class Activity 2 (Additional File 7) in case instructors wanted to make this another activity to be done out-of-class. However, we do suggest that the first in-class activityand the discussion of the Synthesis and Review questions be implemented during class time. We provide further information on how to break the components of this project down to best fit other styles and lengths of classes in the Variations on Activities section.

We found that students struggled with two parts of this project. The first occurred in both the In-Class Activity 1 and Out-of-Class Activity when students tried to construct a character matrix and the tree. Students struggled with how to assign ancestral and derived character states (“0” versus “1”).To address this, we created additional lecture material that used a step-by-step approach to coding outgroup and ingroup character states using winged insects (Additional File 1) or the hypothetical organisms from In-Class Activity 1 (Additional File 3) as examples   Along with this, students struggled with the idea of gain and loss of traits. To address this, we mapped trait gain and loss using the example of winged insects or with the hypothetical organisms (if this issue was brought up while building the tree during In-Class Activity 1). We also demonstrated how numerous trees could be generated from a data matrix that may have more trait “steps” on certain trees. This also allowed us to further discuss the idea that the most parsimonious tree is not always the most evolutionarily accurate.

The second challenge for students was troubleshooting the bioinformatic tools . The main problem occurred with trying to BLAST samples through GenBank and/or the GenBank plugin in MEGA. Oftentimes the function would time out or simply run indefinitely. The first thing we tried was to simply reload the page or restart the BLAST plugin. Sometimes if there were too many open windows in the plugin, it would stall or stop working. If the issue continued, we had students (1)

BLAST the sequence directly through GenBank on their web server or (2) search GenBank (through the GenBank plugin in MEGA) for the species and gene that they were looking for (e.g., "*Gryllus firmus* 16S" under a "nucleotide" search). This would bring them to a window of all 16S genes that they could choose from to upload into their sequence. This second work around does not allow the students to fill in the BLAST table in the second in-class activity. However, students still got an understanding of how BLAST and GenBank worked.

Assessment

Finally, we did not grade our students' assignments for correctness since much of this exercise is subjective (rubric provided as Additional File 8). Instead, we graded students on (1) the completeness of the project, (2) their ability to articulate their understanding of the topics covered in the exercise and (3) their understanding of common misconceptions in tree-thinking and tree building (assessed during the Synthesis and Review question discussion). Instructors who choose to use this activity as a whole or in part can be as free or strict with grading as they like and can create grading rubrics as they see fit.

Variations on Activities

In recognizing that this project was designed for a small class with a two hour class period , we provide some variations on how best to implement this activity in larger and/or shorter classes.

1. If the class period is shorter (50-80 minutes) and paired with a lab, we suggest carrying out the introduction lecture prior to the lab and the first in-class and out-of-class activities during

the lab. The second in-class activity can either be turned into an out-of-class activity or it can be carried out in a second lab period along with the Synthesis and Review questions.

2. If the class period is shorter (50-80 minutes) without a lab, we suggest making this a multi-day activity. The introduction lecture would be given on the first day and the first in-class activity and introduction to the out-of-class activity on the second day. The second in-class activity could either be done on a third day or as an out-of-class activity. If it is turned into an out-of-class activity, we suggest either carrying out the introduction lecture to that material (Additional File 6) during class or providing a recorded lecture. It is also possible to omit the first in-class activity and only carry out the activities that include the case study.
3. If the class size is larger (>40 students) and paired with a lab, groups can be created in individual lab sections and the activity can be carried out as suggested in #1.
4. If the class size is larger (>40 students) and not paired with a lab, the project can be either independent or completed as an out-of-class group project.
5. For introductory level courses (undergraduate or high school), we suggest using just the first in-class activity, just the case study, or just the first part of the case study. Instructors could also provide students with the premade trait list/matrix for the out-of-class activity and/or a premade sequence alignment for the second in-class activity.

Extensions of Activities

1. To evaluate students' comprehension before or after the lesson, instructors can administer the Basic Tree-Thinking Assessment (Baum et al. 2005), "quiz" portions of The Tree Thinking tutorial (Novick et al. 2012), or an evaluation of their own making. Instructors can also

evaluate comprehension during the first lecture using real-time questions via think-pair-share, clickers, or other methods (we have included examples in Additional File 2).

2. As a more in-depth follow up to the conclusion or as a replacement to the Synthesis and Review questions, students can give an oral presentation of their results in which they could (1) discuss morphological and molecular trees, (2) interpret and compare both methods and trees, and (3) identify caveats that could have arisen during the case study. This final point is crucial, especially as a metric to see whether students grasped the exercises and gained a deeper understanding of tree-thinking. Here, students can discuss the limitations of their methodology that could have, in turn, affected their results including how altering traits in their character-based matrix could alter their character-based tree, or how using a different gene (e.g., nuclear versus mitochondrial) or set of genes could alter their neighbor joining tree and other genetic distance metrics.
3. As a more in-depth introduction or follow up to the activity, papers can be assigned that cover aspects of tree-thinking (Gregory 2008- reading evolutionary trees and misconceptions of them; Halanych 2004- influence of molecular data on the tree of life; Novick et al. 2012- an introduction and self-paced tutorial to tree-thinking). These papers can be discussed later in the course or evaluated for completion and comprehension on exams.
4. To reinforce other topics from the first lecture (e.g., rotating nodes, monophyly, etc.) students can be asked to change tree topologies or identify monophyletic/paraphyletic/polyphyletic groupings from the trees they generated during the case study.

Conclusion

There is overwhelming evidence that active- and project-based learning are highly effective teaching strategies when compared to traditional lectures because students become active participants in their own learning (Alters and Nelson 2002; Ballen 2020; Ballen et al. 2017; Estrada et al. 2016; Freeman et al. 2014; Gardner and Belland 2012; Haak et al. 2011; Hake 1998; Nelson 2008; Smith et al. 2005; Theobald et al. 2020). The use of interactive and cooperative styles of teaching engages students in deeper critical thinking and has been at the forefront of recommended standards for teaching biology (AAAS 2011; Nelson 2008; NGSS Lead States 2013). The project-based activity presented here emphasizes the importance of using an integration of both student-centered and instructor-led techniques, as well as combining hypothetical scenarios with a case study to engage students in the scientific process. Because the future of biological sciences is becoming strongly associated with bioinformatics, we used this activity to explore aspects of bioinformatics (e.g., data mining and alignment, tree building algorithms) and as a pathway to training undergraduates in basic bioinformatic tools. From student responses and discussion during Session 3 (discussion of Synthesis and Review questions) it was clear to us that students were thinking more deeply and concretely about the material compared to when it was first presented, as well as how the material and methods they learned could be extended and used in many branches of biology (e.g. conservation). We evaluated the effectiveness of this exercise from student feedback (final question of the Synthesis and Review questions). A majority of the students found the project useful at "conceptualizing how evolutionary trees are built and interpreted", "learning how different data types impact building evolutionary trees", and "recognizing how [they were] incorrectly thinking about trees due to their abstractness." Students were positive about the project in both the activity evaluation and course instructor evaluations, and in particular liked the hands-on aspect of the project, group-work, and the introduction to bioinformatic tools. This activity is well-aligned with the broader goals of biology education and has been designed to have students critically think about evolutionary trees from theory to practice and to have them identify and face misconceptions in their own learning when it comes to the subject of tree-thinking. After this activity, students should have a deeper understanding of tree-thinking and evolution, as a whole.

References

AAAS. Vision and change in undergraduate biology education: A call to action. 2011.

Alexander RD. The taxonomy of the field crickets of the eastern United States (Orthoptera: Gryllidae: Acheta). Ann. Entomol. Soc. Am. Oxford Academic; 1957;50(6):584–602.

Allen D, Tanner K. Infusing active learning into the large-enrollment biology class: seven strategies, from the simple to complex. Cell Biol. Educ. 2005;4(4):262–8.

Alters BJ, Nelson CE. Perspective: Teaching evolution in higher education. Evolution. 2002;56(10):1891–901.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J. Mol. Biol. 1990;215(3):403–10.

Avise JC. Evolutionary pathways in nature: A phylogenetic approach. Cambridge University Press; 2006.

Ballen CJ. Enhancing Diversity in College Science with Active Learning [Internet]. Active Learning in College Science. 2020. p. 873–87. Available from: http://dx.doi.org/10.1007/978-3-030-

33600-4_54

Ballen CJ, Wieman C, Salehi S, Searle JB, Zamudio KR. Enhancing diversity in undergraduate science: self-efficacy drives performance gains with active learning. CBE Life Sci. Educ. [Internet]. 2017;16(4). Available from: http://dx.doi.org/10.1187/cbe.16-12-0344

Baum DA, Offner S. Phylogenies & tree-thinking. Am. Biol. Teach. University of California Press; 2008;70(4):222–9.

Baum DA, Smith SD. Tree thinking. Citeseer; 2013.

Baum DA, Smith SD, Donovan SSS. Evolution. The tree-thinking challenge. Science. 2005;310(5750):979–80.

Bell S. Project-based learning for the 21st century: Skills for the future. The Clearing House: A Journal of Educational Strategies, Issues and Ideas. Routledge; 2010;83(2):39–43.

Berchiolli B, Movahedzadeh F, Cherif A. Assessing student success in a project-based learning biology course at a community college. Am. Biol. Teach. University of California Press; 2018;80(1):6–10.

Bilgin I, Karakuyu Y, Ay Y. The effects of project based learning on undergraduate students' achievement and self-efficacy beliefs towards science teaching. Eurasia J. Math. Sci. Technol. Educ. Modestum Limited; 2015 Apr 29;11(3):469–77.

Blacquiere LD, Fawaz A, Hoese WJ. Who's related to whom? Use published phylogenies and make customized tree-thinking assessments. Evolution: Education and Outreach. BioMed Central;

2020;13(1):1–24.

Blumenfeld PC, Soloway E, Marx RW, Krajcik JS, Guzdial M, Palincsar A. Motivating project-based learning: Sustaining the doing, supporting the learning. Educ. Psychol. Routledge; 1991;26(3-4):369–98.

Brewer CA, Zabinski C. Simulating genetic change in a large lecture hall: The ultimate bean counting experience. Am. Biol. Teach. [University of California Press, National Association of Biology Teachers]; 1999;61(4):298–302.

Buckberry S, Burke da Silva K. Evolution: improving the understanding of undergraduate biology students with an active pedagogical approach. Evolution: Education and Outreach. BioMed Central; 2012;5(2):266–73.

Burks RL, Boles LC. Evolution of the chocolate bar: A creative approach to teaching phylogenetic relationships within evolutionary biology. American Biology Teacher. National Association of Biology Teachers; 2007;69(4):229–37.

Byerly AR, Firneno TJ Jr, Beard R, Larson EL. The role of community science in orthopteran research. Journal of Orthoptera Research.

Chen C-H, Yang Y-C. Revisiting the effects of project-based learning on students' academic achievement: A meta-analysis investigating moderators. Educational Research Review. 2019 Feb 1;26:71–81.

Chen X SM. STEM attrition: college students' path into and out of stem fields. NCES 2014-001, Washington DC; 2013.

Cherif A, Adams G, Movahedzadeh F, Martyn MA, Dunning J. Why do students fail? Faculty's Perspective. In: A Collection of papers on creating and supporting learning environments. The Higher Learning Commission; 2014.

Darwin C. On the origin of species by means of natural selection, or, the preservation of favoured races in the struggle for life. London: John Murray; 1859.

David AA. Using project-based learning to teach phylogenetic reconstruction for advanced undergraduate biology students: Molluscan evolution as a case study. Am. Biol. Teach. University of California Press USA; 2018;80(4):278–84.

Dees J, Momsen JL. Student construction of phylogenetic trees in an introductory biology course. Evolution: Education and Outreach. 2016;9(1):3.

Dees J, Momsen JL, Niemi J, Montplaisir L. Student interpretations of phylogenetic trees in an introductory biology course. CBE Life Sci. Educ. 2014;13(4):666–76.

Eddy SL, Crowe AJ, Wenderoth MP, Freeman S. How should we teach tree-thinking? An experimental test of two hypotheses. Evolution: Education and Outreach. BioMed Central; 2013;6(1):1–11.

Estrada M, Burnett M, Campbell AG, Campbell PB, Denetclaw WF, Gutiérrez CG, et al. Improving underrepresented minority student persistence in STEM. CBE Life Sci. Educ. [Internet]. 2016;15(3). Available from: http://dx.doi.org/10.1187/cbe.16-01-0038

Freeman S, Eddy SL, McDonough M, Smith MK, Okoroafor N, Jordt H, et al. Active learning increases student performance in science, engineering, and mathematics. Proc. Natl. Acad.

Sci. U. S. A. 2014;111(23):8410–5.

Gardner J, Belland BR. A conceptual framework for organizing active learning experiences in biology instruction. J. Sci. Educ. Technol. 2012;21(4):465–75.

Gibson JP, Hoefnagels MH. Correlations between tree thinking and acceptance of evolution in introductory biology students. Evolution: Education and Outreach. BioMed Central; 2015;8(1):1–17.

Gray DA, Weissman DB, Cole JA, Lemmon EM, Lemmon AR. Multilocus phylogeny of Gryllus field crickets (Orthoptera: Gryllidae: Gryllinae) utilizing anchored hybrid enrichment. Zootaxa. 2020 Mar 12;4750(3):328–48.

Gregory TR. Understanding evolutionary trees. Evolution: Education and Outreach. BioMed Central; 2008;1(2):121–37.

Guo P, Saab N, Post LS, Admiraal W. A review of project-based learning in higher education: Student outcomes and measures. Int. J. Educ. Res. 2020;102:101586.

Haak DC, HilleRisLambers J, Pitre E, Freeman S. Increased structure and active learning reduce the achievement gap in introductory biology. Science. 2011;332(6034):1213–6.

Hake RR. Interactive-engagement versus traditional methods: A six-thousand-student survey of mechanics test data for introductory physics courses. Am. J. Phys. American Association of Physics Teachers; 1998;66(1):64–74.

Halanych KM. The New View of Animal Phylogeny. Annu. Rev. Ecol. Evol. Syst. Annual Reviews;

2004;35:229–56.

Halverson KL. Improving tree-thinking one learnable skill at a time. Evolution: Education and Outreach. 2011;4(1):95–106.

Heddy BC, Nadelson LS. The variables related to public acceptance of evolution in the United States. Evolution: Education and Outreach. BioMed Central; 2013;6(1):1–14.

Ingram EL, Nelson CE. Relationship between achievement and students' acceptance of evolution or creation in an upper-level evolution course. J. Res. Sci. Teach. Wiley; 2006;43(1):7–24.

Kanter DE, Konstantopoulos S. The impact of a project-based science curriculum on minority student achievement, attitudes, and careers: The effects of teacher content and pedagogical content knowledge and inquiry-based practices. Sci. Educ. Wiley; 2010 Sep;94(5):855–87.

Kooloos JGM, Klaassen T, Vereijken M, Van Kuppeveld S, Bolhuis S, Vorstenbosch M. Collaborative group work: effects of group size and assignment structure on learning gain, student satisfaction and perceived participation. Med. Teach. 2011;33(12):983–8.

Kumar S, Nei M, Dudley J, Tamura K. MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. Brief. Bioinform. 2008 Jul;9(4):299–306.

Larmer J, Mergendoller J, Boss S. Setting the standard for project based learning. ASCD; 2015.

Lents NH, Cifuentes OE, Carpi A. Teaching the process of molecular phylogeny and systematics: a multi-part inquiry-based exercise. CBE Life Sci. Educ. 2010;9(4):513–23.

Maddison WPADRM. Mesquite: a modular system for evolutionary analysis [Internet]. 2021.

Available from: http://www.mesquiteproject.org

Mahasneh AM, Department of Educational Psychology, Faculty of Educational Sciences, The Hashemite University, Zarqa, Jordan, dahmadmahasneh1975@yahoo.com, Alwan AF, Department of Educational Psychology, Faculty of Educational Sciences, The Hashemite University, Zarqa, Jordan. The effect of project-based learning on student teacher self-efficacy and achievement. Int. J. Instr. Media. Modestum Limited; 2018 Jul 25;11(3):511–24.

McCullough EL, Verdeflor L, Weinsztok A, Wiles JR, Dorus S. Exploratory activities for understanding evolutionary relationships depicted by phylogenetic trees: United but diverse. Am. Biol. Teach. University of California Press; 2020;82(5):333–7.

McKeachie WJ, Lin Y-G, James Strayer. Creationist vs. evolutionary beliefs: effects on learning biology. Am. Biol. Teach. [University of California Press, National Association of Biology Teachers]; 2002;64(3):189–92.

Mead LS, Clarke JB, Forcino F, Graves JL. Factors influencing minority student decisions to consider a career in evolutionary biology. Evolution: Education and Outreach. BioMed Central; 2015;8(1):1–11.

Meir E, Perry J, Herron JC, Kingsolver J. College students' misconceptions about evolutionary trees. The American Biology Teacher [Internet]. National Association of Biology Teachers; 2007 [cited 2022 Nov 5];69(7). Available from: https://bioone.org/journals/the-american-biology-teacher/volume-69/issue-7/0002-7685_2007_69_71_CSMAET_2.0.CO_2/College-Students-Misconceptions-About-Evolutionary-Trees/10.1662/0002-

7685(2007)69[71:CSMAET]2.0.CO;2.short

Meisel RP. Teaching tree-thinking to undergraduate biology students. Evolution Education and Outreach. 2010;3(4):621–8.

Nelson CE. Teaching evolution (and all of biology) more effectively: Strategies for engagement, critical reasoning, and confronting misconceptions. Integr. Comp. Biol. 2008;48(2):213–25.

NGSS Lead States. Next generation science standards: For states, by state. National Academies, Washington, DC; 2013.

Novick LR, Catley KM. Reasoning about evolution's grand patterns: college students' understanding of the tree of life. Am. Educ. Res. J. American Educational Research Association; 2013;50(1):138–77.

Novick LR, Catley KM. Fostering 21st-century evolutionary reasoning: teaching tree thinking to introductory biology students. CBE Life Sci. Educ. [Internet]. 2016;15(4). Available from: http://dx.doi.org/10.1187/cbe.15-06-0127

Novick LR, Catley KM, Schreiber EG. Understanding evolutionary history: An introduction to tree thinking [Internet]. 2012. Available from: https://cdn.vanderbilt.edu/vu-my/wp-content/uploads/sites/2396/2017/02/14141743/TT_instruct_Novick_Catley_8-12.pdf

Prince M. Does active learning work? A review of the research. J. Eng. Educ. Wiley; 2004 Jul;93(3):223–31.

Sandvik H. Tree thinking cannot taken for granted: challenges for teaching phylogenetics. Theory

Biosci. 2008;127(1):45–51.

Smith KA, Sheppard SD, Johnson DW, Johnson RT. Pedagogies of Engagement: Classroom-Based Practices [Internet]. Journal of Engineering Education. 2005. p. 87–101. Available from: http://dx.doi.org/10.1002/j.2168-9830.2005.tb00831.x

Sokal RR. A Phylogenetic Analysis of the Caminalcules. I. the Data Base. Syst. Biol. Oxford Academic; 1983a;32(2):159–84.

Sokal RR. A Phylogenetic Analysis of the Caminalcules. II. Estimating the True Cladogram. Syst. Biol. Oxford Academic; 1983b;32(2):185–201.

Sokal RR. A phylogenetic analysis of the Caminalcules. III. Fossils and classification. Syst. Biol. [Internet]. academic.oup.com; 1983c;. Available from: https://academic.oup.com/sysbio/article-abstract/32/3/248/1611365

Sokal RR. A Phylogenetic Analysis of the Caminalcules. IV. Congruence and Character Stability. Syst. Biol. Oxford Academic; 1983d;32(3):259–75.

Tamura K, Stecher G, Kumar S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol. Biol. Evol. 2021;38(7):3022–7.

Theobald EJ, Hill MJ, Tran E, Agrawal S, Arroyo EN, Behling S, et al. Active learning narrows achievement gaps for underrepresented students in undergraduate science, technology, engineering, and math. Proc. Natl. Acad. Sci. U. S. A. 2020;117(12):6476–83.

Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994;22(22):4673–80.

Weissman DB, Gray DA. Crickets of the genus Gryllus in the United States (Orthoptera: Gryllidae: Gryllinae). Zootaxa. 2019;4705(1):zootaxa.4705.1.1.

Weissman DB, Rentz DCF, Alexander RD, Loher W. Field crickets (Gryllus and Acheta) of California and Baja California, Mexico (Orthoptera: Gryllidae: Gryllinae). Trans. Am. Entomol. Soc. American Entomological Society; 1980;106(3):327–56.

Author Contributions

T.J.F. and E.L.L. conceptualized and designed the activity. T.J.F, G.T.W., P.J.N., and E.L.L. implemented the activity. All authors created, compiled, and edited additional files. T.J.F. wrote the manuscript with input and editing done by G.T.W., P.J.N., and E.L.L. All authors read and approved of the final manuscript.

Acknowledgements

We would like to thank the undergraduate students in E.L.L.'s Evolution course that took part in this activity and gave us valuable and critical feedback to help hone this project-based activity. We would also like to thank Brandon Woo for sharing photographs of *Gryllus firmus* and *Gryllus pennsylvanicus* used in the case study slides.

Competing Interests

The authors declare that they have no competing interests.

Ethics Approval and Consent to Participate

Not Applicable.

Funding

This work was supported by the National Science Foundation to E.L.L. (DEB 2012041 and IOS 2015976) and to T.J.F. (DBI 2208825), as well as Orthopterists' Society Theodore J. Cohn Research awards to T.J.F. and G.T.W.

Additional Files

Additional file 1 - activity worksheets
Additional file 2 - example introduction lecture
Additional file 3 - introduction to in-class activity 1 and case study
Additional file 4 - MESQUITE tutorial
Additional file 5 - character bank
Additional file 6 - introduction to in-class activity 2
Additional file 7 - MEGA tutorial
Additional file 8 - rubric

Tables

Table 1. Summary of the misconceptions of tree-thinking.

Misconception	**Description**	**Source**
The Great Chain of Being	Evolution has progressed from simpler to more advanced organisms	Meisel 2010, Gregory 2008, Kummer et al. 2016, Schramm and Schmiemann 2019
Reading across tips	Use relative order of tips to make conclusions about species relatedness	Meisel 2010, Gregory 2008, Kummer et al. 2016, Schramm and Schmiemann 2019
Clade density	Species-poor clades are “primitive” while species-rich clades are “advanced”	Meisel 2010, Schramm and Schmiemann 2019
Node Counting	The more nodes that separate species, the more distantly related they are	Meisel 2010, Gregory 2008, Kummer et al. 2016
Main line and side tracks	Human evolution forms the main line of the tree, and all other branching species are side tracks	Gregory 2008
Similarity vs. relatedness	Group organisms based on phenotypic similarity rather than relatedness	Gregory 2008, Kummer et al. 2016, Schramm and Schmiemann 2019
Sibling vs. ancestor	The common ancestor of two contemporary groups is very similar to one of these two groups	Gregory 2008
Long branch implies no change	Interpreting a long branch to mean that a species is more similar to the root ancestor than the other contemporary species	Gregory 2008, Schramm and Schmiemann 2019
Difference lineage ages for contemporary species	Conflate taxon age with lineage age	Gregory 2008, Schramm and Schmiemann 2019

Backwards time axes	Read time from tips as being oldest an root being youngest	Gregory 2008
Change only at nodes	Assuming node represents exact moment of change	Gregory 2008

Figures

Figure 1. Workflow and timeline of project-based activity for upper-level undergraduate evolution course.

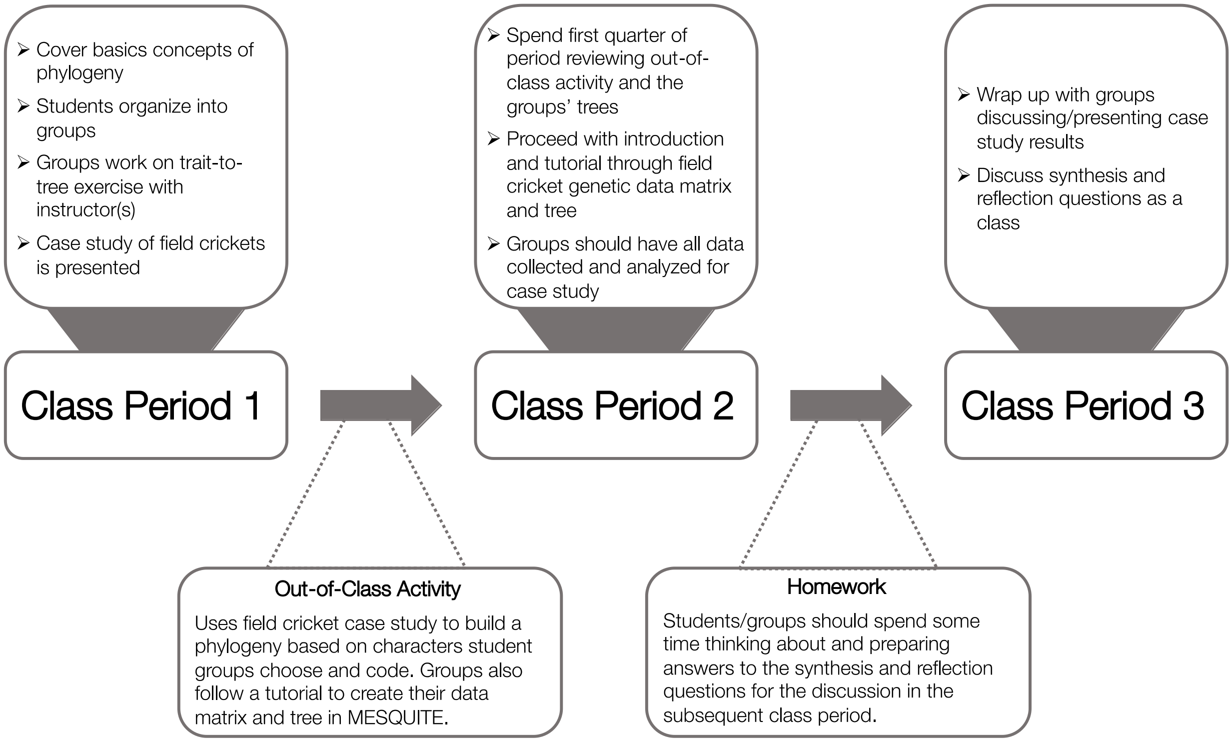


Figure 2. Example of a student's character trait list, matrix and tree generated for the first in-class activity with hypothetical organisms. This student showed both events of (1) trait evolution and then loss (red) and (2) separate trait evolution across taxa (blue). We used this tree example to discuss these events (trait gain/loss) with the class since it was often a place where students got stuck or caught up while making their trees.

Character List

1: Wings: 0 = absent, 1 = present

2: Feet: 0 = absent, 1 = present

3: Eyes: 0 = simple, 1 = compound

4: Mouth Parts: 0 = absent, 1 = present

5: Antennae: 0 = short, 1 = long

6: Body: 0 = two parts, 1 = three parts

7: Body Pattern: 0 = linear, 1 = curved

Character Matrix

		Characters						
		1	**2**	**3**	**4**	**5**	**6**	**7**
OTUs	**OG**	0	0	0	0	0	0	0
	A	1	1	1	1	1	0	1
	B	1	0	0	0	1	0	1
	C	1	1	1	1	0	1	1
	D	1	0	1	0	0	0	0
	E	1	1	1	1	1	1	1

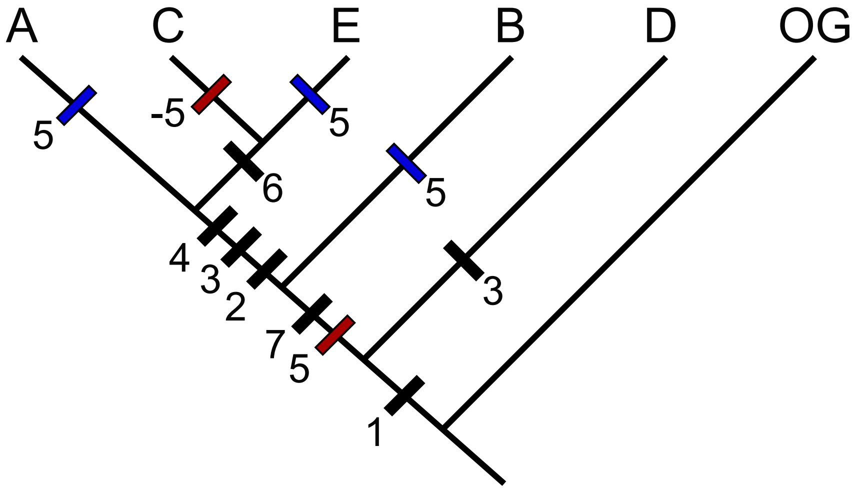
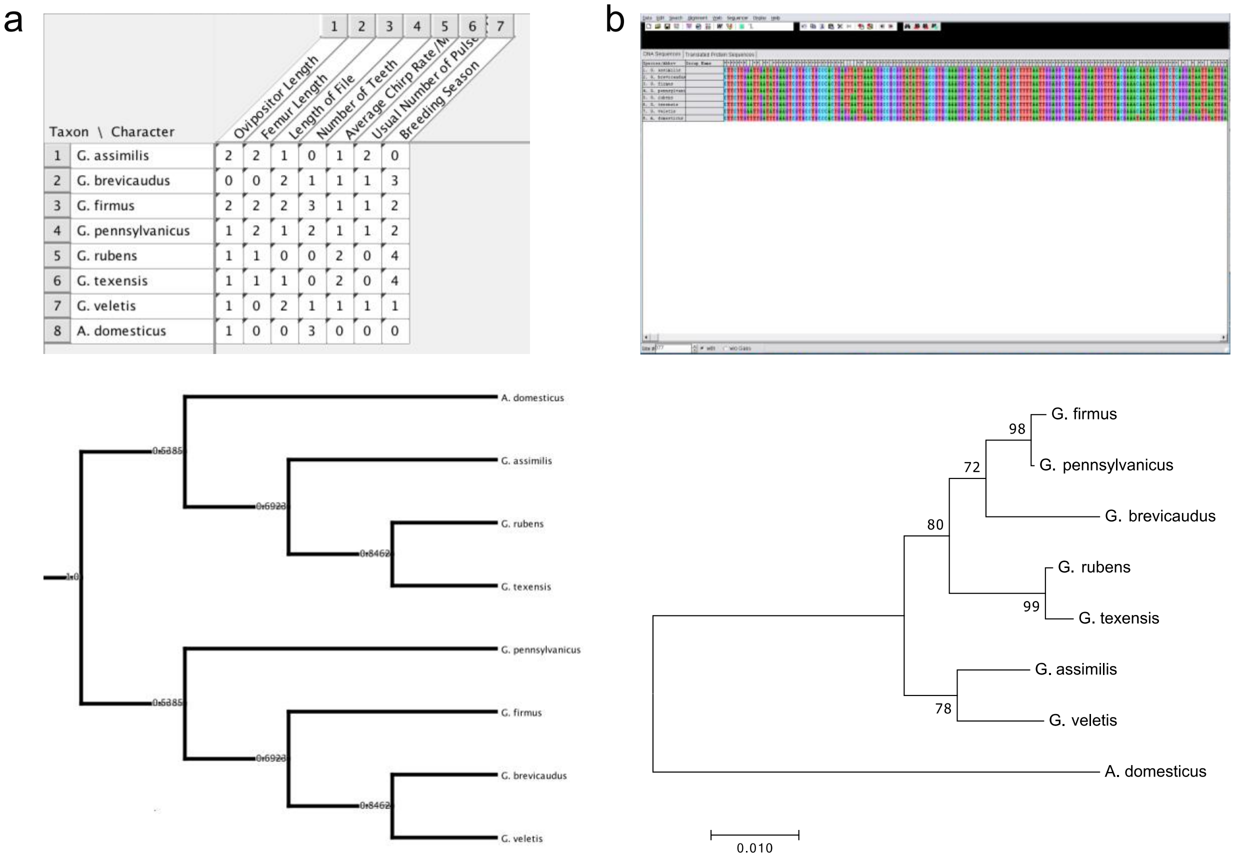


Figure 3. (**a**) Example character matrix and tree generated in MESQUITE, and (**b**) example 16S sequence alignment and the neighbor joining tree generated in MEGA for the field cricket case study.



Taxon \ Character	1 Ovipositor Length	2 Femur Length	3 Length of File	4 Number of Teeth	5 Average Chirp Rate (M	6 Usual Number of Pulse	7 Breeding Season
1 G. assimilis	2	2	1	0	1	2	0
2 G. brevicaudus	0	0	2	1	1	1	3
3 G. firmus	2	2	2	3	1	1	2
4 G. pennsylvanicus	1	2	1	2	1	1	2
5 G. rubens	1	1	0	0	2	0	4
6 G. texensis	1	1	1	0	2	0	4
7 G. veletis	1	0	2	1	1	1	1
8 A. domesticus	1	0	0	3	0	0	0