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Theme Issue: Darwin and Evolution

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About Our Cover

***Acrocanthosaurus* Footprints**

Our cover this month honors our theme by featuring evidence of a creature who is no longer with us but some of whose descendants evolved to become the birds of today. Here we see recently exposed footprints of the giant two-legged dinosaur *Acrocanthosaurus* (think *Tyrannosaurus*), preserved in the Glen Rose Formation now part of the riverbed of the Puluxy River. These footprints and those from a four-legged sauropod identified as either *Pleurocoelus* or *Sauroposeidon* (something like a *Brontosaurus*) have been known since a flood exposed them in 1909. However, the opposite phenomenon—a drought—recently revealed an even longer trackway in Dinosaur Valley State Park southwest of Fort Worth in central Texas.

These animals lived during the Lower (Early) Cretaceous about 113 million years ago and so existed long before the asteroid impact that heralded the end of the age of dinosaurs about 66 million years ago. There are many trackways visible in the park, depending on the water level in the river, so visitors are all but guaranteed to see the signs of these giant beasts.

The trackways found here and throughout many exposures of the Glen Rose Formation, running from northeast to southwest central Texas, were first studied by paleontologists from nearby Southern Methodist University. They were brought to national attention in 1937 by Roland T. Bird of the American Museum of Natural History in New York City. Bird wrote a popular account for the May 1954 issue of *National Geographic* in which he discussed how the tracks can be interpreted to reveal new insights into the lifeways of these animals. In addition, he excavated a long trackway with both types of prints, which now graces the dinosaur hall at the museum, beneath a mounted sauropod skeleton.

The *Acrocanthosaurus* stood about 15 ft (4.5 m) tall and weighed nearly 7 tons (6350 kg), while the *Sauroposeidon* was even larger: 60 ft (18.3 m) tall and weighing about 44 tons (40,000 kg). The two-legged dinosaur was an aggressive meat-eating predator, while the sauropod was a more docile plant-eating giant. One can only imagine what interactions might have occurred between these animals and others living along the shores of a shallow sea millennia ago.

This digital image was recorded with a Nikon D810 camera using a 28–300mm image-stabilized zoom lens. The photographer is William F. McComas, editor of *The American Biology Teacher* and Parks Family Professor of Science Education and director of the Project to Advance Science Education at the University of Arkansas (mccomas@uark.edu).

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GUEST COMMENTARY

GLENN BRANCH

Why Are There Still Misconceptions about Evolution?

There's a question that's all too familiar to biology educators: "If we evolved from monkeys, why are there still monkeys?" It's so prevalent that the anthropologist Jonathan Marks mischievously entitled a recent book *Why Are There Still Creationists?* (2021) without bothering to explain the joke. Sometimes the question is posed as a stumper, in the mistaken belief that it exposes a decisive failure of evolution; sometimes, perhaps more often, it is more of a cry for help, expressing sincere puzzlement. Either way, of course, the claim of evolution is not that humans evolved from monkeys but that humans and monkeys share a common ancestor that lived millions of years ago in the Oligocene.

Unfortunately, it's usually not sufficient just to correct the misclassification of that common ancestor. A useful way to continue the conversation: assuming that the questioner claims to be of, for instance, Irish descent, ask, "If you're descended from Irish people, why are there still Irish people?" The answer is that the Irish of yesteryear didn't become Americans en masse: rather, some remained in the Auld Sod while some immigrated to the Land of Opportunity. So too with the ancestors we have in common with monkeys: it's not that they all evolved into modern monkeys or modern apes; rather, some of their descendants evolved into modern monkeys while some of them evolved into modern apes, including humans.

The question thus reflects the common misconception that evolution is a linear progression, in which earlier species are replaced wholesale by later species. In actuality, evolution is a branching process, in which earlier species produce multiple successor species. The misconception is not new. The zoologist William K. Gregory once began a lecture by observing, "I suppose if you have talked to people about evolution they have said: 'Well, if monkey-like animals evolved into men at one time, why did not all monkeys evolve into men, and why are there any monkeys alive at the present time?'" He was speaking in 1917, but there's nothing, except for the gendered terminology, dated about his observation.

There are plenty of misconceptions about evolution. Project 2061, a long-term project launched in 1985 by the American Association for the Advancement of Science to help to improve American science education, identified no fewer than 27 misconceptions about evolution discussed in the science education research literature. Even so, that list was incomplete, with the idea of evolution as a linear progression surprisingly missing. But if misconceptions about evolution have been recognized as obstacles to the understanding and acceptance of evolution by scientists and science educators for more than a century, why are there still misconceptions about evolution?

The chief misconceptions about evolution appear to be rooted in intuitive notions about biological phenomena grounded in the nature of human cognition. In the case of "If we evolved from monkeys, why are there still monkeys?" the psychologist Andrew Shtulman suggests in *Scienceblind* (2017), the culprit is a form of essentialism, "according to which all members of a species evolve together, their fates intertwined by a common essence. In such a view, it makes no difference whether a population has been split in two, because all members of the population are united by a common essence. The only way a new species could emerge from an old one is if the old species metamorphosed into a new one."

There are still misconceptions about evolution, largely because there are constantly new students, who come equipped, by nature and nurture, with such intuitive notions. Biology educators need to teach accordingly, by recognizing the range of misconceptions and then identifying, addressing, and helping their students to overcome their faulty notions about evolution. A growing body of research suggests that the approach of misconception-based teaching yields better engagement and retention in general. And for a topic such as evolution that is socially, though not scientifically, controversial, overcoming the misconceptions that stand in the way of student understanding is particularly important.

A collection of five model lesson sets developed by the National Center for Science Education (NCSE) (freely available at https://ncse.ngo/evolution-lesson-sets) takes the point to heart. Designed by and for teachers and aligned with the Next Generation Science Standards, each focuses on a set of common misconceptions about evolution and is geared toward helping the instructor guide students to overcome them by examining the evidence, replacing them with the correct scientific understanding. Lesson 4, No More Monkeying Around, focuses on three misconceptions about human evolution, including in particular "If we evolved from monkeys, why are there still monkeys?"

To pose a final "why are there still" question: why are there still biology educators who aren't teaching evolution effectively? There isn't a single answer, of course. Among these educators, there are still teachers who don't accept evolution (although happily their numbers are dwindling), teachers who are concerned about the possibility of backlash in their communities, and teachers who have not received adequate preparation. But hopefully, equipped with resources like NCSE's model lesson sets that help them to guide their students to overcome their misconceptions about evolution, more and more biology educators will be empowered to teach evolution effectively.

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FEATURE ARTICLE

Teaching Evolution Using Semester-Long Student Investigations of Adaptation by Natural Selection

GREGORY HAENEL

Abstract

Case studies are valuable tools for instruction but are often limited to a single topic and a single class period. Courses such as evolution that synthesize multiple concepts around a common theme, however, can use a single case study type project that extends over the entire semester to develop and link core concepts. A central theme in evolutionary biology is determining if complex biological traits represent adaptations that arose by natural selection. The instructional model presented here engages students in a step-by-step process to answer this question of adaptation for a trait of their choosing. In this process, the instructor first introduces the major concepts required to address adaptation. As each major concept is developed in class, students apply this concept to their particular trait, using information gathered from published studies. Students then report their research back to the class. At the end of the semester, each group synthesizes their evidence into a paper developing an argument as to whether or not their trait fits the criteria of being an adaptation. This project provides students with ownership of course material, gets students to act as practicing scientists, and helps them integrate and apply theoretical material to real questions.

Key Words: *natural selection; fitness; active engagement; collaborative groups; evidence; case study.*

○ Introduction

Active engagement in course material can enhance student learning, but getting and maintaining student engagement in the classroom can be challenging. When students have the opportunity to use and apply new information, they tend to better understand and retain the lessons (Gormally et al., 2009; Schank et al., 1999). One common and highly successful method of actively engaging students in learning is through case studies (see National Center for Case Study Teaching in Science; https://www.nsta.org/case-studies). Most case studies are of short duration and focus on a single topic leading to potentially high-quality but short-term engagement in a single topic. The instructional model presented here takes the key components of case study instruction and expands the process over the majority of the semester. This provides an overall course structure that actively engages students with core course content at a deep level and provides student ownership of the material. A sense of ownership is important for student success (O'Neill, 2005). This unique course structure was designed and implemented in an upper-level undergraduate evolutionary biology course and will be described here in this context. However, this model may be applicable to other courses where there is a major overarching question central to that field that can be broken into multiple conceptual steps.

The semester-long case study model presented here for examining the process of adaptation by natural selection helps students contextualize and apply many major concepts in evolution.

Getting students to understand evolution is important because the concept of evolution links all of biology together and is fundamental to understanding biology (Dobzhanski, 1973). However, students tend to have a poor understanding of this core idea in science (reviewed in Gregory, 2009), and cognitive biases can significantly interfere with student learning of evolutionary concepts (Barnes et al., 2017). For example, teleological reasoning, or explaining something by its end result rather than what caused it, often plays a large role in impairing student understanding of natural selection. As evolution is a unifying, cross-disciplinary concept in science (Gould, 2002), it also requires students to understand and apply material from other fields such as genetics. Yet genetic mechanisms important to understanding evolution, such as mutation and random variation, can also be particularly difficult for students to grasp (Morabito et al., 2010).

In the instructional model presented here, students work in groups to develop and present arguments for five different major conceptual issues that apply to whether a phenotypic trait should be considered an adaptation. The students' arguments are based on evidence they find in the primary literature. Lecture and lab exercises add additional content

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while scaffolding the material to help students make conceptual connections between their individual research goals and specific course content. When argumentation is made an explicit part of instruction, it appears that understanding of content can improve (Asterhan & Schwarz, 2007; Zohar & Nemet, 2002). Students also develop arguments based on evidence that crosses disciplinary fields, such as applying genetics to development of phenotypes.

The process of adaptation by natural selection, how complex traits of organisms develop and come to fit their environment so well, is a core concept in evolutionary biology and remains an active area of investigation. Over the course of the semester, each group of students finds and evaluates evidence for the hypothesis that a particular biological trait is an adaptation that arose by natural selection. Figure 1 presents a visual summary of the timeline of different activities outlined here and provided in detail in the course description section below.

At the beginning of the semester, each of several student groups picks a different complex biological trait to investigate. Examples of traits students have used are provided in Table 1. Evidence required to test the hypothesis that a trait is an adaptation is broken into five main criteria that also represent major conceptual areas in evolutionary biology and major learning goals of the course (Table 2; Brandon, 1991). During the semester, each group applies each of these five major concepts one by one to their particular trait.

Groups follow a jigsaw format to investigate and present each criterion as it is introduced and developed over the semester. Following the in-class introduction to each criterion, one student from each group takes the lead and researches the primary literature, looking for studies that investigate that particular concept/criterion with respect to their group's trait. These lead students review the available data and develop an argument based on that evidence as to whether or not that criterion supports the hypothesis that their trait is an adaptation that arose by natural selection. Then the student who did the research from each group presents their argument to the class. First, these students work together to give a brief panel presentation providing an overview of the importance of that criterion to the process of adaptation. Then each of these lead students presents the evidence they found for this particular criterion as it applies to their group's specific trait. This process proceeds in

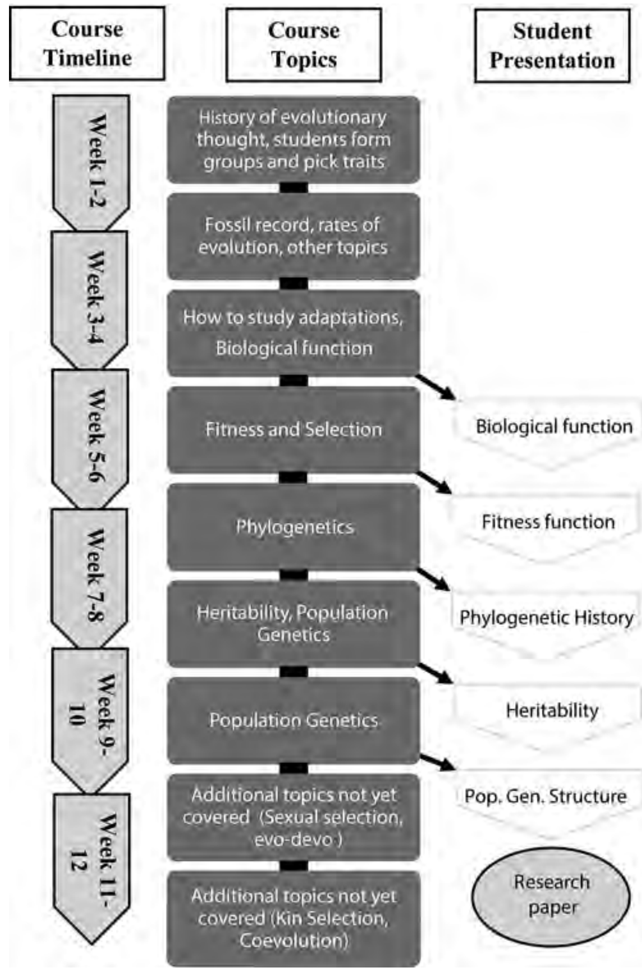

Figure 1. Course structure. The first column gives the approximate time in the semester each event takes place. The second column gives the order of content presented in the course. The third column gives the criteria for adaptation under study and approximate timing of each set of student presentations.

Table 1. Topic choices: A sample of potential traits for this case study. Many phenotypic traits could be used in this case study. The trait should be fairly unique and distinct enough to allow students to clearly define the phenotype. It is important that there are published studies to provide enough information for the students to develop some conclusions.

Trait	Description
Rattlesnake rattle	One group of pit vipers has developed a modified tail that makes noise.
Siblicide	Booby (blue-footed and Nazca) chicks will kill their younger brothers and sisters. How can this be an advantage?
Human speech	One of the things that really sets us apart.
Paedomorphosis in salamanders	Sometimes adult tiger salamanders do not turn into terrestrial adults but stay in their larval form.
Alternative mating strategies in male sunfish	Some males are territorial while others use sneaky strategies to mate.
External testes in some mammals	Most vertebrates have internal testes. However, in some mammal species, the testes move out of the body during development.
Altruism in humans	Why do some people risk their lives to help others?

Deciduous leaves	Oaks (for example) occur in both the temperate and tropical regions. Here they drop their leaves all at the same time. Is “fall” adaptive?
Flower color	We mostly think about plants as being green. However, the reproductive organs of many (flowers) are very brightly colored.
Armor in male stickleback fish	These little fish, found in both salt water and fresh water, are covered in armor-like plates and have spines that stick out.
Feathers	One of the characteristics associated with birds and their relatively unique ability to fly are feathers.

Table 2. Criteria used to make an argument that a trait is an adaptation that arose by natural selection (Brandon, 1991). The only real restriction to the order is that biological function needs to come first so that the phenotype under study is clear to all following group members.

Criteria	Brief Descriptions of Criteria
1) Biological function	Description of the phenotype students will be studying. What is the physiological, physical manifestation of the trait? This is also known as proximal function or how the trait functions in current ecological time.
2) Fitness function	How does the trait impact survival and/or reproduction?
3) Phylogenetic history	Is the trait evolutionarily new to the group under study? What was the ancestral state of the trait, and what is the distribution of the trait across the phylogenetic history of the groups that possess the trait?
4) Heritability	Is there a genetic basis for variation in the trait that can allow it to respond to selection?
5) Population genetic structure	Do past or current selective environments support the selective arguments being made above?

a stepwise (jigsaw) manner through the semester until each of the five conceptual areas has been developed formally in lecture and lab exercises, researched by the students, and finally presented to the class by a student from each group. Providing students with clearly defined roles within their group like this enhances the effectiveness of team building within groups (Salas et. al., 1999; Theobald et al., 2017).

Once students present on the last criterion, each group synthesizes its individual research findings on their trait into a single paper. This final paper presents all their accumulated evidence and evaluates how well the evidence from each conceptual component supports the hypothesis that their particular trait is an adaptation that arose by natural selection.

This teaching technique has been used in both an upper-level undergraduate evolution course for biology majors (class sizes have ranged from 8 to 26) and a second-year honors seminar course (20 students, not limited to science majors).

○ Course Description: Details of the Process Applied to Evolution

At the beginning of the semester, the instructor presents a brief overview of the case study goals and objectives to the students. The instructor also provides a description and introduction to each of the traits on the list the class will pick from to study (see Table 1 for a list of potential traits). Students then have the opportunity to form groups based on topic choice preferences and preferred group members. As there are five criteria, groups of five students work best so that each student can focus on a separate criterion. Students can also decide at this point whether they want to investigate a trait not on the list. The students must propose the trait for approval by the instructor, who then evaluates the appropriateness of the trait for this process. If it is a trait the instructor is not very familiar with, the instructor will need to do enough of a survey of the literature to determine whether there are sufficient studies available for this trait that address the key criteria for this study of adaptation.

The instructor then introduces the concept of adaptation and major approaches to studying adaptation. Discussions of papers like “The Spandrels of San Marco and the Panglossian Paradigm: A Critique of the Adaptationist Programme” (Gould & Lewontin, 1979) and the first three chapters of *The Blind Watchmaker* (Dawkins, 1986) support these objectives well. As the case study process does not cover all course content, those topics not covered, such as the history of evolutionary thought, geologic record, rates of evolution, kin selection, evo-devo, coevolution, and sexual selection, can be bookended to the beginning and end of the semester. This can be done with lectures and presentations, as preferred by the instructor, and help provide a framework for what evolution is about before jumping into the details of natural selection and adaptation.

The instructor then introduces the specific format of the case study model they will be utilizing for the rest of the semester to examine the process of adaptation (see Figure 1). This model is based around five criteria or levels of evidence required to determine whether a trait is an adaptation that arose by natural selection as outlined by Brandon (1991). In brief, these five criteria for the traits are (1) biological function, (2) fitness function, (3) phylogenetic history, (4) heritability, and (5) population genetic structure. These five criteria are defined in Table 2, and a more detailed handout of these definitions can be found in the Supplemental Material available with the online version of this article. Each student within the different groups picks one of these five criteria and will take the lead on researching and presenting it with respect to their group's trait.

After choosing a topic to investigate, students begin by reading general introductions to their topic area (e.g., textbooks, book chapters, web pages) and collecting sources. Each group then works together to write a prospectus outlining how they propose their trait will fit each of the criteria. The prospectus is brief and simply involves writing about the five major questions that must be addressed to establish that a trait is evolved and how those questions are to be phrased with respect to their topic. This document is intended to help students focus on what they will be looking for in their reading.

Biological function is the first criterion to develop, as it defines the phenotype (structurally and functionally) on which the rest of the group members will be focusing their efforts. The focus in this first section is on describing the proximal function of the trait. Once provided with the conceptual background, students who picked the biological function criterion search for primary literature that describes the details of the phenotype of their trait. The material presented in class, meanwhile, moves on to the concept of evolutionary fitness, the second major criterion. Clearly separating the proximal biological function of a trait from the fitness implications of the trait helps students break the cycle of teleological thinking about natural selection. By having to clearly articulate how the phenotype interacts with the environment to promote fitness, students can better understand the context dependence of selection.

About one and a half weeks into the evolutionary fitness material, students who picked the biological function criterion present their research findings to the class. Presentations begin with a short panel where the presenters from all the groups work together to give a brief explanation of what the criterion is and how it fits into the context of the study of adaptation. In the individual presentations that follow, students present the specific evidence they found in the literature to show how this criterion for adaptation is supported for their trait. Typically, individual presentations last 8–12 minutes. These first presentations on biological function are fairly descriptive in nature but are very important for setting the stage for the other researchers in their groups by clearly describing the phenotype and how it functions biologically in the environment.

After completing classroom material on the second conceptual criterion, evolutionary fitness function, the student from each group who selected this criterion has a basis from which to now look for and understand studies measuring fitness and selection on their trait. Meanwhile, lecture and lab topics move onto phylogenetics. Students learn about phylogenetic tree construction, ancestral state reconstruction, and phylogenetic comparative methods, with a break for the presentations by the students who researched the fitness function criterion. As students gain an understanding of phylogenetics, the ones who chose the phylogenetic history criterion research the origins of their trait, where it arose in the phylogeny, how the trait is distributed across the phylogeny, and what the ancestral condition was likely to have been. The goal here is to see if the trait arose in the group in which it is proposed to be an adaptation. As the course content moves onto topics of heritability and population genetics, the phylogenetic students present their findings to the class. Heritability can be a challenging topic, and additional lessons in genetics may be appropriate to add here.

Classroom instruction supporting the final criterion, population genetic structure impacting the process of adaptation, can focus on processes of genetic drift, gene flow, mutation, and/or inbreeding. Students developing this criterion may focus their research in a variety of different directions depending on what studies are available for their trait and what material has been developed in previous presentations. In some cases, direct tests of gene flow and genetic drift may be available and appropriate. In others, students can use a historical perspective to develop an argument for whether or not selective environments proposed to have led to the trait were present at the time and place the trait was thought to have arisen (building on the phylogenetic history material presented previously).

After covering population genetic topics, the remainder of the semester in class can be dedicated to treating any additional areas of evolutionary biology not yet covered. After about a week and a half into this material, the students who were researching population genetic structure present their findings to the class, thus completing the presentation part of the case study.

Following the last presentations, students focus on the second major product of this case study, the group papers. These papers are a synthesis of the research each group member did during the semester. The papers typically consist of seven sections: an introduction that explains the overall model used here for studying adaption, five separate sections presenting the evidence for each different criterion, and a conclusion that summarizes the group's final argument for how well their trait met the criteria for being an adaptation that arose by natural selection. Each student is expected to act as lead author of the section on which they presented but is also expected to help the others with the conceptual synthesis and writing. The more students work together on this final synthesis, the more opportunity there is for reinforcement of the concepts for which they were not lead author.

○ Supporting Student Learning and Assessment

This project has numerous "checkpoints" built into it, where the instructor provides direction, feedback, and support. Since student ownership is a core part of this learning experience, guidance is often built into the feedback on different parts of the assignment that can be applied to the next step, rather than presented to the students as up-front directions. Table 3 presents the relative point value of each of the assignments and whether it is graded as an individual or group project.

The prospectus is an early group assignment designed to help group members begin to articulate their understanding of their topics at this early stage. Feedback on this assignment allows the instructor an initial opportunity to clarify misunderstandings students may have of the five questions and provide direction to their research. This is an opportunity for the instructor to suggest key search terms and important authors to the students.

Reading primary literature is challenging. Early in the semester, papers from the literature on adaptation are assigned and discussed in class. While learning about how we study adaptation is a key goal of this activity, during these paper discussions students are also asked to examine the structure of the scientific papers and are guided toward how to find the key evidence of the study. To build

Table 3. Breakdown of grading of major assignments. Some assignments are graded individually, while others represent group efforts. The case study represents 39% of the overall grade in the course, and this is further broken down in the third column.

Assignment	Individual/Group	% of Total Grade
Prospectus	Group	1
Annotations	Individual	4
Presentation	Individual	12
Questions on presentations	Individual	1
Adaptation paper section draft	Individual	2
Final paper	Group	15
Group development of trait	Group	4
Other assignments from class (exams, debate, paper discussions, worksheets, participation)	Mixture	61

on these initial lessons about reading primary literature, students complete three article annotations that are due two class periods prior to their presentations. For these article annotations, students pick what they think are the three most relevant research papers they found so far in their research. A sample article annotation worksheet (available in the Supplemental Material online) functions to help guide students in their reading of the articles. Students first write a summary of the key points of the paper. Students then answer questions that focus their reading of the articles on how each criterion in the study of adaptation was specifically addressed, if it was at all. The annotation form further asks them to identify literature cited in the paper that looks useful for their own specific goals and the goals of the other members of their group. From these annotations, the instructor can (1) determine if the student found appropriate papers and, if not, direct them toward the correct literature before they give their presentation; (2) see if the student can summarize the main points of the paper correctly and, if not, provide feedback to help them see where these points were; and (3) see if the student can identify and articulate the points that are most relevant to their specific goals. The timing of these annotations allows the instructor to see if students are finding the appropriate sources while judging the students' level of understanding and also to provide the above feedback before their presentation, when this feedback is directly applicable to their giving a successful presentation. A fourth article annotation is due later in the semester, before the written paper is due, giving students a chance to use the prior feedback and gain further directed practice in reading the relevant literature.

In a typical case study, the instructor supplies the material and questions. Allowing students to explore the literature to find the key papers on their own more closely aligns this to how science is practiced. Similar to what scientists do when they are developing projects, the students must be able to clearly articulate the question and how the criteria they are investigating apply to their trait, figure out what type of data they need to address that question, and find the appropriate literature resources that show what is already known about the topic. It is important for the instructor to be familiar enough with the traits to be able to help guide the students to the appropriate key words and sources. An alternative to this more open approach is to only use traits the instructor has already researched and provide the key literature sources directly to the students. While this eliminates the research component, the instructor can focus on more directly guiding the reading and extracting of information from the literature.

Presenting scientific evidence clearly is an important skill for scientists. Before the first presentations (on biological function of the traits), the instructor provides details about how to give a scientific presentation to the entire class, along with specific expectations. By showing examples of high-quality and low-quality presentation slides, the instructor can help model these expectations to the students. After each set of presentations, the instructor provides detailed written feedback to those students who presented, to help them understand the strengths and weaknesses of their research presentations, with an eye toward what they need to fix or add to the written component of the project (see sample presentation rubric in the Supplemental Material online). This is another point in the process where the instructor can correct any errors in logic or recommend any important literature these students missed. These issues can then be addressed by the students in the final written paper. Students are more likely to incorporate feedback when it includes points they will need to address in a later assignment and the feedback can focus on ideas and concepts, rather than lower-order issues such as presentation style or grammar (Szymanski, 2014). Students are encouraged to share the feedback with their group members so they all benefit and are less inclined to repeat any mistakes.

To support student practice of critical thinking during presentations, each nonpresenting student submits to the instructor three questions they came up with during the presentations. These questions, if deemed appropriate by the instructor, can be incorporated into the instructor's written feedback to the presenters. These student-generated questions provide feedback from the perspective of their peers and often help uncover misconceptions of both presenters and observers. For example, if students submit questions that show they did not understand a key component of the presentation, the instructor can clarify that point to the entire class.

Communicating scientific evidence and arguments through writing is another important skill scientists use. Each group produces a final paper that synthesizes all their evidence, with each student acting as the primary author for their own section. To support this effort, all the students who presented a given section read that section from all the groups. Students submit drafts to the instructor for feedback, and a rubric is provided to help guide the students' writing. Students are also reminded to use feedback from their presentations. A sample paper grade sheet/rubric is available in the Supplemental Material online.

Much science is done by working in collaborative groups. Successful completion of this project requires collaboration among group members. This component is assessed in part through peer review of group members that focuses on each group member articulating their own contribution to the overall project and their perception of the contributions made by the other group members. The instructor also looks for how much each group worked together and contributed to individual presentations and how well the final paper integrates each independent section into a coherent whole. For example, if a mistake is pointed out in feedback in an early presentation and the same mistake is repeated in a later presentation, it is apparent the feedback was not shared among group members. This falls under the grade category "group development of the trait."

○ Potential Learning Benefits

The semester-long case study model presented here for examining the process of adaptation by natural selection helps students contextualize and apply many major concepts in evolution. Students are encouraged to become active researchers in evolutionary biology. A strong case has been made for evolution instruction to not only integrate instruction in supporting fields such as genetics, but also include science practices (Beardsley et al., 2011; Catley et al., 2005). Researchers found that when students were given the opportunity to use science practices in evolution instruction, positive impacts on learning were observed (Glaze & Goldston, 2015). Students observe and read how researchers develop and apply evidence in evolutionary biology, then model this process using that evidence to develop their own arguments. Table 4 provides a brief summary of major learning benefits not necessarily specific to evolution that students felt they gained from this course design.

By applying the course material they recently learned about in class to their own particular trait and having to explain it back to the class along with supporting evidence they found, students benefit from reinforcement of core lessons, and misconceptions become apparent and can be addressed. Having a clear and unique goal for each student's research also moves the challenging process of reading primary literature from just-in-case to just-in-time learning (Schank et al., 1999). Research papers are being read not because the instructor assigned them, but rather because the student needs the information to answer their own unique question that they are responsible for to their group and have to present to their classmates.

Since groups work on a single trait throughout the semester, and since workload is split among members of the group, with each student focusing on a single component of the argument, a strong sense of ownership of course material can develop without it being all-consuming. Support of learning across each conceptual topic is provided by within-group collaborations (on the same trait but across different criteria). For example, many published studies do not focus on one student's particular goal but may actually provide information about multiple criteria. Students benefit by sharing and discussing these papers with other group members who also have a stake in understanding those research papers. Presentation class periods provide powerful reinforcement for each concept, as each presentation addresses the same major evolutionary concept but applies it to a different trait, so students watching the presentations see a variety of approaches and applications for each major concept.

In preparing their presentations, students practice critically evaluating evidence (data) found in primary literature and developing an argument using that evidence. They are encouraged to present and explain evidence from original figures found in the research papers. This also provides students practice interpreting and presenting data in graphical format. Collaborating with group members who focused on different types of evidence helps students synthesize ideas from different fields of biology. By having to link different types of evidence to address a single large question, students learn how to frame very specific arguments into a conceptual whole. The final paper in particular helps students build a multilayered complex argument from many detailed, often very specifically focused, studies that had different goals from those of the students.

Table 4. A summary of major learning benefits not necessarily specific to evolution that students felt they gained from this course design. These points were taken from discussions with students who took the course.

Contextualizing and applying major concepts in evolution
Increasing ownership of course material
Applying knowledge from lecture/lab directly to their question
Becoming active researchers in evolutionary biology
Reading the primary literature with a purpose (just-in-time not just-in-case learning)
Critically evaluating evidence (data) presented in primary literature
Interpreting and presenting data in graphical format
Presenting evidence about hypotheses in which they are invested
Collaborating with team members to synthesize ideas from different fields of biology
Learning to frame a specific argument into a conceptual whole
Building a multilayered complex argument from many detailed, specific studies

Flexibility of the Instructional Model

This case study model can be readily integrated into an evolution course currently taught with a lecture format. Each of the five major conceptual criteria are themes that are typically stressed in undergraduate evolution courses, and thus little or no content should be lost when adopting this model. Main adjustments to a lecture-based course may include changing the order of the presentation of topics, and changing five lecture days, or partial lecture days, to presentation days.

While ideal group size is five, smaller or larger groups can be accommodated by having students collaborate on one or more of the criteria. Larger class sizes can be accommodated by having shorter presentations, by not having every group present during each presentation day, or by having poster presentations that would allow a concept to be presented for many different traits at the same time. It may also be possible to apply this approach to students earlier in their academic development by supplying the groups with key papers to focus on (with question sets designed to help scaffold the material) rather than having them go into the primary literature on their own.

The course structure described here works for evolution in part because evolution is a topic that synthesizes information from across different areas of biology. A similar approach could be developed in other disciplines, provided there is an overarching question that can be partitioned into discrete units and the question can be applied to different situations/traits that allow each group to explore in their own direction.

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Supplemental Material

- Five Criteria to Test Adaptation Hypothesis
- Presentation Grade Rubric
- Paper Grade Sheet/Rubric
- Article Annotation Worksheet

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FEATURE ARTICLE

A Day in the Life of Carlton Smith: The Bombardment of Evolution Misconceptions

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Abstract

The United States still has one of the lowest evolution acceptance rates in the world. Biology educators have been diligent in their methods to increase evolution acceptance and knowledge, with much success. However, misconceptions still arise through education, textbooks, and even social and religious settings that may influence our citizens' low evolution acceptance rates. Much research has been done on such misconceptions. But with new technology and electronic advances, we now have a wide variety of information available at our fingertips. Advents such as social media, popular culture, and smart devices may incorporate new and unique misconceptions not seen before in evolution education. We see a younger generation raised in an era where they may experience a bombardment of evolution misconceptions daily, from the games they play to the posts or memes they read on social media. Research into these effects is lacking but may be essential to push the boundaries of evolution education into the future.

Key Words: *evolution education; misconceptions; popular culture; education; social media.*

○ Introduction

Evolution is the central theme in any biology class (Dobzhansky, 1973; American Association for the Advancement of Science, 2011; Brownell et al., 2014). However, the United States has one of the lowest evolution acceptance rates globally (Miller, 2006). We see different acceptance levels throughout the general U.S. population, from about 45% to 60% (Pew Research Center, 2012; Gallup, 2017), which can change based on how questions are asked (Pew Research Center, 2019). College students show a similar pattern, with about 41% of college students in the United States rejecting human evolution (Barnes et al., 2008), which is different from scientists' views on human evolution: 98% acceptance (Pew Research Center, 2012). There seems to be a difference in evolution acceptance rates between people not specialized in science and those most informed.

In an effort to help teachers be prepared for the potential misconceptions and barriers they may face in their students, we follow a hypothetical high school student, Carlton Smith, through a typical day.

One reason for nonacceptance among college students in the United States is religiosity (Coyne, 2012): one's level of commitment to religious practices and principles focusing on their belief in a god (Cornwall et al., 1986). Students with higher religiosity are more likely to reject evolution (Heddy & Nadelson, 2013). Students reject evolution when they find it incompatible with their religious beliefs or feel it contradicts what they have been taught (Cobern, 1994; Dagher & BouJaoude, 1997; Downie & Barron, 2000; Stanger-Hall & Wenner, 2014). If teaching students evolution threatens their beliefs, they will stick with what they know (Meadows et al., 2000) and refuse to learn evolution (Nadelson & Southerland, 2010). Kostas Kampourakis argues in his book *Understanding Evolution* (Kampourakis, 2014) that part of the problem is that experts see evolution as easy to understand, while many students find evolution challenging. Understanding students' views on evolution knowledge and acceptance seems essential in evolution education (Barnes, Dunlop et al., 2020; Ferguson & Jensen, 2021).

Students may enter a classroom with an understanding of a concept or principle that differs from the generally accepted views or interpretations of that principle. In our review, we will define misconceptions this way. Leonard et al. (2014) highlighted the idea that determining the origin and structure of different ideas commonly held by students is crucial for future work in science education. Understanding how students go from misconceptions to scientific knowledge is essential for teachers to help students overcome these misconceptions through conceptual change (Scott et al., 1992). However, the prevalence of misconceptions is not necessarily unexpected, given that many secondary teachers themselves hold misconceptions (Yates & Marek, 2014; Glaze et al., 2015), and many

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shy away from the topic, based on their own religious discomfort (Meadows et al., 2000; Plutzer & Berkman, 2008) or their lack of confidence in their ability to teach these concepts in the face of potential student conflict (Meadows et al., 2000; Griffith & Brem, 2004; Meadows, 2009; Glaze et al., 2015; Borgerding, 2017; Tolman et al., 2021). Thus, teachers may be facing not only their own discomfort and potential lack of knowledge, but also a fear born of ignorance of what their students are bringing to the table. How little do their students know? What misconceptions do they hold? From where are these misconceptions stemming? What barriers are teachers going to have to help the students overcome? How have these barriers formed?

It is to these last questions that we address this review. In an effort to help teachers be prepared for the potential misconceptions and barriers they may face in their students, we follow a hypothetical high school student, Carlton Smith, through a typical day. Though this story is fictional, it is based on actual experiences that have been shared with us from students willing to share their personal interactions with evolution through interviews, office hours, and classroom discussions. The dialogue is our own, but the experiences Carlton has with evolution are real. And through this story, we will introduce teachers to the literature that has shed light on these issues and to the potential evidence-based solutions to each of these issues that exist. It is our goal that, armed with this understanding and this evidence based in an authentic scenario (i.e., a typical day for a typical student like Carlton), teachers can enter the classroom with more confidence, better preparation for what they are facing, and better tools for how they can best help their students overcome these barriers and accept the foundational theory of biology.

○ Carlton in His High School Biology Class

Carlton enters his eight o'clock biology class with a few minutes to spare. He sits next to one of his best friends, who is quietly giggling to himself.

"What is so funny?" Carlton asks.

His friend slowly shows him an image he was viewing on his phone. It is a picture of a chimpanzee shrugging its shoulders with its arms raised above its head. In white letters, it says, "If we came from monkeys, why are there still monkeys?"

"It is so dumb that people believe in evolution," his friend says. "I am interested to see how today goes; it should be a good laugh."

Carlton's friend went back to viewing his phone. "That's right," Carlton thought. "Today we are learning about evolution."

In the last class, their teacher had said, "The state requires us to learn about evolution, but I will make it as quick and painless as possible."

When class started, Carlton's teacher talked about Charles Darwin, "the scientist who came up with the idea of evolution," which he called natural selection. Throughout his class, Carlton heard phrases such as "survival of the fittest," "organisms must adapt to survive," "Darwin's theory," and "organisms choosing to change." After the lecture, the instructor asked students to spend the last few minutes reading a textbook page. The text went into a little more detail on natural selection and how the environment determines the evolution of any given species on Earth. After the textbook reading, the teacher dismissed the class, and students went about their day.

○ Misconceptions in Learning and Teaching

Let us pause for a moment and discuss Carlton's class. We get the impression from Carlton's teacher that she does not want to teach evolution in the class but must because of state standards. Teaching evolution in high school is problematic for some teachers, to the point that they try to avoid it at all costs. As shown in Rutledge and Mitchell's study (2002), 43% of teachers characterized their teaching of evolution as avoidance or said that it was briefly mentioned. This can be especially problematic for teachers in religious populations; sometimes, they opt out of teaching evolution and teach creationism instead. As Berkman and Plutzer (2011) showed in their study of 926 high school teachers across the United States, 13% taught creationism instead of evolution. Although the number of teachers teaching creationism in the classrooms is decreasing, teaching creationism in a science classroom is still present and problematic (Plutzer et al., 2020).

Studies suggest that evolution in high school biology may be absent or fraught with misinformation (Eglin, 1983; Johnson, 1985; Roelfs, 1987; Shankar & Skoog, 1993; Rutledge & Mitchell, 2002; Plutzer et al., 2020). When teachers hold misconceptions about evolution, students may acquire more misconceptions about evolution even though they may feel more confident in their evolution knowledge. Yates and Marek (2014) showed that students had more misconceptions about evolution after taking a high school biology class, and they noticed more misconceptions among students whose teachers held more misconceptions. A significant concern about evolution education lies with the association of misconceptions with words such as *theory*, *fact*, and *proven* (Bybee, 2001), as these words can impede a student's ability to learn biological concepts (Rector et al., 2013). This can lead to misconceptions (Zukswert et al., 2019). Preservice science teachers also hold misconceptions about evolution, such as the idea that evolution always selects the healthier, better, and perfect individual or that creation and evolutionary theories are conflicting (Karataş, 2020). Secondary and high-school-level biology classes may even create misconceptions, as Karataş in her 2020 study showed; 30% of students had evolution misconceptions, some of them possibly coming from their classes. The teachers, however, cannot and should not be the only ones to blame.

Misconceptions may be found throughout textbooks and sometimes can go undetected (Tshuma & Sanders, 2015). One study in a medical school concluded that seven different anatomy textbooks inaccurately described eye anatomy (Wood et al., 2020). If experts or educators rely on textbooks for accuracy, this can be concerning. In their 2015 study, Tshuma and Sanders found these misconceptions about evolution among students: "individual organisms evolve," "organisms adapt during their lifetimes," and "environmental change is essential for evolution"; they found these same misconceptions in the textbooks (Sanders & Makotsa, 2016). Another misconception students have is about the centrality of evolution to the study of biology. We, as biologists, claim evolution to be the central theme (Dobzhansky, 1973) in our biology classes. Still, our discussions of evolution within other topics are lacking (Nehm et al., 2009), especially our discussion of macroevolutionary processes (Padian, 2008, 2010). In summary, in an educational setting, there are many ways that students can develop misconceptions. This is concerning, but there are proposed ways to address misconceptions in the classroom.

○ Potential Solutions

One simple solution to combat misconceptions is to use a survey instrument that measures misconceptions among students. A survey can help educators find what misconceptions students are holding, and there are a few surveys that could be beneficial for educators. The first is the Biological Evolution Literacy Survey developed and tested by Yates and Marek (2014), which is a 23 item survey that has been used on high school students, college students, and high school teachers to determine evolution misconceptions. Educators could also use the Conceptual Inventory of Natural Selection (CINS) developed and tested by Anderson et al. (2002), which is a 20 item survey measuring common conceptions about natural selection. Both surveys have shown reliability, validity, and readability, which is important for students taking the survey. There are also many resources that you can use in the classroom that can help students learn about evolution; one source of these is HHMI BioInteractive (https://www.biointeractive.org). This website can be used for kindergarten through college students and may be a helpful resource for students and educators.

Workshops have also been shown to be effective. In one workshop attended by secondary teachers and religious teachers, Kaloi and colleagues (Kaloi et al., 2022) had activities for the teachers in which they measured hominin skull features and predicted relatedness. This workshop also allowed discussion about how we know what we know about hominid evolution and what things we do not yet know about hominid evolution. After the workshop, teachers were significantly more confident in their ability to teach human evolution (Kaloi et al., 2022). Although this example may not specifically deal with misconceptions, it can help educators build confidence in their ability to educate students and correct misunderstandings of evolution.

○ Carlton in Social Settings

Let us continue following Carlton throughout his day.

On the way home, Carlton sits next to his older brother William on the bus.

"What are your thoughts on evolution?" He asks his brother.

"Evolution … like Pokémon?" replies his brother.

"No, like scientific evolution," says Carlton with a bothered look on his face. "You know, how people say we 'evolved' from monkeys and such."

"Don't worry about it too much," says William, comforting his brother. "It's just a theory, and they don't have much evidence for it. It is just a bunch of atheists that want us to stop believing in God. Every year, when they talk about evolution in school, many kids have questions just like you. So tonight, at youth group, Pastor Dan will talk about evolution and hopefully help you feel better about what we believe. I had similar questions when I learned about evolution as well, but the youth group was helpful."

"Thanks, William, that makes me feel better. I am looking forward to tonight," Carlton says with a smile on his face.

That night at the youth group, Carlton and a group of kids sat in their church building's side room. Their chairs formed a half circle, and Pastor Dan stood in the middle at his portable podium right underneath the single light in the room as the kids all listened and watched.

Pastor Dan smiled and said, "I know many of you have questions about what you learned in school today. Evolution can be confusing and difficult to understand. I am here to help you make sense of evolution as best I can." He grabbed his portable podium and started walking around within their arc of chairs. He praised science for its accomplishments. He said, "We know a lot of stuff about this world because of science. Science is the pursuit of understanding God's handiwork. I love science, and I love religion. But, when science says we morphed from monkeys, it simply is not true. When science says that animals and plants can become different "unique" organisms, it simply is not true. It is possible that animals can adapt to new environments, and science seems to get that part right, but not much else in terms of the creation of the Earth." Pastor Dan speaks more about God and the design and urges them, when confused, to start with the Bible and choose God. Pastor Dan said all these things in confidence that the Bible is the word of God and holds the truth of the creation. After it was over, Carlton's mom was waiting to take him home.

She asked as he got into the car, "How was it, dear?"

"Fine, I guess," he said exhaustedly. "It's just a lot to take in right now. Evolution seems silly, and I think I have spent too much time thinking about it today. I just want to go home and relax."

"It is silly," his mom said. "Don't think about it anymore than you need to; it is a waste of time. Just remember God lives, and the Bible is true."

○ Misconceptions in Social Settings

When Carlton was talking about evolution on the bus, his brother said evolution was "just a theory." In American vernacular, a theory is often considered an imperfect fact (Gould, 1981) or "hunch" (Nelson et al., 2019). When the word *theory* is viewed by students using the everyday vernacular meaning, and when their teachers use it with its scientific meaning, it may cause confusion, leading to misconceptions. And many students struggle when they learn that science is an ever-changing field, especially when the word *theory* is involved. Larochelle and Désautels (Larochelle & Désautels, 1991; Nelson et al., 2019) interviewed students 14 to 16 years old and found that they thought of science as one person's opinion (especially when *theory* was used) instead of as a collaboration by many people. In another study, Dagher and BouJaoude (2005) interviewed 15 students. Out of the 15 students, 3 were uncertain about evolution theory. These students had misconceptions about both evolution and scientific theories. Social interactions and the use of words with different meanings in everyday speech than in the scientific field can undoubtedly lead to misconceptions.

One of the biggest reasons students struggle with accepting evolution is religion (Coyne 2012) and the perceived conflict between religious beliefs and evolution (Barnes, Supriya et al., 2021). Many studies show that the more religious someone is, the less likely it is they will accept the theory of evolution (Ha et al., 2012; Rissler et al., 2014; Glaze et al., 2015; Barnes, Brownell, & Perez, 2017; Barnes et al., 2019; Dunk et al., 2017). The problem is not a single religion or religious belief but how religious people view science. According to Barnes and colleagues (Barnes, Dunlop et al., 2020), 48% of religious students included in their study said you have to be an atheist to accept evolution, indicating students would have to give up their belief in God to accept evolution. In Carlton's case, his pastor said the same thing, religion and human evolution are incompatible. Some scientists suggest a similar view: science and religion are not compatible, and science can disprove a god(s) (Harris, 2005; Dawkins & Ward, 2006; Krauss, 2015; Coyne, 2016).

Although many educators do not hold views like those of Carlton's pastor or the above scientists, they still do not feel it is their responsibility to increase evolution acceptance (Barnes & Brownell, 2016). With most U.S. populations having a Judeo-Christian religious belief (Pew Research Center, 2016a), this can be troublesome for religious students taking science classes. The misconception that students have a presumed dichotomy in their lives, that they must choose their religious beliefs or accept evolution, is not scientific, nor is it helpful.

○ Potential Solutions

As educators, we still have students struggle with accepting, understanding, and learning about evolution, but there are many scientists and educators who are effectively teaching evolution in their classrooms. Barnes et al. (2017a) discuss the importance of scientists and teachers becoming culturally competent in educating students about evolution. They highlight important practices used to incorporate culturally competent methods in classrooms successfully, such as acknowledging a possible conflict between different parts of a student's beliefs on evolution (Dagher & BouJaoude, 1997; Deniz et al., 2008), having students explore their personal views on evolution (Manwaring et al., 2015; Lindsay et al., 2019), providing students with a role model (Barnes, Elser, & Brownell, 2017; Holt et al., 2018; Ferguson & Jensen, 2021), and focusing on the nature of science (Dunk et al., 2017, 2019). These methods of teaching students have been shown to help decrease religion-evolution conflict while increasing evolution knowledge and acceptance, and most are relatively easy to incorporate into the classroom. One study showed that a six-minute discussion about religion and science compatibility was enough to change students' views about evolution (Truong et al., 2018). It may also be helpful for educators to remember the *bounded nature of science* due to which only natural phenomena can be investigated. Teachers can also help students understand that science is agnostic and not atheistic, which has been shown to help religious students overcome the misconception that religion conflicts with science (Barnes, Dunlop et al., 2020) and thus help them keep their religious identity and become more accepting of evolution.

○ Carlton and Popular Media

Let's return to Carlton one more time. That night, Carlton finally has some time to relax and unwind from his long day at school and the youth group meeting. He decides to sit down and play one of his favorite games of all time, Pokémon. While playing, Carlton can make his favorite Pokémon evolve to its final and strongest evolutionary form by having it battle with another Pokémon. After he spends some time playing, he wonders if Pokémon's evolution is like evolutionary theory. So, he logs in on Facebook and posts a question asking his friends their thoughts on Pokémon evolution and evolution theory. He gets a few responses. Some of his friends say, "Sounds the same as we learned in class today." Others say that it is "more like a metamorphosis." Confused, he decides to search Google and finds a lot of information about Pokémon evolution. One blog he reads talks about other possible theories on how Pokémon evolution happens and its plausibility. He also finds a published scientific paper about Pokémon evolution, where they used "trees" to build the Pokémon's evolutionary history (Shelomi et al., 2012). He also finds many other blogs claiming that Pokémon evolution does not help people understand the theory of evolution. With all this information, he decides to take a break and watch TV.

○ Misconceptions in Popular Media

This last scene shows how students may come across misrepresentations of evolution even during relaxing times. With the wide variety of popular media accessible to students, they may enter a science classroom with various illusions obtained through popular media. A popular book, *Jurassic Park* (Crichton, 1991), turned into a movie by Spielberg in 1993, is based on scientists' ability to extract ancient DNA from dinosaurs, fill in missing fragments with frog DNA, add the DNA to ostrich eggs, and simply bring dinosaurs back to life. The fact that the dinosaurs live, breathe, and thrive is a biological implausibility glossed over quickly and casually (Van Riper, 2003), and many students think it is possible. Popular media may affect how people view science in general. Still, evolution is a subject often brought up in popular media, not always accurately, due to its controversial nature. Evolution is discussed in many popular media scenarios, from *The Simpsons* (see the 2019 Bosky essay on science and religion for more details) to other movies, series, and even popular kids' shows and video games like Pokémon.

As a franchise, Pokémon is the highest-grossing video game franchise of all time (Burwick, 2018). In the Pokémon world, players collect and catch organisms (Pokémon; the word is both singular and plural) and use them in battles. A Pokémon battle, where two or more Pokémon fight each other, allows your Pokémon to become stronger. When you help your Pokémon become stronger, they can sometimes "evolve" into bigger and more powerful Pokémon. One Pokémon may evolve and get bigger, grow a pair of wings, or even lose limbs. When a Pokémon evolves, it happens instantly, but it is more akin to metamorphosis than the process of evolution. When students have been watching shows like *Pokémon*, where the word *evolution* is frequently used, albeit differently than in science, this exposure may affect how students perceive the theory of evolution. Some articles have claimed that using the word *evolution* in *Pokémon* may negatively influence how people learn about evolutionary theory in science classrooms (Chamary, 2016). Naturalish (2018) used scientific ideas such as enzymatic reactions and DNA recombination to explain Pokémon evolution's plausibility better. Recently, a researcher and a big Pokémon fan tried to reconstruct the evolutionary history of Pokémon by creating a Pokémon phylogeny (Shelomi et al., 2012; Fortuna, 2016). These last few examples explain Pokémon evolution using scientific methods and may do more harm than good. As students come across inaccurate evolution statements, research shows, they may develop misconceptions and may have trouble following along with the educators (Osborne & Freyberg, 1985; Smith & Abell, 2008).

Popular media is not the only thing that may cause misconceptions about evolution. Social media is taking up most people's time every day, especially among our younger populations. When Carlton had a question, he reached for his phone, wrote a post on Facebook, and waited to hear what his friends on Facebook had to say. He also decided to Google his question. Carlton found some articles and blogs that gave him some answers, but they often contradicted one another. He got similar results from his friends on Facebook. About 62% of adults get their news and updates from social media (Pew Research Center, 2016b), even though about 65% of Americans think the news on social media is inaccurate (Blatchford, 2018). This can promote misconceptions, especially considering that misinformation moves around faster and more in depth on social media than on news media (Vosoughi et al., 2018). We are seeing a new generation of students entering our classrooms. These students have grown up with smartphones and tablets. They have learned how to find information differently than past students because it is more readily available.

Let us consider Carlton again. At the beginning of our story, Carlton's friend shows him a meme he found about evolution. The meme states this: "If we came from monkeys, why are there still monkeys?" This statement is a misconception about evolution. This meme falsely gives the idea that evolution teaches that we come from monkeys. Is it possible that social media posts and memes influence students' evolution acceptance?

○ Potential Solutions

Currently, we do not know the answer, and this is something worth studying. Very little research has been done on misconceptions in social media, and even less (i.e., none) has been done on potential interventions and solutions for correcting these. It is possible that the culturally competent methods used recently in teaching evolution (Barnes, Brownell, & Perez 2017) could also be applied to popular media. Cultural competence can help bridge the cultural gaps between students and educators and make science more inclusive (Barnes, Supriya et al., 2020; Barnes, Maas et al., 2021), and with the amount of time students are spending watching TV or scrolling through social media, popular media might be influencing how students view and interpret the world. Thus, cultural competence is important, but it is only one way to potentially solve the problem. Using popular media in our classrooms may be another solution.

Van Riper (2003) argues that although popular media sometimes gets it wrong, discussion can turn these incorrect moments into genuinely teachable moments. Talking about the complexity of *Jurassic Park* or the inaccuracies of Pokémon evolution in a classroom may increase student engagement, while allowing for a class discussion on the scientific flaws or accuracies. In ecology, conservation, and wildlife biology classrooms the Pokémon GO app has become popular because of its similarity to the natural world (Dorward et al., 2017; Lupton, 2017; Deslis et al., 2019). For example, when walking along a river in nature, you are likely to see birds, bugs, and fish; Pokémon GO would follow a similar pattern. While playing the game along a river, you would most likely see Pokémon that resemble birds, bugs, and fish. This app has been shown to benefit schools in urban areas where nature is not readily available to observe and collect data from (Lupton, 2017).

Educators need to be aware of the bombardment with evolution misconceptions students are obtaining through popular media. Using popular media references of evolution in our classrooms may make learning evolution more engaging and enriching for the students. It is also a way Of approach the inaccuracies of these references in a way that is culturally competent without attacking students' cultural beliefs. The research on the effect of popular media on the acceptance of evolution is lacking, and with the amount of time students spend viewing popular media, using it in the classroom may be a good starting point to help engage students to think about what they view on popular media differently.

○ Conclusion

This review was written as a guide for teachers to better understand where their students are when they enter the classroom—what misconceptions they have and where these misconceptions came from. Hopefully, teachers who use this review will also have gained some new and better tools to overcome the cultural barriers that exist within their classrooms. Thus, they can enter the classroom with confidence to teach evolution most effectively. Using culturally competent practices and including popular media in our classrooms may be a unique way to approach students' bombardment with evolution misconceptions.

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FEATURE ARTICLE

Evolutionary Medicine: An Introduction

CHAY BEESON, ERICA KOSAL

Abstract

Evolutionary medicine is a growing area of research and practice; however, it is not widely discussed in introductory biology courses. Because of the interest in human biology, using evolutionary medicine is a great way to hold students' interest, placing topics in context, making the subject of evolution relevant. Too often students lose interest in learning about evolution because they think it is not pertinent. The pedagogical technique of using case studies in the classroom engages students in a way that will grow their understanding of topics, in this case evolution, as well as helping students with critical thinking and process skills, growing their scientific literacy.

The following case study is appropriate for an introductory biology course that focuses on evolution, an AP biology course, or an introductory evolution course for undergraduates. The case focuses on the evolutionary perspectives of what might be causing human ear infections, as well as the role of beneficial species of gut bacteria in maintaining a healthy immune system. It is advantageous for students to know about natural selection and coevolution before using the case study.

Key Words: *evolutionary medicine; evolution; natural selection; case studies; ear infections; bacteria.*

○ Introduction

Case studies are stories based on data that can be used in the classroom to paint a picture of relevance to students. Instead of giving a traditional lecture to a class, using a case study is more engaging and interactive, with students becoming involved in solving parts of the case study, working through different problems and scenarios. To students, it is like solving a puzzle of sorts. Case studies can be fun for students, providing relevance for topics, and helping students learn concepts more fully as a result. Case studies have been shown to help develop a student's critical thinking skills (Popil, 2011). Additionally, there is evidence that case studies are more effective than classroom discussions and textbook reading when it comes to students learning key biological concepts and that students find they have gained in oral and written communication skills as well as the ability to see connections to aspects of life (Bonney, 2015).

If traits of humans are influencing the population of a pathogen via coevolution, how might this affect how physicians treat a pathogen that is causing a bacterial infection?

The following case study is for implementation and use in the classroom. The focus is on evolutionary medicine and consists of two parts to work through in class, followed by a third part for homework to reinforce the concepts covered in the case study. Evolutionary medicine is a field where questions surround why the human population is susceptible to particular diseases or ailments due to human evolutionary history, in hopes of providing medical solutions to these problems. Instead of solely focusing on treating a disease or illness once acquired, evolutionary medicine tries to be proactive in understanding how to prevent diseases based on natural selection. For this reason, some people refer to evolutionary medicine as Darwinian medicine, in reference to the intersection between evolution and medicine. This field broadly addresses why natural selection, over evolutionary time, has given us traits that leave us vulnerable to disease and illness.

This case study is appropriate for an introductory biology course that focuses on evolution or for an introductory evolution course, both of which are likely at the 100 or 200 level for undergraduates. It may also work well for an AP biology course in high school where an emphasis is placed on evolution.

See the Supplemental Material available with the online version of this article for an answer key to the questions asked in this lesson.

Learning Objectives

Students who successfully complete this case study will be able to do the following:

- Explain the value of using evolutionary medicine to tackle a medical problem.

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- Describe briefly the evolution of the human ear that lends itself to fluid buildup and ear pain today.
- Explain how coevolution works, specifically with respect to human ear infections and bacteria.
- Describe from an evolutionary perspective why beneficial species of bacteria are necessary to keep human systems in balance.
- Interpret data from graphs.

○ Part I: How the Past Can Affect the Present

Dr. Penny Selin had the treatment for every disease and infection. The common cold? She knew all the right remedies for a speedy recovery. A broken foot? An easy fix. Yet, above all else, Dr. Selin's expertise was treating bacterial infections. For as long as she had been a doctor, Dr. Selin had prescribed antibiotics, medications that either kill bacteria or hinder their growth and reproduction. Some of the antibiotics that she prescribed include cephalosporins and fluoroquinolones. Cephalosporins treat strep throat, while fluoroquinolones treat pneumonia, urinary infections, and other infections (Lewis, 2021). And, most of the time, the bacterial infection would completely disappear, never to return!

However, sometimes the antibiotic treatment would not work, as was the case for her most recent patient, Ray Z. Stance.

Ray Z. Stance had a terrible ear infection, even after many doctor visits and prescriptions. Dr. Selin prescribed him clarithromycin, a common antibiotic used to treat ear infections (Lewis, 2021). However, she was surprised when her prescription of clarithromycin did not cure Mr. Stance. Perhaps she should have prescribed a higher dosage of clarithromycin? Or, maybe she should have told the patient to take the drug twice a day, rather than once a day? Unsure of what the problem might be, Dr. Selin consulted her colleagues, who reminded her about evolutionary medicine—a field that considers medical problems through an evolutionary lens. In other words, it is the study of the root causes of why the human body evolved the way that it did.

Dr. Selin's colleague explained how evolutionary medicine might be used to study the origins of, and subsequent problems found with, another organ or structure. "Let me use the illustration of the appendix," the colleague started. He explained that the appendix, a blind sac that is found at the junction of the large and small intestines, was traditionally cited as a "vestigial structure"—a structure that was inherited from an ancestor but, on a superficial level, is no longer serving a necessary function for the body.

"Because a traditional belief was that the appendix was not vital, a common medical practice was to remove the appendix if it became inflamed or irritated. Recent research, however, has called this into question, and we now know that the appendix is designed to protect good bacteria in the gut."

Study of the appendix detected lymphoid tissues inside; essentially, these tissues are sites of lymphocytes and other white blood cells. The lymphocytes are important because they determine the specificity of the immune response. The appendix can therefore protect good bacteria by attacking harmful pathogens. Researchers have found that appendixes of species that contain high average concentrations of lymphoid tissue promote the growth of beneficial gut bacteria in the digestive tract, aiding in the body's immune response (Smith et al., 2017).

Dr. Selin was glad for the reminder and thought, "Evolutionary medicine offers a perspective on how to treat diseases that may lead to insights overlooked without the use of an evolutionary lens. This gives me some great new ideas for my patient. Because the bacteria causing the ear infection seem to be showing antibiotic resistance, I need to consider other contributing factors and how the design of the ear itself may be contributing to the problem."

According to natural selection, an environmental pressure is a factor exerted on a population that allows organisms with a certain genotype within the population to reproduce more and pass on that genotype to the next generation. The individuals with this genotype have an advantage over the other members in the population that do not have this genotype. In other words, the individuals with this genotype reproduce more, resulting in an increase in alleles producing those traits in the next and subsequent generations. How might this help us understand the evolution of ear infections?

An ear infection is an inflammation of the middle ear that occurs when fluid builds up behind the eardrum. It is usually caused by bacteria (Figure 1). The middle ear is located between the eardrum and the inner ear. Small amounts of fluid are normally produced in the middle ear and then drained through the eustachian tube, which connects to the nasopharynx, the upper part of the throat. If this fluid gets trapped, it can cause an earache. This fluid can accumulate when allergies cause inflammation or cause mucus to block the eustachian tubes. And often, an ear infection is preceded by a respiratory infection that is caused by bacteria. These bacteria can move to the middle ear, which can stimulate the buildup of fluids.

Children tend to get ear infections more than adults, due to the smallness of their eustachian tubes, which makes it difficult for fluid to drain out of the ear. This issue is compounded when a child

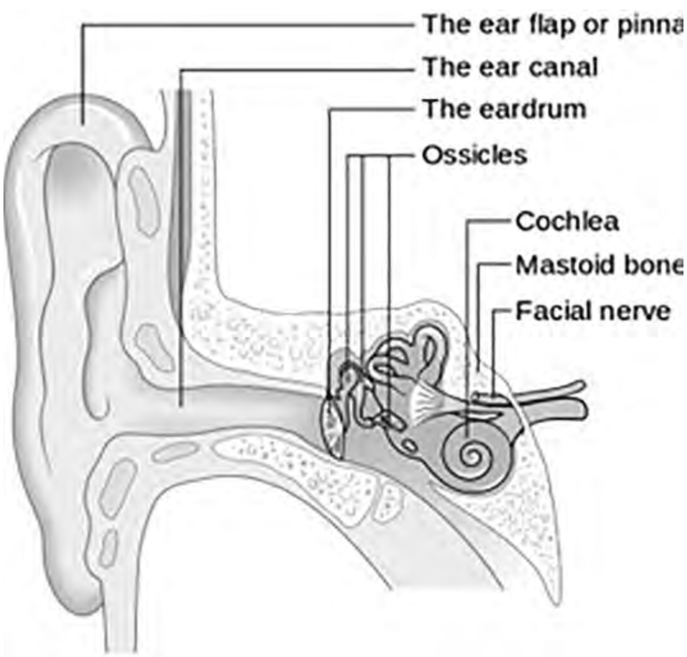

Figure 1. The parts of the human ear. Notice that the eardrum marks the boundary between the outer and middle ear—this is where fluid can become trapped, causing an earache. The cochlea is part of the inner ear, which produces nerve impulses in response to sound vibrations, therefore assisting with hearing. *Cancer Research UK, 2014; available on Wikimedia Commons.*

has a cold or a respiratory illness, because mucus can block the tubes and prevent the fluid from draining at all. Additionally, the immune system of a child is still developing, which makes it harder for children to fight infection compared with adults.

Why did this evolutionary design happen? The evolution of the eardrum and the middle ear is what has allowed mammals to hear through the medium of air. In vertebrate ancestors, the three bones that make up the middle ear were instead parts of the jaw. These reptile-like mammals (called cynodonts) had a primary jaw joint that served for both chewing and hearing. The multiple skeletal remains of an ancient species of mammal have revealed that hearing and chewing separated as a definitive mammalian middle ear was evolving, which likely improved hearing (Mao et al., 2019). Modern mammals have three tiny bones in the middle ear that help them hear. The old-style jaw joint of the earliest ancestors of mammals has evolved to become part of the mammalian middle ear. Many early mammals were small insectivores that probably hunted at night, and having more bones in the middle ear would have improved their hearing, allowing them to detect their prey and to avoid predation themselves. With this improved hearing, mammals could likely detect higher-frequency sounds than other types of animals such as reptiles (Kitazawa et al., 2015). The cavity of the middle ear is thought to form as an extension of the pharynx, which connects to the middle ear with the eustachian tube, and its diameter is dictated by growth of the brain and head anatomy (Tucker, 2017).

Questions

1. Define evolutionary medicine in your own words. Why might it be beneficial to study evolutionary medicine alongside traditional medical fields like immunology or pathology?
2. How do antibiotics influence a bacterial population?
3. What would happen if a few individuals in an infection-causing bacterial population have a gene that allows them to withstand antibiotics? Moreover, what would happen to the bacterial population over several generations?
4. What will happen when bacteria develop antibiotic resistance?
5. How are antibiotic resistance and evolutionary medicine connected?
6. Over evolutionary time, the human middle ear evolved. Summarize the major change that occurred with the middle ear and how that led to a predisposition for ear infections.
7. If eustachian tubes had evolved larger than their current sizes, what possible consequences might be the result of this change. Would ear infections still occur? Regardless of your answer, elaborate.
8. Do you think natural selection will influence the human body such that it will eventually evolve to prevent ear infections? Explain.

○ Part 2: How the Present Can Affect the Future

After collaborating with her colleagues, Dr. Selin prescribed a different antibiotic, amoxicillin, for Ray Z. Stance. Soon enough, the antibiotic worked, and the ear infection that plagued Ray eventually vanished.

A few days later, Ray Z. Stance's sister Prudence showed up to Dr. Selin's office, concerned about the possibility of developing an ear infection herself. So far, she had no medical history of ear infections, due to her cautious nature, yet her brother Ray, as well as her other siblings and parents, had all had ear infections in the past. Attentive to Prudence's concerns, Dr. Selin considered other ways to treat ear infections. Dr. Selin knew there was a relationship between gut bacteria and humans that could influence how a human's immune system functioned, and she wondered if there might be some connection to ear infections.

Coevolution is a form of natural selection in which two species (in this case, humans and bacteria) evolve based on the adaptations of each to the other. Many species of animals coevolve with bacteria that live in their gut. In one study, a group of researchers looked at fecal samples from humans and 59 other mammalian species and sequenced the DNA of bacteria found in those feces. By analyzing the DNA of a sequence of a gene found in every organism (16 S ribosomal RNA), they found that mammalian host diet and phylogeny (evolutionary relationships among species) influenced bacterial diversity. They found the *most* bacterial diversity with herbivorous mammals and the *least* bacterial diversity among carnivorous mammals. Modern humans are omnivorous, and our gut biomes closely match those of other omnivorous primates (Ley et al., 2008). The bacteria living in the guts of humans can act as selective agents on humans, and adaptations of humans can act as selective agents on these bacteria as well. For example, reduced fiber in the diet of humans results in microbes competing for this limited resource. Since fiber is a major factor that defines niche space in the gut, if fiber is decreased, the number of microbe species decreases as well. Antibiotics can also reduce the microbes in the human gut, reducing the number of species present. In turn, the reduced niche space and biodiversity in the gut seem to destabilize how the host can respond and recover from illness. For example, in a study with mice, a reduced-fiber diet led to a decrease in microbe diversity, and the mice failed to recover from antibiotics (see Venkatakrishnan et al., 2021). Dr. Selin was pondering all of this as well and putting things together. She then turned to consider whether replacing some species of bacteria in the host could be beneficial to restore "balance" and therefore help with minimizing ear trouble.

Since ear infections are caused by harmful species of bacteria, with the inflammation and fluid buildup in the middle ear resulting in pain, could a coevolutionary relationship be present here? Could additional beneficial species of bacteria outcompete the harmful ones and minimize ear infections? Dr. Selin was considering as many angles as she could possibly think of. She recalled an article that discussed how inflammation caused by the immune system responding to infection can be aided with probiotics—beneficial species of bacteria found in pill form that supplement the natural microflora of the human gut.

When the gut microflora is harmed, beneficial bacteria naturally found in the human gut are destroyed and cannot aid in healing certain infections, including respiratory ones (Shahbazi et al., 2020). To replace these missing bacteria, probiotics are used. As Dr. Selin was contemplating her patient, she remembered a recent conference she attended where research was presented on the use of probiotics. A group of researchers assessed whether probiotics could help prevent the occurrence and reduce the severity of middle ear infections in children. Seventeen randomized controlled trials with children up to 18 years, with comparisons of probiotics and placebo, were included in the study that involved 3488 children. In this large sample, the number of children who presented with an ear infection was lower for those taking probiotics than for the control kids, especially for children who were not prone to infections in the first place. Additionally, probiotics decreased the proportion of children taking antibiotics for any infection (Scott et al., 2018). Dr. Selin concluded that probiotics may help keep the gut biome healthy, which is advantageous, as a healthy gut biome aids with the immune system overall and helps prevent pathogen invasion (Shahbazi et al., 2020); various researchers have pointed out the correlation between probiotics and reduced ear infections, but Dr. Selin knew of one study in particular that stood out to her. In this case, the probiotic bacterial species *Lactobacillus rhamnosus* was likely boosting the immune system and preventing the growth of harmful bacteria known to cause ear infections (Gasta et al., 2017).

By evolutionary design, the middle ear is susceptible to fluid buildup in small eustachian tubes. To help prevent harmful bacteria from taking hold in the ear and setting up a chain of events that leads to ear infections, probiotics may be part of the solution.

By studying the ear and ear infections through the lens of evolutionary medicine, Dr. Selin was reminded that keeping the entire human body in homeostasis through any means possible, including introducing beneficial bacteria to the human gut via probiotics, is a key to staying healthy.

With all of this information in mind, Dr. Selin recommended to Prudence that she take several probiotics in hopes that they would make Prudence less susceptible to ear infections. Prudence thanked Dr. Selin multiple times for the visit, feeling at ease knowing that she could take preventative measures against possible ear infections.

Questions

1. As mentioned above, the gut biome is filled with bacteria that can influence the effectiveness of the human body's immune system against pathogens. Describe a plausible mechanism by which probiotics can help with ear infections.
2. The data in Figure 2 focus on a species of bacteria, *Pseudomonas fluorescens*, and its predator, *Tetrahymena thermophila*, a species of protist (Friman et al., 2013). The optical density of the bacteria population (an indirect measurement reflecting the overall size of the population) was measured for six hours at one-hour intervals via bacterial dilutions.
    a. What can be concluded from the data? Does population density/size tell us anything about coevolution despite frequencies of traits or alleles not being present on the graph? Justify your thoughts.
    b. If more data were collected over a longer period of time, for example several months, would they show that coevolution had occurred? If so, what patterns would you expect to see on the graph? Would you need more data or different types of data to make this determination? Explain.
3. If traits of humans are influencing the population of a pathogen via coevolution, how might this affect how physicians treat a pathogen that is causing a bacterial infection?

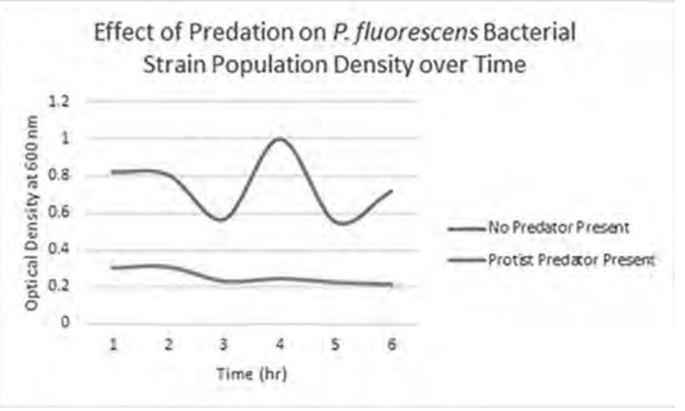

Figure 2. Optical density of *Pseudomonas fluorescens* with (bottom) and without (top) the presence of *Tetrahymena thermophila*.

○ Part 3: Homework

1. To help summarize what has been learned through this case study, create an infographic that covers at least three of the main topics that have been covered (coevolution, evolutionary medicine, ear infections, probiotics, etc.). Be as creative as possible!

○ Helpful Video Clips

The Traits That Spawned the Age of Mammals from PBS, https://www.youtube.com/watch? v=R7IaRQPJHf4

This Ancient Mammal's Ears Were Built for Chewing from SciShow, https://www.youtube.com/watch? v=3jXdKcqkeQ4

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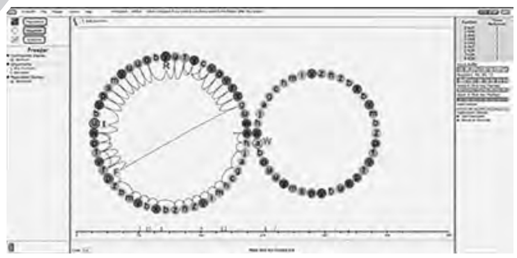
FEATURE ARTICLE

Using Avida-ED Digital Organisms to Teach Evolution and Natural Selection Benefits a Broad Student Population



DELBERT S. ABI ABDALLAH, CHRISTOPHER W. FONNER, NEIL C. LAX, MATTHEW R. BABEJI, FATIMATA PALÉ

Abstract

In instructional settings, evolution and natural selection are challenging concepts to teach, due to the fact that these topics are difficult to observe in the laboratory or lecture hall. In the past few years, Avida-ED has emerged as an innovative tool for teaching evolutionary principles. It allows students to directly observe effects of evolution by changing different variables, such as environmental conditions and genetic sequences. In our study, we used pretest and posttest questionnaires to investigate the use of Avida-ED in undergraduate coursework. We showed that students demonstrated similar improvement in evolutionary understanding irrespective of major, undergraduate level, sex, or final end-of-course grade. These results reinforce the idea that Avida-ED can facilitate learning of evolution in all student populations.

Key Words: *Avida-ED; evolution; natural selection; digital evolution.*

Based on our results, Avida-ED has great potential in being utilized in a variety of different contexts and settings, such as in lower grade levels, community science outreach events, and a great number of high school and undergraduate institutions.

○ Introduction

The principles of evolution and natural selection continue to be among the more difficult concepts to teach to students. Between the lack of acceptance by the general public (Pobiner, 2016) and the inability to easily observe the process in the lab (Johnson & Lark, 2018), evolution continues to face many hurdles in the classroom. Specifically, previous studies have attempted to identify the factors influencing the acceptance of evolution and have found that an understanding of the nature and process of science is associated with the ability to accept evolution (Dunk et al., 2017). Moreover, all classes are composed of students with different backgrounds, majors, demographics, social attributes, and performance abilities. Many of these parameters have been found to influence students' understanding and acceptance not only of evolution, but also of scientific concepts in general. For example, cultural factors can act as barriers to scientific learning in classroom settings; with appropriate interventions and strategies, these barriers to evolutionary acceptance can be reduced (Green & Delgado, 2021). In addition, a study analyzing the effects of numerous factors on evolutionary acceptance found that parental pressures or attitudes and religiosity were significant predictors of whether students accepted the theory of evolution (Barnes et al., 2017). Furthermore, a 2020 study by Siciliano-Martina and Martina demonstrated that in an online, nonscience-major evolution course, gender as well as psychological and social parameters (including peer or political pressures and negative perceptions of evolution) were significant factors influencing evolutionary acceptance. Interestingly, religious factors were not a significant predictor of evolutionary acceptance in the post-surveys, thus indicating that exposure to an online evolutionary curriculum can potentially reduce barriers to acceptance of evolutionary theory (Siciliano-Martina & Martina, 2020). Clearly, further investigations regarding new methods of teaching evolution in different student populations are vital for increasing the acceptance of evolutionary theory.

Not surprisingly, science majors have a greater acceptance rate and knowledge of evolution than nonscience majors (Partin et al., 2013). Among nonscience majors, as few as 59% have been shown to accept the theory of evolution, and among those, only 6% could accurately explain the principles (Robbins & Roy, 2007). Moreover, nonscience majors and even upper-level biology majors rely heavily on incorrect Lamarckian and teleological reasoning to explain changes in organisms over time (Stover & Mabry, 2007). In terms of sex differences, the literature is not as clear. There seems to be an interaction between the subdisciplines of biology, such as natural

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selection, genetics, and evolution, and question types, such as open-ended, multiple choice, and visual spatial reasoning, such that males outperform females in some of these disciplines and question types, while females outperform males in others (Federer et al., 2016). Other researchers have found that males generally outperform females in science learning, which can be attributed to greater quantitative and visuospatial abilities in males than in females (Halpern et al., 2007). Moreover, the use of various instruments, including the Measure of Acceptance of the Theory of Evolution (MATE) and the Generalized Acceptance of Evolution Evaluation (GAENE), has shown that white and male individuals possessed higher degrees of evolution acceptance (Sbeglia & Nehm, 2018). The use of simulations and other active learning strategies has been shown to improve these gaps. For instance, using interactive animations of complex geological principles related to plate tectonics can effectively eliminate the differences in science learning between males and females (Sanchez & Wiley, 2010). Others have found that using active learning techniques to teach the intricacies of DNA replication in undergraduate biology classes can have different effects on confidence and retention of material, based on sex (Lax et al., 2017). The use of novel learning tools in the classroom is needed to improve these disparities.

Despite the many hurdles in evolution education and the many differences that exist among students in a classroom, educators have developed tools to help bridge the gap in evolutionary thinking and understanding. Many simulation software programs have been developed in recent years to help students grasp these difficult concepts in a hands-on way. One in particular, known as Avida, was first developed at Caltech in the late 1990s and was further developed as an educational tool, known as Avida-ED, at Michigan State University (Ofria & Wilke, 2004; Pennock, 2007). This program allows students to track asexual self-replicating virtual organisms (known as Avidians) in a virtual environment. Our group and others have found that Avida-ED is very effective in helping undergraduate students gain knowledge about the principles of evolution and natural selection, as well as in increasing the acceptance of evolutionary theory (Speth et al., 2009; Abi Abdallah et al., 2020; Lark et al., 2018). Avida-ED would be most beneficial if it could provide many different types of students from various backgrounds and contexts with a better understanding of the concepts of evolution and natural selection; however, studies to date have not conducted more in-depth assessments of the effectiveness of this program. Given this, we decided to measure how the software affects the understanding of evolution in the various populations of students found in any given classroom. Specifically, we measured student performance, on multiple choice and open-ended questions related to evolution, before Avida-ED instruction began, and we did the same after instruction. From the before and after scores we calculated fold change. The analysis was conducted by grouping the students' scores on the basis of major, sex, class year, and overall performance (final grades) in the course. Overall, we found that the Avida-ED software benefited all groups of students, demonstrating how this digital evolution tool can be helpful to a broad base of students.

○ Materials and Methods

The materials and methods are identical to our first study published in 2020. Here, we provide a brief materials and methods section. Detailed materials and methods can be found in our previous study at the following link: https://doi.org/10.1525/abt.2020.82.2.114.

○ Student Population

The student population comprised undergraduates at Thiel College, a liberal arts college located in Greenville, Pennsylvania. The Avida-ED lab was part of an undergraduate biology course (foundational level, with significantly more freshmen than students in other years) taken by biology and nonbiology majors (to fulfill a core curriculum requirement). $N = 125$ across 10 different sections of the course from five different semesters and taught by six different instructors. Data were collected between 2016 and 2018. The lab component using the Avida-ED platform involved the pre-course instruction of evolutionary principles (students had not been taught evolution in the course before they performed this lab but may have had evolution taught in other courses or in high school).

Avida-ED Software

The Avida-ED software used in this study is available for free from the Michigan State University and can be found here: https://avida-ed.msu.edu/avida-ed-application/.

Avida-ED Experiments and Laboratory Design

Students followed the Avida-ED curriculum that was generated by the design team at Michigan State University. For our study, we implemented the model lessons found in the Avida-ED lab book and curriculum, which can be found here: https://avida-ed.msu.edu/curriculum/. The curriculum focused on the basic principles of natural selection, including variation by random mutations, fitness, functions, and selection, as well as preadaptive versus postadaptive mutations. The exercise titles are described below. We spread the exercises over a two-week period. Students were tested for evolutionary knowledge before introduction of the Avida-ED program with a pretest survey. Subsequently in week 1, we introduced the software, and students ran their first experiment: "Exercise 1: Understanding the Introduction of Genetic Variations by Random Mutation." In week 2, the students ran experiments 2 and 3: "Exercise 2: Exploring Fitness, Functions, and Selection" and "Exercise 3: Exploring Mutations and Selection: Pre-adaptive or Post-adaptive?" We ended week 2 with a posttest survey (which was identical to the pretest). A timeline of the surveys and Avida lab activities is shown in Figure 1. All Avida-ED exercises were performed and finished before any formal instruction of evolution occurred in the lecture portion of the course.

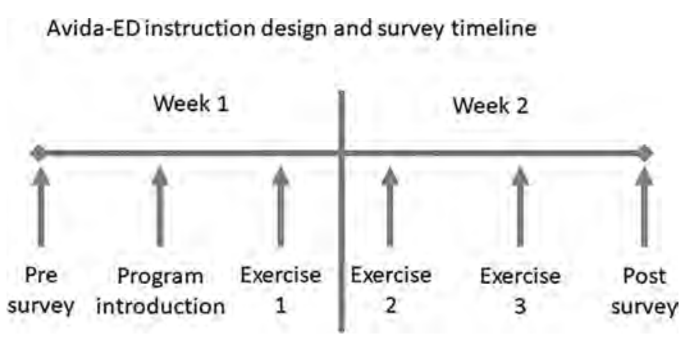

Figure 1. In week 1 of the timeline, students were given a survey to assess their knowledge of evolutionary concepts. The Avida-ED program was introduced and one exercise was performed. In week 2, two more exercises were performed and a post-program survey was conducted to test gains in knowledge and understanding.

Survey (Pre- and Posttest)

The student surveys were conducted at the beginning of week 1 (pretest) and at the end of week 2 (posttest). The survey is based on questions from the Conceptual Inventory of Natural Selection (CINS) (Anderson et al., 2002). The survey was given in the form of hard copies face-to-face with no access to other resources. The students had to complete the survey in class. The students were given the option of whether they wanted to participate in the survey or not (the study was approved by the Institutional Review Board Committee of Thiel College), and several students opted out. Participant compensation was not available.

Survey Analysis, Data Entry, and Statistics

Student answers were graded by professors or teaching assistants. Answers were scored blinded to student names, and a strict rubric was used to ensure reliability between graders. Mann-Whitney *U* and Kruskal-Wallis tests were used to analyze data. Data were analyzed and graphed by using Excel or GraphPad Prism 7.04 (San Diego, California).

○ Results

Student responses to both multiple choice and open-ended questions before and after Avida-ED instruction were analyzed based on various characteristics of the student population (N = 125 students who took the course between 2016 and 2018). First, students were divided into science versus nonscience majors. Science majors included students who were declared biology, chemistry, neuroscience, or physics majors (all possible science majors) when the course was taken (N = 91). Nonscience majors included all math, humanities, and social science students (N = 33). Fold change in correct answers from pre-Avida-ED instruction to post-Avida-ED instruction showed no statistically significant difference. Specifically, the fold change for science majors was 1.453 while the fold change for the nonscience majors was 1.367 (Figure 2, Mann-Whitney *U* test, P = 0.595). Similarly, when the effects of Avida-ED instruction were analyzed based on class year, no statistically significant differences were observed. Freshmen (N = 93), sophomores (N = 5), juniors (N = 6), and seniors (N = 20) had an average fold change of 1.426, 1.507, 1.171, and 1.460, respectively (Figure 3, Kruskal-Wallis test, P = 0.408). Next, the students were divided based on sex. As was seen with major and class year, Avida-ED instruction was shown to be equally effective in both male and female student populations. The average fold change for males (N = 59) was 1.457 and was 1.406 for females (N = 59) (Figure 4, Mann-Whitney *U* test, P = 0.796). All of these data suggest that the Avida-ED instruction helps all student populations.

After the effects of Avida-ED instruction were analyzed based on major, class year, and sex, students in the class were divided into groups based on their performance in the course. In this analysis, the fold change was matched with the final grade earned in the course overall. As was seen with the other parameters, the final grade earned in the course did not correlate with the effectiveness of Avida-ED instruction. Students who earned an A (N = 32) had an average fold change of 1.549; B students (N = 32) had an average fold change of 1.237; C students (N = 31) had an average fold change of 1.439; D students (N = 9) had an average fold change of 1.592; and F/W students (N = 20) had an average fold

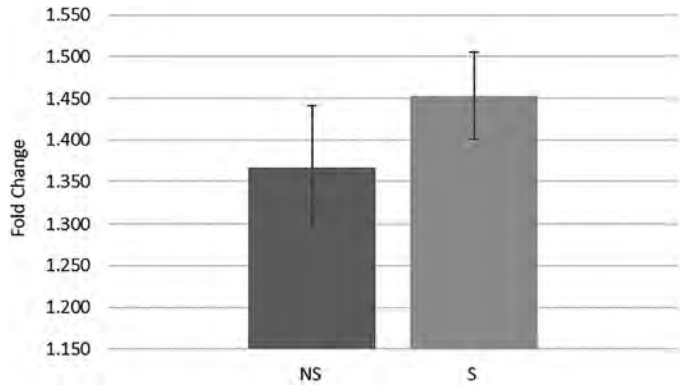

Figure 2. Fold change in pre- and posttest scores for nonscience (NS) and science (S) majors. Nonscience majors had a 1.367-fold improvement while science majors had a 1.453-fold improvement. No statistical difference between nonscience and science majors was observed.

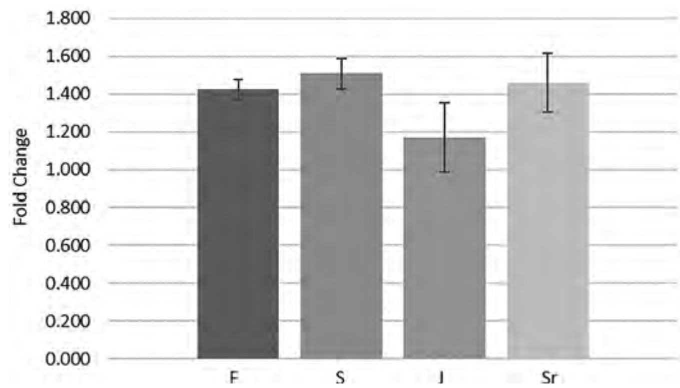

Figure 3. Fold change in pre- and posttest scores for freshmen (F), sophomores (S), juniors (J), and seniors (Sr). Fold change averages: freshmen = 1.426; sophomores = 1.507; juniors = 1.171; and seniors = 1.460. No statistical difference was observed between groups.

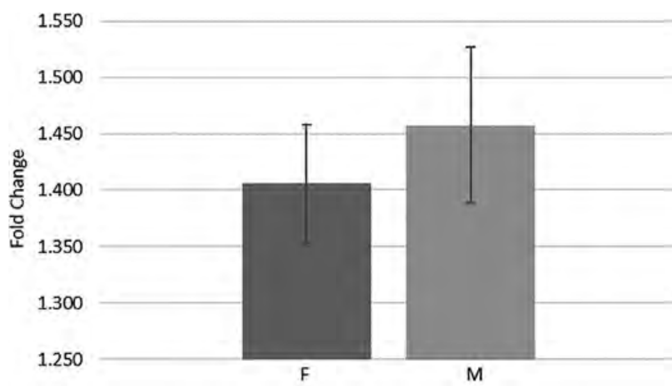

Figure 4. Fold change in pre- and posttest scores for females (F) and males (M). Females had a 1.406-fold improvement compared with a 1.457-fold improvement in males. No statistical difference was observed between the two groups.

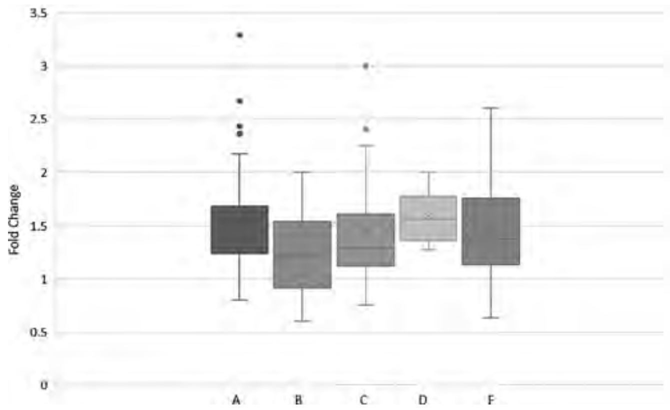

Figure 5. Fold change in pre- and posttest scores according to final course grade. Fold change averages: students earning an A = 1.549; students earning a B = 1.237; students earning a C = 1.439; students earning a D = 1.592; and students earning an F/W = 1.461. No statistical difference was observed between groups.

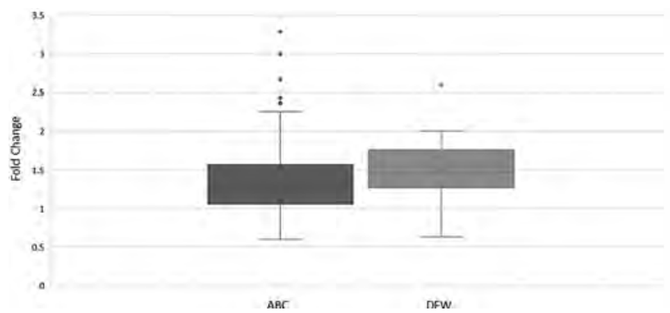

Figure 6. Fold change in pre- and posttest scores according to "passing" and "failing" final course grades. Fold change averages: students earning an A/B/C = 1.408; students earning a D/F/W = 1.501. No statistical difference was observed between groups.

change of 1.461 (Figure 5, Kruskal-Wallis test, P = 0.659). For most students, to receive credit for the course, they need to earn an overall grade of at least a C. Therefore, the next analysis combined all of the students who earned an A, B, or C final grade ("passing") and compared them with D, F, or W students ("failing"). As with individual letter grades, no statistically significant differences were found. Students who passed the course (N = 95) had an average fold change of 1.408 while students who failed the course (N = 29) had an average fold change of 1.501 (Figure 6, Mann-Whitney U test, P = 0.155). However, it should be noted that there were some interquartile differences in this analysis. The first quartile value ($Q1$) for "passing" students was 1.057 while for "failing" students it was 1.27. The third quartile value ($Q3$) for "passing" students was 1.57 while for "failing" students it was 1.75. Thus, the interquartile range ($Q3 – Q1$) for the "passing" students was 0.5135 while it was 0.48 for the "failing" students. This may indicate that "failing" students actually benefit more from the Avida-ED instruction than "passing" students, who would have been expected to do well regardless.

○ Discussion

The development of the Avida-ED software allows for numerous possibilities in assessing the performance and understanding of students in regard to evolutionary concepts. Our previous study (Abi Abdallah et al., 2020) showed that the use of Avida-ED technology significantly enhanced the understanding and retention of complex evolutionary principles in introductory biology students. In order to further examine specific differences in this student population in this regard, we used pre- and posttests to analyze potential changes in understanding based on several factors, including major, undergraduate level/year, sex, and final end-of-course grade. Our results demonstrated that there were no significant differences in pre- to posttest fold changes in student performance for any of these factors. Overall, these results indicate that, regardless of major, undergraduate level, sex, or final end-of-course grade, students exhibited similar degrees of improvement in evolutionary understanding, thus supporting the idea that Avida-ED can be used to enhance evolutionary knowledge in all student populations.

Several studies have demonstrated the effectiveness of Avida-ED in enhancing evolutionary knowledge in both high school and college-level students (Smith et al., 2016; Lark et al., 2018; Abi Abdallah et al., 2020). Additionally, changes in student understanding and retention of a variety of evolutionary concepts (genetic variation, carrying capacity, biotic potential, heritability of genetic variation, and origin of species, among other factors) through use of this software can be assessed with the use of the pre- and posttest questionnaires (based on the Conceptual Inventory of Natural Selection; Anderson et al., 2002). In order to further assess the applicability of Avida-ED, we conducted more in-depth analyses of the data from an introductory undergraduate biology course according to sex (male vs. female), undergraduate level/year (freshman, sophomore, junior, and senior years), major (science vs. nonscience majors), and end-of-course grades, and we observed no significant differences in improvement of evolutionary knowledge within these factors. These results demonstrate that, regardless of the type of student and different student populations, Avida-ED can similarly enhance the retention and understanding of complex evolutionary knowledge. In addition to the results of our previous study (Abi Abdallah et al., 2020), the results of the current study further highlight the effectiveness of Avida-ED in advancing evolutionary knowledge in various student populations. This represents a significant advantage of the Avida-ED software in providing an innovative, hands-on, straightforward method for teaching evolutionary concepts in multiple different settings and groups, whether in educational environments or potentially in larger venues, such as scientific community outreach events.

With respect to the final course grade analyses, it should be noted that there were interquartile differences when the A/B/C group data were compared with the D/F/W group data. Specifically, both the Q1 and Q3 values for the D/F/W students were greater than those of the A/B/C students, thus indicating that there was a trend for D/F/W students to exhibit a greater increase in knowledge than the A/B/C students did. This result indicates a possible added benefit of this program, that is, the weaker/at-risk students may be the ones to gain the most knowledge regarding complex scientific concepts, such as evolutionary theory. Moreover, this further supports the use of Avida-ED as a potential active learning tool or strategy that can help struggling students to better comprehend and/or retain information. Various factors can indicate whether a student will struggle with a specific course and be deemed “at-risk,” including (but not limited to) student engagement (Ayala & Manzano, 2018; Christenson, 2009) and previous high school educational experiences (Bulger & Watson, 2006; Cyrenne & Chan, 2012; Ficano, 2012). Avida-ED may represent an effective instructional tool that can be specifically used for such at-risk students to better engage them and improve their academic performance, in addition to aiding better-performing students to succeed. Future studies are required to further elucidate whether significant improvement can be observed in poorer-performing students with the use of Avida-ED.

Another potential benefit that may be indicated by our current results concerns the use of Avida-ED to enhance the acceptance of evolutionary theory in different groups of individuals. Although our current experiment did not assess changes in the acceptance of evolutionary theory among students with varying biblical and political views or educational statuses, prior studies have identified these as factors associated with the rejection of evolutionary theory by a high number of adults in the United States (Miller et al., 2006; Hokayem & BouJaoude, 2008; Plutzer & Berkman, 2008; Nelson, 2012; Newport, 2012; Pobiner, 2016). Additionally, previous studies examining the relationship between evolutionary knowledge/understanding and acceptance have yielded conflicting results (Rice et al., 2011; Akyol et al., 2012; Nehm & Schonfeld, 2007; Lark et al., 2018). Clearly, more research and strategies for applying instruction techniques to enhance both evolutionary understanding and acceptance are needed. Based on our results, Avida-ED has great potential in being utilized in a variety of different contexts and settings, such as in lower grade levels, community science outreach events, and a great number of high school and undergraduate institutions. It is possible that if Avida-ED is utilized in multiple different groups to instruct evolutionary concepts in a straightforward, yet interesting and applicable, manner, more individuals will begin to accept the theory of evolution as being valid. Future studies should examine the degree of acceptance of evolution as a result of Avida-ED use in various contexts with parameters similar to those used in this study.

An additional application of the Avida-ED software pertains to its use in online learning environments. Due to the outbreak of the novel coronavirus and the resulting pandemic and school closures, online learning strategies have become vital for numerous courses, especially courses with laboratory components. Moreover, due to the uncertainty regarding school reopenings and the future use of online/hybrid learning for “at-home” students, it is important to consider innovative and simple-to-use strategies for teaching these students. Avida-ED represents an efficient and easily applicable learning tool for teaching a complex subject like evolution in an online “laboratory” setting, with the application of Avida-ED in a digital curriculum having already been demonstrated in a previous study (Smith et al., 2016). Based on these reasons and on our current results, a set of digital laboratory experiments (or even an entire evolution curriculum) can potentially be designed and utilized by virtual-only students in the future.

Similar to our previous study (Abi Abdallah et al., 2020), several limitations were present in the current study. First, both sets of questions in our pretest and posttest (the multiple choice and open-ended questions) only focused on basic, general evolutionary principles. In order to more accurately assess the breadth of knowledge of students concerning evolutionary theory, both “concept knowledge” (e.g., knowledge of a specific evolutionary term or idea) and “process knowledge” (e.g., knowledge about how an evolutionary action or mechanism is elicited) should be analyzed with more in-depth techniques. Additionally, both pre- and posttest questionnaires were identical in terms of the makeup of questions, which may have sensitized students to the assessed concepts, thus resulting in the “practice” effect and increased response rates in the posttest. Moreover, the fact that this course is an introductory biology course means that a significant majority of the students are freshmen, which limits the sample size available to examine how effective Avida-ED is on upper-level students. Indeed, our sample sizes for sophomores and juniors were 5 and 6, respectively. This limits the extent to which our conclusions can be applied to all academic years. Another limitation is the lack of comparison to other instructional strategies. Our study focused exclusively on the use of Avida-ED as an instructional tool, but it is possible that greater learning gains would have been achieved by other means. Finally, as has been previously described, our assessments did not examine the degree of acceptance of evolutionary theory. Future studies should utilize multiple different assessment techniques to analyze various degrees of evolutionary knowledge that may be gained through the use of Avida-ED, and they should focus on different factors, including the acceptance of evolution as a scientific theory, in order to gain a more comprehensive understanding of the benefits of this software.

In conclusion, our results clearly demonstrate that Avida-ED can be applicable and beneficial for a wide variety of undergraduate students, regardless of factors such as sex, educational level, or specific major. These results encourage the continued use of Avida-ED for instructing and enhancing evolutionary knowledge for different populations. Its use may help alleviate issues concerning the rejection of evolution as a scientifically sound theory throughout the United States. Future studies that fully address the impact of this software on the enhancement of evolutionary knowledge and acceptance may predict that use of Avida-ED can have far-reaching implications in both educational and community settings.

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RESEARCH ON LEARNING

Sexual Selection as a Tool to Improve Student Reasoning of Evolution

SARAH K. SPIER, JOSEPH T. DAUER

Abstract

There is an emphasis on survival-based selection in biology education that can allow students to neglect other important evolutionary components, such as sexual selection, reproduction, and inheritance. Student understanding of the role of reproduction in evolution is as important as student understanding of the role of survival. Limiting instruction to survival-based scenarios (e.g., effect of food on Galapagos finch beak shape) may not provide students with enough context to guide them to complete evolutionary reasoning. Different selection forces can work in concert or oppose one another, and sexual selection can lead to the selection of trait variants that are maladaptive for survival. In semistructured interviews with undergraduate biology students (n = 12), we explored how leading students through a sequence of examples affected student reasoning of evolution. When presented with an example where sexual selection and survivability favored the same variant of a trait, students emphasized survival in their reasoning. When presented with a scenario where sexual selection selected for trait variants that were maladaptive for survival, more students described how two different selection forces contributed to evolutionary outcomes and described reproductive potential as a part of fitness. Moreover, these students considered how the maladaptive traits were inherited in the population. Scenarios where sexual selection and survival-based selection were opposed improved student ability to reason about how factors other than survival impact evolutionary change. When instructors introduce students to scenarios where survival-based selection and sexual selection are opposed, they allow students to change their reasoning toward inclusion of reproduction in their evolutionary reasoning.

Key Words: *evolution; sexual selection; biology education research; student reasoning.*

○ Introduction

Biology students who effectively engage in reasoning about evolutionary mechanisms readily recognize (1) trait variation in a population, (2) differential inheritance of the variable traits based on how those traits affect fitness, and (3) the impact of certain traits on an organism's ability to survive *and* reproduce (Gregory, 2009; Harms & Reuss, 2019). Traits that affect survival are commonplace in introductory biology, and many introductions to evolution use scenarios with traits that impact organisms' abilities to perform behaviors like feeding and predator avoidance (Maan & Seehausen, 2011). When asked about fitness, university biology students emphasize survival in their reasoning (Kampourakis & Zogza, 2008; Beggrow & Nehm, 2012; Perez et al., 2013). However, a trait's effect on the ability of an organism to reproduce is an equally important factor to consider when assessing fitness and making evolutionary predictions (Scheuch et al., 2019). To make complete predictions about future generations, students must include reproduction and inheritance in their reasoning in addition to their reasoning about survival.

Informal introductions to evolutionary ideas may contribute to an overuse of survival-based evolutionary reasoning (Nehm et al., 2010). Terms like *adaptation* and *survival of the fittest* can be misinterpreted in biology classrooms, based on their use in everyday conversation (Bishop & Anderson, 1990). For example, *survival of the fittest* suggests that fitness is based on survival alone and probably influences students to overemphasize survival in their reasoning (Ferrari & Chi, 1998; Gregory, 2009). Some students explain that if an organism has a trait that benefits survival (e.g., improved antipredator response, strength, access to food), the organism will survive longer, providing more opportunities mate (Bishop & Anderson, 1990). While survival is an important component of fitness, students who rely on survival to evaluate fitness are missing the equal importance of reproduction in their reasoning. Few students describe how traits that directly improve an organism's ability to mate may also benefit fitness by providing more opportunities to reproduce and pass on their traits (Nehm & Reilly, 2007). Therefore, examples that emphasize the role of reproduction in fitness may provide opportunities for students to improve their ability to incorporate reproduction into their evolutionary reasoning.

In addition to emphasizing survival, students may reason that individuals (instead of populations) sometimes modify a trait to satisfy a need (e.g., avoid a predator, obtain resources) and pass the modified trait to offspring (Bishop & Anderson, 1990; Harms & Reiss, 2019). This reasoning does not address the genetic basis of traits, how variation in traits arises, and how traits are typically

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inherited. Students who equally include reproduction in their reasoning are considering inheritance when they predict evolutionary change. Students who do not also consider the role of reproduction in their reasoning may not consider the important role of inheritance in their predictions. They may incorrectly reason that an individual evolves certain traits to meet a need, rather than that the alleles for the trait are differentially inherited in the population. Therefore, improved student reasoning of the role of reproduction in evolution may help them recognize that traits are differentially inherited over many generations and do not change in a single lifetime (Kampourakis & Zogza, 2008; Harms & Reiss, 2019).

Sexual selection can serve as an alternate explanation for variation and differential fitness in populations. Sexual selection is selection based on the ability of an individual to mate. Examples that include sexual selection allow instructors to introduce variation and fitness in a way that is similar to the way they are introduced in examples that include survival-based selection. However, sexual selection examples may allow students to better relate reproduction and inheritance to evolutionary change. Sexual selection can select for the same type of trait as survival-based selection, causing strong selection for that trait variant. For example, bright red skin color in strawberry poison dart frogs (*Oophaga pumilio*) serves as a warning sign to ward off predators, but it is also attractive to females, so both survival-based selection and sexual selection select for the bright red skin trait (Maan & Seehausen, 2011). Alternatively, sexual selection can select for traits that do not benefit survival, but rather benefit an individual's ability to mate (Ritchie, 2007; Chenoweth et al., 2015). For example, in long-tailed widowbirds (*Euplectes progne*), males with short tails are better at escaping predators, but females prefer to mate with males with long tails. Conflicting selection pressures led to males having much longer tails than females in the population. This example describes a trait that does not benefit survival, because males with longer tails are preyed upon at higher rates (Andersson, 1982). It also provides an example of sexual dimorphism, when males and females of the same species have different phenotypes. Sexual dimorphism is a result of sexual selection acting on the two sexes differently. Introducing students to scenarios where sexual selection does not benefit survival may help students recognize that survival-based reasoning alone does not explain all mechanisms of evolutionary change (Scheuch et al., 2019).

A study that explored the gaps in biology education research revealed the need for more studies on sexual selection, with only four published at the time (Ziadie & Andrews, 2018). One study explored sexual selection in a lab experiment, but the examples in the study were limited to adaptive traits (e.g., aposematic skin coloring in frogs) (Eason & Sherman, 2003). In another study, undergraduate students engaged with maladaptive traits (like widowbird tail length) in lab experiences, and they included reproduction in their explanations of why the trait would evolve (Bouwma-Gearhart & Bouwma, 2015). Another study developed a learning progression across many topics related to evolution, including survival-based and sexual selection. As learning progressed, students accurately included mechanisms of evolutionary change in sexual selection examples (Scheuch et al., 2019). Our study differs from the Scheuch et al. 2019 paper in that we started with an example where selection of a trait could be for both survival and reproduction. Then we introduced dissonance, where the selection of a seemingly maladaptive trait improves reproduction. This design allowed us to qualitatively describe how students changed their reasoning of fitness and inheritance in scenarios that differ in how sexual selection is presented.

We used interviews to assess student ability to describe how traits impact fitness through mating success and also student proclivity to include inheritance as part of the process of evolution. Students were asked to describe the evolutionary implications of different scenarios; in some, survival-based selection and sexual selection reinforced one another, and in others, they opposed one another. Responses were qualitatively analyzed to determine how the context of different selection forces affected student ability to (1) describe how mating success influences fitness and (2) include inheritance in their descriptions of evolutionary change over time. We predicted that when students were presented with scenarios where survival-based selection and sexual selection selected for opposing traits, more students would include how the ability to acquire a mate affects fitness and evolutionary change over time. Additionally, we predicted that when students were presented with these scenarios, more students would directly include inheritance in their evolutionary reasoning. We propose that instruction that progresses from survival-based selection, to reinforcing selection, to opposing selection will support the inclusion of reproduction and inheritance in student reasoning of evolutionary mechanisms.

○ Methods

To explore student reasoning of the role of mate choice and sexual selection in evolution, we interviewed introductory biology students at a large Midwestern university following instruction of evolutionary concepts. Students were recruited from two large lecture sections (about 250 students total), and of the 20 students who responded, 12 students were interviewed. Semistructured interviews were conducted orally, with images pertaining to the questions provided on paper. Students were presented with four scenarios: (1) Darwin's finches, (2) poison dart frogs, (3) long-tailed widowbirds, and (4) noise pollution and black-capped chickadees. The students were then questioned about fitness and evolution. Prior to this study, students had only been introduced to Darwin's finches in class instruction. The order of the scenarios follows a progression of increasingly more apparent (to the researchers) presentations of sexual selection. Students were prompted to describe the evolutionary processes in these scenarios.

The focus of this paper follows two prompts that introduced students to factors that may affect the evolution of a population. The two prompts differed in how both sexual selection and survival-based selection acted on the population. The first scenario included the skin color trait of strawberry poison dart frogs. Frogs with skin that is brighter red deter more predators than those with paler red skin, and female frogs are attracted to males with brighter red skin (Maan & Cummings, 2009). Therefore, the bright red skin color trait benefits both survival and reproduction for the frogs. In a second scenario, with long-tailed widowbirds, males with shorter tails are less likely to be captured by predators, and males with long tails are more likely to attract females (Andersson, 1982). The widowbird scenario provides an example where a trait (i.e., long tail) may be beneficial for attracting mates but maladaptive for survival. The two scenarios were chosen based on their different presentations of survival-based selection and sexual selection, to determine whether there is an effect on student reasoning. Students were presented with the scenarios in the same order so that the widowbird scenario would not prime students to detect sexual selection where they usually would not (i.e., the frog example). While this does have the shortcoming of a possible order effect, due to small sample size, we chose this method to determine the effect of sexual selection examples on student reasoning of evolution.

For part 1 of each scenario, students were shown a picture of a male and female next to one another and asked the following questions:

1. Are there observable differences between the male and female frogs/widowbirds? Why?
2. Do you think a predator would avoid males or females more? Why?
3. How does skin color/tail length affect fitness?

For part 2 of each scenario, students were shown a drawing of a population of about 50 male frogs that varied by skin color, and about 50 male widowbirds that varied in tail length. Students were then asked two questions:

1. What do you observe about the male population?
2. Describe how evolution has acted on this population to cause it to appear as it does currently.

Students' responses indicated whether students recognized that males and females of the same species can have variation in traits and that male fitness may be impacted by mate choice. Student responses to part 1 were evaluated at three levels (Table 1): (level 1, low) the student used survival-based reasoning alone (no students used mating-based reasoning alone to describe fitness); (level 2, medium) the student applied mating to the assessment of the fitness of an organism; or (level 3, high) the student described how the ability to acquire a mate influenced evolutionary change over time. Student responses to part 2 of each question were also coded based on the description of the effects of inheritance on changes in a population over time (Table 1): (level 1, low) the student did not include any inheritance in their response, (level 2, medium) the student described a connection between reproductive potential and change over time, and it could be inferred that the student considered inheritance as the link between the two; (level 3, high) students directly described the passing on of genes, traits, or characteristics in the context of evolutionary change in a population.

We used the constant comparative method to qualitatively analyze student interview responses, first building a profile of the students' responses and then comparing student responses for each example (Boeije, 2002). The two authors reviewed a sample of responses from two students and compared them with a rubric developed by Salter and Momsen (2018). This produced a preliminary coding rubric. Then, another small sample of responses (four random fitness responses and four random evolution responses) were coded by the same two individuals. Codes were compared, discussed, and revised to address the minimal discrepancies. Then one author (SS) coded the remainder of student responses with the revised coding rubric. Statistical analyses were not applied to the results, as sample size limited the strength of conclusions that might be drawn from statistics.

○ Results

Fitness

In the frog scenario, all 12 students described sexual dimorphism when presented with images of a male and female frog. Mate choice (female mating preference) influences the difference in skin color between male and female frogs. However, most students included only survival in their descriptions of how skin color affected fitness in male frogs. When students were asked how skin color affected fitness, all students described how brighter color increased the survival of male frogs by deterring predators: "If you have a brighter color, you have a better chance of surviving because they're not going to eat you." Most students connected fitness to survival, although

Table 1. The coding rubric used to evaluate descriptions of fitness and inheritance in student responses to questions about fitness and evolutionary change over time. Themes and example student descriptions were ranked at level 1, level 2, or level 3 for application of mate choice and inheritance.

	Fitness	Inheritance
Level 1 (Low)	**The student used survival-based reasoning only.** *It affects fitness because it helps them survive. It's camouflage or, in the male's case, an alert. It helps them ward off predators.*	**The student did not include inheritance in their response.** *It brought along more dark red males because it was successful in surviving against predators, and the paler red weren't as successful surviving so they died off and the red frogs kept going.*
Level 2 (Medium)	**The student described how the ability to acquire a mate can impact fitness.** *It makes you easier to spot, which makes you more likely to be caught by a predator, but it also helps you find a mate and pass on your traits, which would make you a more fit organism.*	**The student included a connection between reproduction and change over time; inheritance can be inferred.** *More red ones are able to reproduce, and so that causes the population to be kind of shifted towards the red side.*
Level 3 (High)	**The student applied accurate descriptions of fitness to evolutionary change over time.** *The ones with the intermediate tail are better at escaping predators and getting mates so they reproduce more. Then that gene for the intermediate tail would increase [in the population] because those birds have higher fitness.*	**The student directly included inheritance or the passing on of traits.** *They were able to mate and reproduce and pass on their trait of having an intermediate sized tail to their offspring.*

2 students included female mate preference in their descriptions of the fitness of males (Figure 1):

The female frogs know that a brighter color implies a bigger fitness from the male. In that case it will try to mate with the male that has a brighter color, and that way he will be able to produce more offspring than if he didn't have such a bright color.

Overall, students were more likely to include only survival in their descriptions of fitness, even when students recognized a difference in male and female skin color.

Survival-based selection was invoked more often than sexual selection when students described how evolutionary change acted on the frog population. When students were asked how evolution led to a population with more bright red individuals, they explained that increased survival of bright red individuals led to increased prominence of bright red individuals in the population. For example, a student stated:

These organisms with the brighter colors would probably be more successful in finding mates and passing on their genetic traits because if they have a more vibrant color, then predators will avoid them more than say a frog with a lighter red…. The darker red frogs, since they survived to adulthood and were more successful in finding a mate, they were more likely to have their offspring survive, and if their offspring survive, that means their traits survive.

The same 2 students who applied mate choice to fitness were the only two who described how both mate choice and survival influenced the evolution of frogs: "The bright ones have more offspring because they are more successful…. They must be attracting mates. They could help them live longer, but if they're reproducing, then it must be attracting mates." The other 10 students did not include how mate choice contributed to evolutionary change, and they left out sexual selection as a possible selection pressure. When survival-based selection and sexual selection were reinforcing one another and selecting for the same trait (frog skin color), survival dominated students' reasoning of fitness and evolutionary change over time.

The widowbird scenario highlighted opposing selection pressures, with sexual selection and survival-based selection selecting for longer and shorter tail lengths, respectively. As with the frog scenario, all students identified sexual dimorphism. Differences emerged when students were asked about fitness, as 10 students explained how mate choice may influence individual fitness (Figure 1). Eight students that had not included mate choice in their descriptions of fitness in the frog scenario *did* include how mate choice may impact widowbird fitness. Students explained that having a long tail attracted mates but also made it harder to escape predators, applying both survival and mate choice to fitness. For example, when describing how tail length influenced male fitness, a student explained:

It probably helps them get more mates because it's attractive for female birds, but it probably also decreases their chances for survival, at least compared to females, because of them taking up more space and making it easier for predators to catch them.

In contrast to the frog scenario, in the widowbird scenario students described sexual dimorphism *and* described how mate choice influenced fitness.

In their descriptions of widowbird evolutionary change over time, 8 students included how both survival-based selection and sexual selection acted on the population (Figure 1). The students described how female mate choice selected for long tails and survival selected for short tails that led to a higher frequency of males with a medium tail length in the population. For example, one student explained:

The medium-sized tail would be able to get away from a predator easier than the ones with the long tail, but then they would be able to find a mate better than the ones with the short tail, so the medium length tail mutation and gene was passed down more frequently.

Of the 10 students who did not include how sexual selection influenced evolution in the frog scenario, 6 students applied sexual selection to evolutionary change in the widowbird scenario. Students were able to extend the reasoning about mate choice to include the effect mate choice may have on changes in a population over many generations.

Inheritance

Where selection pressures were opposing (widowbirds), students were more likely to reference inheritance of genetic information than where selection favored the same trait (frogs). In the frog scenario, six students included inheritance directly, using "inherited" or "passed on" when describing evolutionary change, and five students included responses where inheritance could be inferred (Figure 2). These five students described how a trait increased reproductive potential or number of offspring and led to an increase of that trait in the population, but they did not directly include inheritance:

I see more red than orange. I guess that the red frogs were more successful in having offspring, so that caused the population to have a change in the alleles so that more of the frogs nowadays are red than they were in the past.

We inferred that the student was describing inheritance, but incompletely. Many students used terms like *gradual*, *eventually*, and *slowly* to describe a change in evolutionary time and had a lack of clarity with regard to changes occurring in the population over generations:

So then slowly as the lighter ones got preyed upon, there would be less of those, so the brighter ones would reproduce more. It would, not overtake, but there would be more compared to the lighter ones.

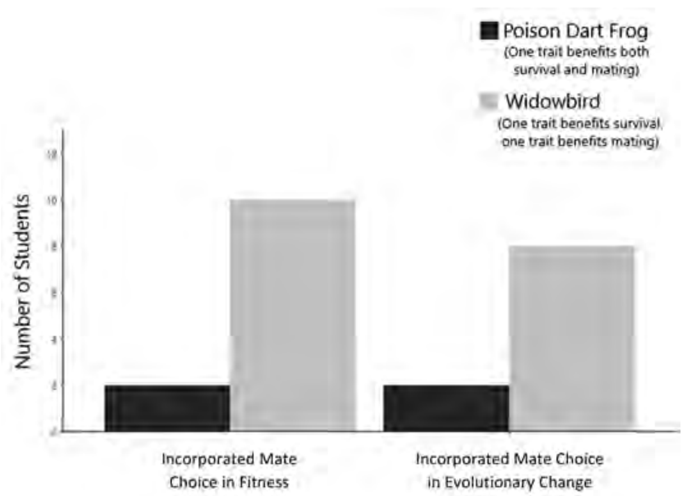

Figure 1. Inclusion of mate choice in reasoning about evolutionary change. Students included mate choice more frequently where survival-based selection and sexual selection were opposed (widowbird tail length) than where they selected for the same trait variant (frog skin color).

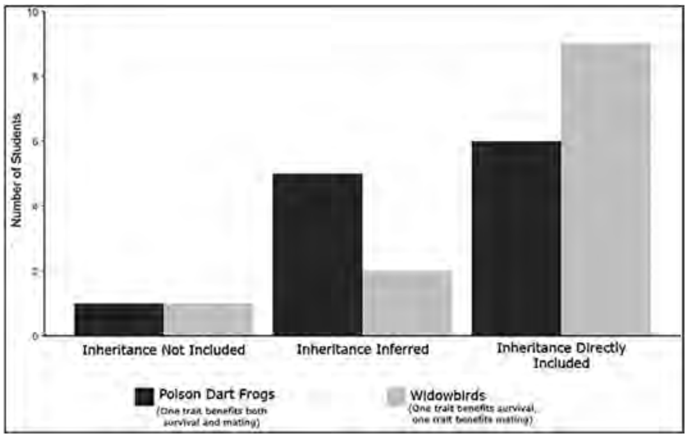

Figure 2. Inclusion of inheritance in reasoning about evolutionary change. Students included inheritance more frequently where survival-based selection and sexual selection were opposed (widowbirds) than where they selected for the same trait variant (frogs). While inheritance was included in most student responses, the widowbird scenario elicited direct statements about inheritance.

Approximately half of the students directly referenced inheritance of skin color when describing population adaptations, and the remainder seemed to imply inheritance was necessary.

In the widowbird scenario, nine students had responses that included inheritance directly, and two students included responses where inheritance could be inferred but was not directly stated (Figure 2). When two selection forces were opposing, inheritance of the intermediate trait entered into the description:

There might be less of the short tails because they couldn't find a mate to reproduce so they couldn't pass on that short tail. Then the long tail, there might be less of them because they were being hunted more often so then they die and can't reproduce…. [The males with intermediate tails] were able to mate and reproduce and pass on their trait of having an intermediate-sized tail to their offspring.

This student described the passing on of traits (inheritance) when two selection forces were selecting for opposing variants of traits. As observed with this student, more students directly included inheritance in their descriptions of evolutionary change over time in their responses to the widowbird scenario than to the frog scenario.

○ Discussion

A common goal of biology instructors is for students to improve their ability to apply important evolutionary components like fitness and inheritance to novel evolutionary scenarios (Gregory, 2009; Harms & Reuss, 2019). Many students emphasize survival in their evolutionary reasoning (Gregory, 2009; Beggrow & Nehm, 2012), which may cause them to leave out other evolutionary components that must be included for accurate evolutionary reasoning. In our study, we observed an emphasis on survival in the frog scenario, where both survival-based selection and sexual selection favored bright red skin color (Figure 1). All students recognized sexual dimorphism in the frogs, but most students did not describe how female mate choice led to males having a brighter skin color. When describing fitness, most students only used survival-based reasoning, leaving out the role of mate choice. The frog scenario also led few students to show a complete application of inheritance to their reasoning of evolutionary change in the population (Figure 2). In our study, showing an example where survival-based selection and sexual selection acted in concert (i.e., frog scenario) was insufficient to generate complete descriptions of fitness and inheritance from most students.

When students were presented with a scenario where survival-based selection and sexual selection selected for opposing traits, students progressed, from simply addressing sexual dimorphism, to describing the mechanisms behind it. In the widowbird scenario, more students included mate choice in their evaluation of male fitness (Figure 1). The progression from reinforcing selection pressures to opposing selection pressures seemed to guide students to consider the role of differential reproduction as well as the role of differential survival. Our results provide evidence that when sexual selection and survival-based selection are opposed, it provides students the opportunity to observe multiple mechanisms of fitness and evolutionary change (Scheuch et al., 2019). One benefit of starting with survival-based selection and reinforcing selection examples is that it meets students where they already are, since many students already have decent knowledge of survival aspects of fitness (Beggrow & Nehm, 2012) and they can build upon that knowledge.

Using scenarios where selection pressures are opposed may also serve to improve student ability to apply inheritance to evolutionary change over time. Most students applied a more complete description of inheritance to their descriptions of evolutionary change in the widowbird population (Figure 2). The close connection between reproduction and inheritance may explain why student responses included inheritance more directly when the survival-based selection and sexual selection selected for opposing trait variants. Inheritance plays an integral role in evolution, as inheritance patterns over generations contribute to changes in the population over time (Gregory, 2009). Many students possess the misconception that traits evolve based on need or use within a lifetime (Bishop & Anderson, 1990; Gregory, 2009; Harms & Reiss, 2019). If students have a better understanding of the role of inheritance in evolution, they are more likely to recognize how a trait that impacts an individual's ability to mate may influence inheritance patterns in the population. Students showed this improvement in the interviews.

The ability for students to better apply mate choice and inheritance when selection pressures are opposed has been observed in other studies, providing further evidence that introducing students to scenarios where survival-based selection and sexual selection select for opposing trait variations promotes important learning gains with regard to understanding the important non-survival-based mechanisms behind evolutionary change over time (Eason & Sherman, 2003; Andrews et al., 2012; Bouwma-Gearhart & Bouwma, 2015; Scheuch et al., 2019). The observations from these studies align with our observations that a student's evaluation of fitness can change from an emphasis on survival ("If a predator was more threatened by the brighter red, that frog would survive. That would probably mean they have higher fitness") to an evaluation that includes survival and reproduction ("The longer length might make it easier for predators … there might be less of the shorter length because it might be trying to attract females"). When making predictions about inheritance and evolutionary change, students can progress from "More red ones are able to reproduce, so that causes the population to be shifted towards the red side" to "Alleles for medium length tail have been the ones who can both get some mates and have a better chance of survival…. And so those are the ones that survive and reproduce, keep passing on their genetic information." Instructors can introduce survival-based selection concepts with typical examples (e.g., Darwin's finches) and then layer on sexual selection through an example where sexual selection is concurrent with survival-based selection, followed by an example where the selection pressures are opposed. One limitation of our study was that the order of the examples did not vary, because we wanted to observe changes in student descriptions. Further studies with larger sample sizes can control for the ordering of the examples, run statistical analyses, and consider application of this reasoning in novel scenarios.

Consideration of multiple selection forces is necessary for making complete predictions about evolutionary change. Activities that guide students to observe the interactions between multiple selection forces provide students with the opportunity to practice more complete reasoning. Instructors who utilize this effective way to introduce students to the roles of reproduction, mate choice, and inheritance in evolution will push students to integrate and apply important evolutionary components. We can use the opposing sexual selection force as a tool to improve student reasoning about how sexual selection may influence differential reproduction and inheritance in a population and to introduce novice biology students to the complexity of selection pressure interactions. As instructors move beyond survival-based selection scenarios, students will be better prepared to more completely reason through increasingly complicated evolutionary scenarios.

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INQUIRY & INVESTIGATION

Connected Biology: Applying an Integrative and Technology-Enhanced Approach to the Teaching and Learning of Evolution in Mendel's Peas

REBECCA ELLIS, LOUISE MEAD, FRIEDA REICHSMAN, KILEY MCELROY-BROWN, JAMES SMITH, PETER WHITE

Abstract

Evolution is often challenging for high school students to understand because it encompasses an array of interlinked processes that occur across a broad swath of biological scales. To help address this, we have developed a set of freely accessible, online, and interactive lessons that focus on the evolution of sweet garden peas from their starchy tasting ancestors. Gregor Mendel first explored the genetics of garden peas in the mid-1800s; our materials help students explore the basis of the R and r alleles from genetic, protein, cell function, artificial selection, and population genetics perspectives. These Next Generation Science Standards (NGSS)–linked lessons integrate concepts across scales and are designed to be used in a flexible order, with support provided to teachers on how to choose a sequence that meets their students' needs. Throughout, students act as scientists as they uncover how multiple processes at disparate scales all worked together in the evolution of sweet and wrinkled peas from ancestors that were starchy and round.

Key Words: *evolution education; genetics; NGSS; Mendel's peas; evolution.*

○ Introduction

Evolution is foundational to biology but a holistic understanding of its mechanisms can be a challenge to teach, and for students to learn. One of the reasons for this is that evolution involves processes that operate at disparate biological scales (Catley et al., 2005). Nucleotide mutations happen within the microscopic realm in the nucleus; natural selection is an interaction between an individual and the environment, compounding in a species across landscapes and over time. In addition, a host of processes happen between and beyond these two levels, from protein formation and function, to biogeographic events. When these biological processes occurring at different scales are taught in isolation from one another, as it is often presented in textbooks (e.g., Nehm et al., 2009), students can struggle to connect them (Lazarowitz & Penso, 1992; Yarden et al., 2004). In an attempt to connect biological processes across scales, White et al. (2013b) developed curricular materials that presented cases of trait evolution where students could explore a phenomenon from the micro to the macro scale; students were guided to investigate a single example of trait evolution, from DNA mutation, to protein function, to phenotype expression, to natural selection, to population-level processes. When doing this, students tended to develop a more complete understanding of the biological underpinnings of evolution (White et al., 2013a). While the approaches of White et al. described above were intended for introductory undergraduate classes, Ellis et al. (2021) recently redesigned some of the materials (exploring the evolution of light fur color in deer mice) to make them more appropriate for high school biology students, including Next Generation Science Standards (NGSS; NGSS Lead States, 2013) performance expectations in an online user-friendly format.

In this paper, we describe new curricular materials we have developed, based on a second example of trait evolution: the evolution of sweet garden peas (*Pisum sativum* ssp. *sativum*). The materials examine how sweet peas arose due to a mutation in a gene responsible for a key starch-forming enzyme, and then how those peas were propagated by artificial selection by early human farmers. The lessons we present here are loosely based on the curricular materials developed by White et al. (2013b). However, our work is unique in that (1) it comprises of a set of unique lessons targeted for high school biology, (2) it involves interactive online simulations that we designed for this project, (3) it includes a teacher portal and teacher support materials, and (4) it expressly connects with NGSS learning expectations. The lessons that we describe below are freely available online at http://learn.concord.org/cbio-peas.

Overview of the Curricular Materials

Today, garden peas come in both starchy and sweet varieties. Historically, wild peas were starchy. This was due primarily to the activity of the starch branching enzyme (SBE1) that converts simple starch (amylose) into complex starch (amylopectin). The starch branching enzyme, SBE1, is transcribed and translated from the starch branching enzyme gene (*sbe1*). Starchy peas, in addition to tasting starchy, also have an interesting biophysical property where they maintain a round shape upon drying down in the pod. In the alternate phenotype—the sweet pea—an approximately 800 base pair insertion into the *sbe1* gene (Bhattacharyya et al., 1990)

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makes the associated SBE1 enzyme nonfunctional. In individuals homozygous for this mutation, the amount of complex starch that is made is greatly reduced, with the excess sugars being converted into sucrose, resulting in a sweeter-tasting pea.

Across our curricular materials (Figure 1: Lesson Map), students investigate the mechanisms and processes that underpin the evolution of the sweet/wrinkled pea phenotype from the ancestral starchy/round state. Students explore and compare DNA sequences, synthesize and compare the proteins, breed pea plants, and more. Along the way, they uncover both the individual and interconnected biological processes involved in the evolution of the garden pea sweet/wrinkled trait. The lessons we have developed can be used in a variety of permutations, as teachers often differ in the order with which they tackle biological topics within the school year. After a recommended Introduction, teachers can work through the materials with their students in the order they see fit. Open structure allows for customization of the curriculum to meet the needs of teachers and students in various instructional contexts.

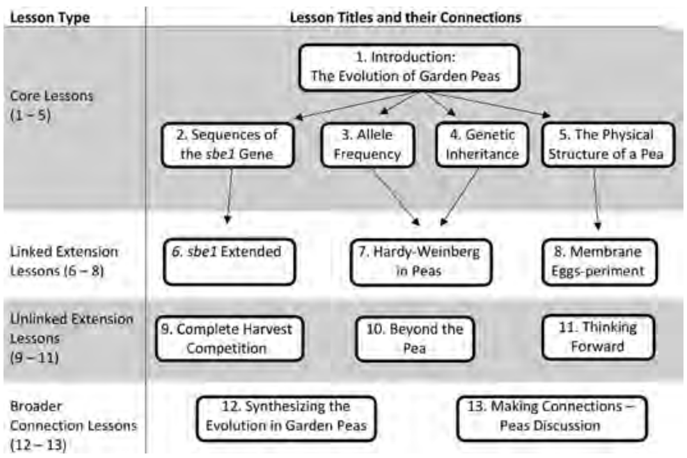

Figure 1. Lesson map. The evolution of garden peas curricular materials are composed of 13 lessons. We recommend that users start with the Introduction (1) before proceeding to *any* of the other core lessons in any order (i.e., 2–5 can be completed in any order). Each linked extension lesson is associated with one or more of the core lessons. Each is optional but can provide deeper learning for the associated core lesson. Further unlinked connection lessons and broader connection lessons can be completed in any order and help emphasize the interconnectedness of the entire set of curricular materials.

Lesson Details

The curricular materials are divided into four sets of lessons. The first set (lessons 1–5) make up the core lessons for the evolution of garden peas. Students are introduced to the biology of pea plants and pea plant reproduction, then explore the genetic and protein basis for the shape of pea seeds. After the Introduction (lesson 1), the subsequent four lessons can be completed in any order, as preferred by the teacher. The second set (lessons 6–8) are linked extension lessons. These lessons are intended as enrichment learning opportunities that can be added on to specific core lessons, at the discretion of the teacher. The third set (lessons 9–11) are unlinked extension lessons, meaning that they can be completed at any point in the lesson sequence, irrespective of whether or not any of the core lessons or linked extension lessons have been completed. The final set (lessons 12–13) are broader connection lessons that focus on building connections between the topics explored in previous lessons.

Core Lessons

1. Introduction

Guiding question: What are the characteristics and history of the plants that Mendel used to study genetics?
NGSS alignment: HS-LS4-2, HS-LS4-4

This Introduction provides students with baseline knowledge about peas, focusing on seed shape and taste. The lesson begins with a brief history on the domestication of peas, where students compare and contrast ancient peas with modern peas. They first analyze the structure of the pea pod; ancient pea pods opened easily, while modern peas stay closed much longer. This lesson introduces the concept of natural versus artificial selection, which plays a role in later lessons. Students then transition to focusing on the trait of round versus wrinkled seeds, with a brief explanation for how the pea shape trait (round vs. wrinkled) is linked to the taste trait (starchy vs. sweet).

Next, students engage in an online simulation called Selective Farmer (Figure 2). Students play the role of the farmer and go through repeated seasons of planting and harvesting a field of round and/or wrinkled peas. As they plant and harvest the peas over many generations, they discover how the pea population changes as a result of their selection choices.

The lesson concludes with an overview of plant reproduction to reinforce for students that plants reproduce sexually and that peas are the "offspring" of pea plants. Students examine diagrams of pea flowers to review anatomical features, watch a time-lapse video of a growing pea plant, and answer targeted questions that call attention to the sexual nature of plants.

2. Sequences of the sbe1 Gene

Guiding question: How do the sequences of DNA influence the proteins formed in peas?
NGSS alignment: HS-LS1 -1

Lesson 2 begins with an overview of two of the different types of starches found in plant cells—amylose and amylopectin—and

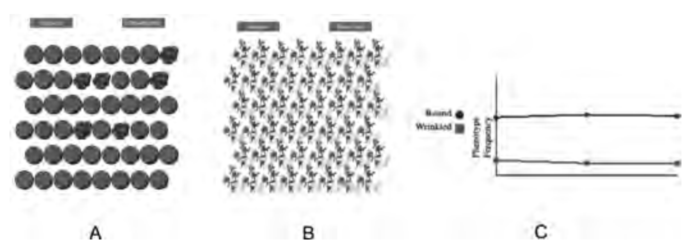

Figure 2. Different student views of the Selective Farmer simulation. In this simulation, students examine and replant peas over many generations. They can (A) inspect the peas to see whether they are round or wrinkled, (B) grow the seeds into pea plants, and (C) keep track of the change in phenotypes overtime by reading the graph that tracks the field.

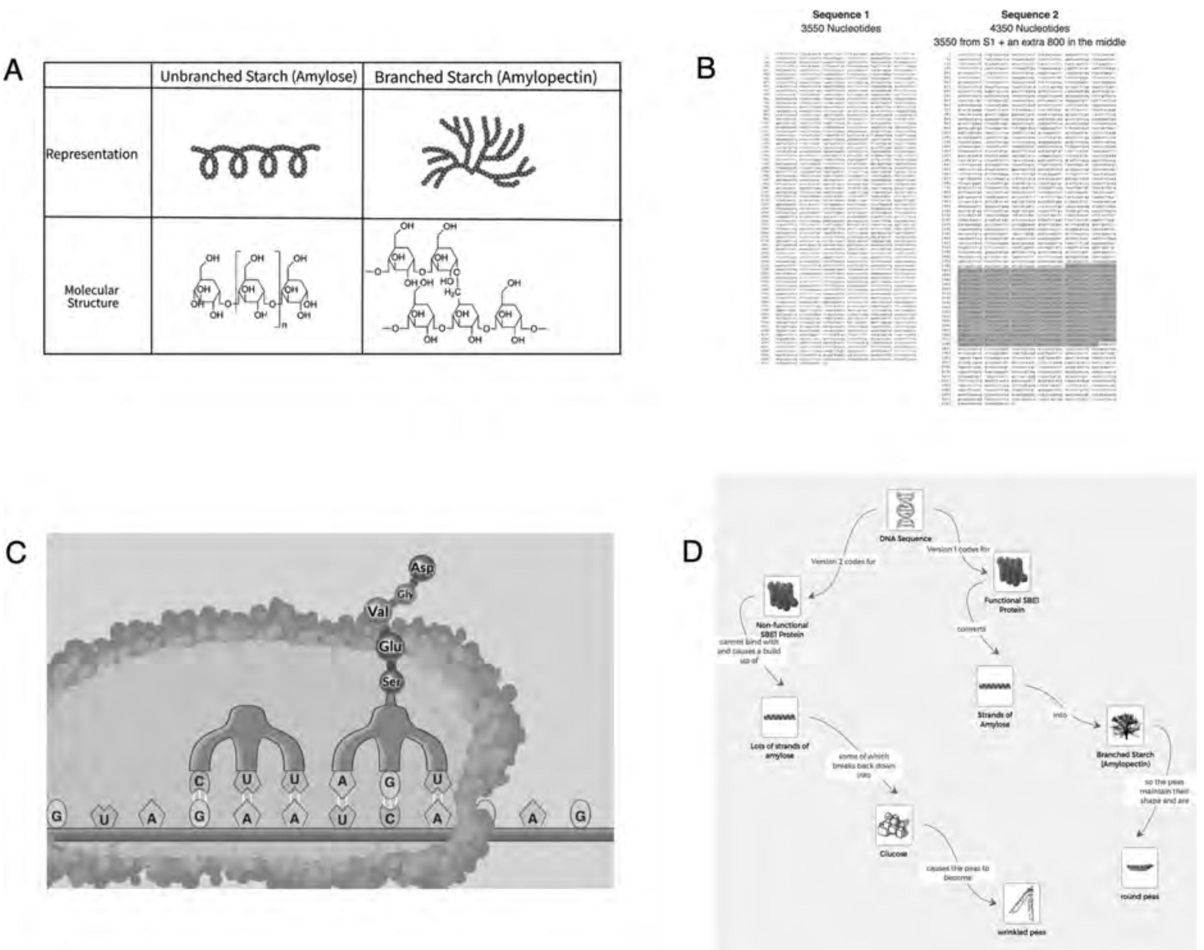

Figure 3. Students study the different DNA sequences that make up the alleles coding for versions of the SBE1 protein. (A) Students compare and contrast the shape and chemical makeup of branched and unbranched starch. (B) Students examine the DNA sequences that code for the starch branching enzyme, noticing a large insertion in one of the alleles. (C)Students use a protein synthesis simulator to transcribe and translate the DNA sequences into amino acid chains. (D) Students use an online modeling tool to develop conceptual models of the pea shape system.

how the starch branching enzyme (SBE1) catalyzes the conversion of one (amylose) into the other (amylopectin) (Figure 3A). Students then investigate the two versions (alleles) of the *sbe1* gene that result in the two versions of the SBE1 protein. These two alleles are the well-known *R* and *r* associated with Mendel's peas. Students discover that the DNA sequence for one of the alleles is longer than the other, due to an 800 base pair insertion mutation (Figure 3B). After predicting what this insertion might do, students use a protein synthesis simulator (Concord Consortium, 2021) to transcribe and translate targeted sections of both sequences and examine the resulting proteins (Figure 3C). Students discover a stop codon within the insertion mutation, which truncates the SBE1 protein and makes it nonfunctional. Students are guided to tie this information back to the amylose and amylopectin starches and to the question of how differences might change the taste of the pea. Combining what they have learned here with the material in the Introduction (above), students use an online model building tool (Sage Modeler; Concord Consortium, 2020) to develop a conceptual model to show how different DNA sequences relate to different protein shapes, to different protein functions, and finally to differences in expressed phenotypes (Figure 3D).

3. Allele Frequency

Guiding question: How can allele frequencies be used as an indicator of evolutionary change in pea plant populations?
NGSS alignment: HS-LS2-2, HS-LS4-3, HS-LS4-4, HS-LS4-5

In this lesson, students are introduced to the concept of random sampling within a population, and the difference between frequency and relative frequency. Students arrange a series of images taken from a very small population of harvested peas over time, and they propose an explanation for how the phenotypes within the population change over time. Next, the students are given definitions for *frequency* and *relative frequency* and discuss when each measure is useful to use. They also compare how these measures differ over time when the measurement is in an entire population versus a smaller sample from that population. Given a sample from a population from three points in time, students calculate the relative frequencies of the phenotypes, and the relative frequencies of the two alleles *R* and *r* (Figure 4). As they model the allele frequencies, students present their data in pie charts, as this gives a visual representation to their numerical results. Students also explore the utility of taking a sample from a population rather than needing to measure a parameter across all individuals.

Figure 4. Students examine samples from a population from three points in time. They first calculate the relative frequency of pea shape and then calculate the relative frequency of alleles. The blue circles distinguish which of the round peas are heterozygous peas.

In the final part of the lesson, students brainstorm ideas for what may cause a population's allele frequency to change over time, connecting their calculations to other concepts they have learned in prior lessons. The lesson concludes with a discussion of how relative frequency of alleles can be a good measure for evolution.

4. Genetic Inheritance

Guiding question: How well does knowing the phenotype of a plant help determine its genotype?
NGSS alignment: HS-LS3 -1, HS-LS3-2, HS-LS3-3, HS-LS4-2

This lesson focuses on how parental genotype is used to determine offspring ratios and explores the molecular and cell biological basis of trait dominance. The lesson begins with a scenario where the students examine three pea plants (Figure 5A, 4B). First, students use a breeding simulator to breed different combinations of flowers from the three plants. The results of

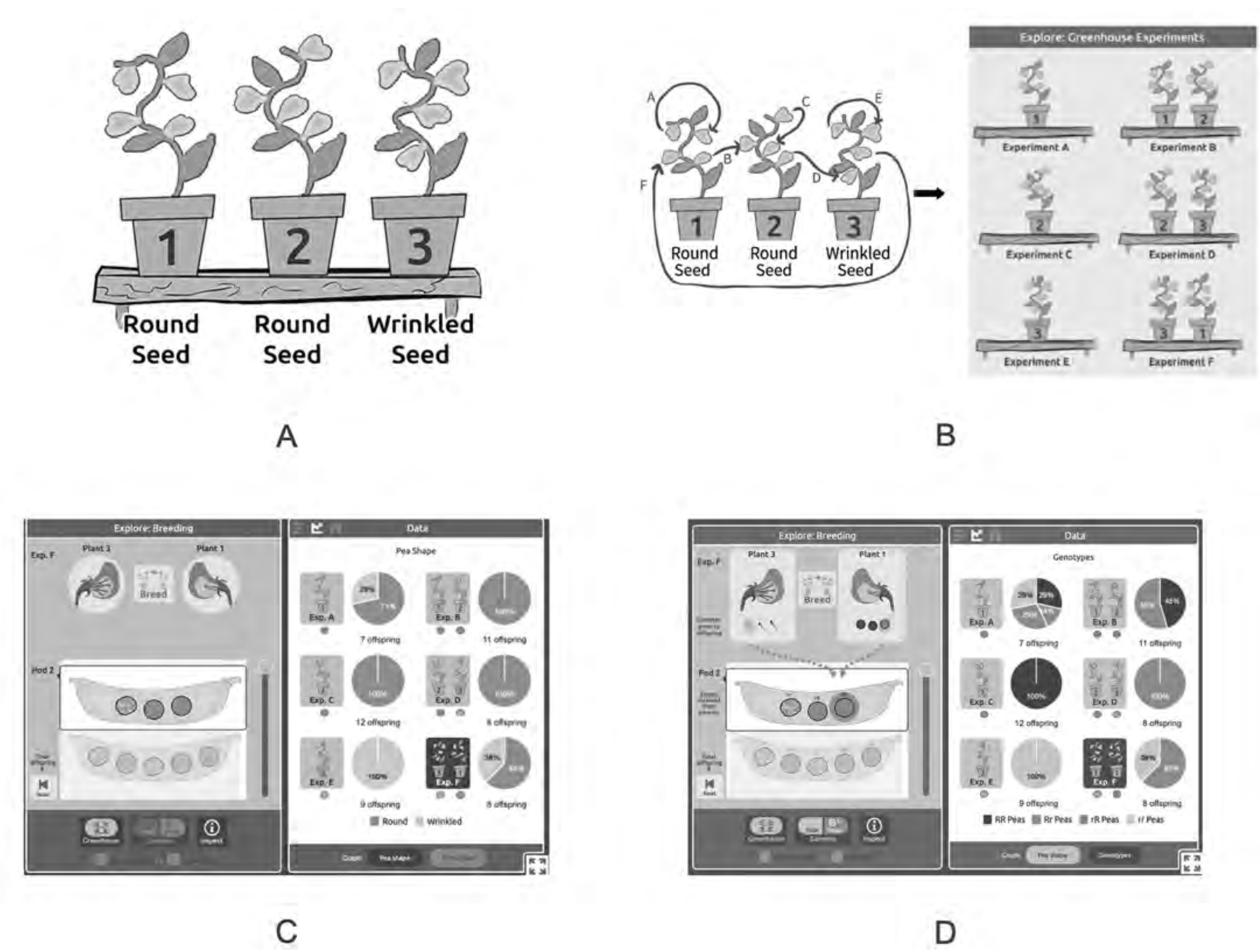

Figure 5. Students explore the genotypes and phenotypes of parents and offspring. (A) Students begin with an inspection of three pea plants, each labeled by the phenotype of the seed that grew it. From there, (B) they use the three plants to set up six different breeding experiments. (C) Students then breed flowers from each plant pair, using a simulation, and examine the phenotypes of the offspring both in the pods and in cumulative pie charts. (D) Students can then inspect the genotype of each offspring pea, using this information to determine offspring ratios and to reverse engineer what the genotypes of the parent plants must be.

the breeding experiments are shown as peas in a pod, and the cumulative totals of peas (they can cross each parent combination multiple times) are displayed in pie charts (Figure 5C). As students examine the phenotypes and phenotype ratios of the offspring, they realize that even though two of the plants grew from round seeds, the differences in offspring phenotypes suggest that the genotypes of the two seeds must have been different (i.e., homozygous dominant vs. heterozygous dominant). Students then examine the genotypes of the offspring and reverse engineer Punnett squares to determine the genotype of the seed that gave rise to each plant (Figure 5D). Once the genotypes of the original three plants have been uncovered, the lesson transitions to an open investigation where students develop their own testable question about pea plants that they can investigate using the embedded simulation.

5. The Physical Structure of a Pea

Guiding question: Why do only some peas wrinkle when dried?
NGSS alignment: HS-PS1-6, HS-LS1-6

This lesson explores the biochemical and physical differences between round and wrinkled peas. It begins with an anecdote about the eighteenth-century French queen Marie Antoinette and her documented preference for sweet peas. Students are prompted to investigate why both sweet and starchy peas are round when freshly picked, but when dried, only the sweet peas wrinkle. Students watch a time-lapse video of peas drying and then use an osmosis simulation to better understand the process. Next, students examine the structures of the molecules in the osmosis simulation (sugar, amylose, and amylopectin) to better understand why only starchy peas maintain their shape after they have dried down (Figure 6). Students are then given the opportunity to critique a video that simplifies the process of peas wrinkling. To complete the lesson, students synthesize the lesson material by developing a conceptual model in the online lesson portal (Concord Consortium, 2020).

Linked Extension Lessons

6. sbe1 Extended

Guiding question: How do insertion mutations impact associated protein structure?
NGSS alignment: HS-LS1 -1

This linked extension lesson builds from the Sequences of the *sbe1* Gene lesson (lesson 2) and examines how the 800 base pair insertion influences the shape of the SBE1 protein. Students hypothesize what would happen to the end of the SBE1 protein if they manually removed the stop codon from the DNA sequence so that the full sequence could be synthesized. Students then simulate this using the protein synthesis simulator (Concord Consortium, 2021). The purpose of this activity is to draw attention to a frameshift mutation associated with the nucleotide insertion, and the consequences thereof. Students discover that, even in the absence of a stop codon within the 800 base pair insertion, the SBE1 protein would contain different amino acids because of a change in the translation reading frame.

7. Hardy-Weinberg in Peas

Guiding question: How can we predict the offspring from a set of pea seeds?
NGSS alignment: HS-LS3-3

This lesson connects to the Allele Frequency lesson and the Genetic Inheritance lesson (lessons 3 and 4). In this lesson, students "visit" the fields of four pea seed suppliers that each use a different planting strategy. Students use an interactive simulator that includes the alleles from the parent generation as they simulate the pollination process that produces the offspring. The lesson transitions to the assumptions needed to use the Hardy-Weinberg equilibrium formulas, and students apply them to the supplier's fields. The students then calculate the likely allele frequencies of the subsequent generation and compare their calculations to the simulated results. This activity is followed by a thought experiment for how finding a population that is not in Hardy-Weinberg equilibrium can be an indicator of evolution. The lesson concludes with a brief summary of what the students have learned and what they still want to know.

8. Membrane Eggs-periment

Guiding question: How does the movement of water across a membrane influence an object's shape?
NGSS alignment: HS-PS1-6, SEP4, SEP5

This lesson is linked to the Physical Structure of the Pea lesson (lesson 5). To support student understanding of osmosis, we provide a lab activity to mimic the change in pea seed shape using

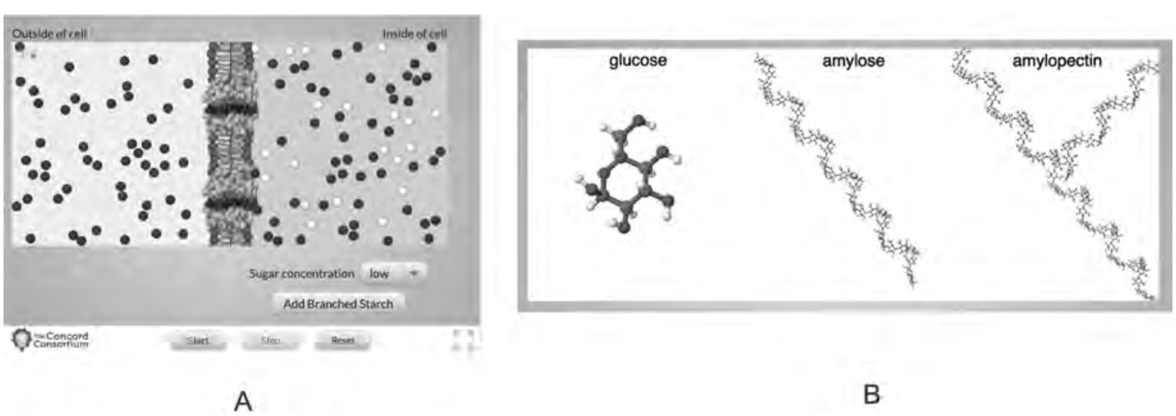

Figure 6. Students explore the molecules in a pea cell and how the molecules influence the cell's osmotic potential. (A) Students control the sugar concentration and whether there is branched starch within the cell. The simulation then models the transfer of water into and out of the cell. (B) Students examine the molecular structures of glucose, amylose, and amylopectin. Students can zoom in and out, as well as rotate the molecules.

chicken eggs. In this system, students examine what happens to eggs when placed into different solutions. Here, the egg plays the part of a pea plant seed, where the direction of water flowing through the membrane is based on the different solutes in the pea cells. This exercise flips the script and uses a constant "cell" (i.e., an egg) and instead changes the solute concentrations in its surroundings. Students can either perform the experiment live in their classroom or analyze prerecorded data that are provided within the lesson. Students graph the data and come to conclusions about the direction of water flow. The activity concludes by linking back to the pea with a claim-evidence-reasoning question.

Unlinked Extension Lessons

9. Complete Harvest Competition

Guiding question: How does the proportion of dominant and recessive alleles change as artificial selection is enacted over time?
NGSS alignment: HS-LS4-5, HS-LS4-2, HS-LS4-4, HS-LS3-3

In this activity, students are presented with two neighboring farmers who have different goals. The first farmer wants to produce only round peas while the second wants to produce only wrinkled peas. As students evaluate the two goals, they are asked to predict who will achieve their goal first. Students are encouraged to think about how this might apply in real-world scenarios and the impact of a heterozygous genotype on offspring phenotypes. This activity can be used as a review, as a quiz, or as a warm-up.

10. Beyond the Pea

Guiding question: What parts of the sweet peas system can be applied to other biological systems?
NGSS alignment: SEP3, CCC1

This activity encourages students to apply what they learned in the pea lessons to other phenomena. Students first brainstorm the concepts they have explored using peas, such as artificial selection or dehydration. Then, they hypothesize where else in nature these processes might occur. For example, students may suggest that the same mechanism that causes a wrinkled pea also causes a grape to wrinkle into a raisin. Finally, students brainstorm how they could investigate their hypotheses. This lesson is open-ended to allow for student creativity.

11. Thinking Forward

Guiding question: What other biological factors might influence pea taste?
NGSS alignment: SEP1

This short lesson stimulates student thinking about other factors that might influence the evolution of pea taste and how they could be investigated. This activity can be used as a bridge between lessons, as the basis for developing a "driving question board," or as a transition from the peas lessons back to other curricular material.

Broader-Connection Lessons

12. Synthesizing the Evolution in Garden Peas

Guiding question: How do the concepts explored in the Introduction and other core lessons work together to help explain why some peas are sweet while others are starchy?
NGSS alignment: SEP2, SEP6, SEP8, CCC1, CCC2, CCC3, CCC4

In this lesson, students use a table to summarize the main points from each lesson and then consider how the materials from pairs of lessons interact to influence pea shape. While many of the connections between lessons have been implied throughout, this is an opportunity to explore evolution as an integrative concept, requiring knowledge of biological principles from a number of topic areas and across different levels of biological organization. Students tie their ideas together using the integrated model building tool (SageModeler; Concord Consortium, 2020) and summarize their work to describe how wrinkled peas evolved over time. Finally, students distill their understanding of evolution by creating an "elevator pitch."

13. Making Connections: Peas Discussion

Guiding question: How does knowledge across different biological scales integrate to influence pea shape and taste?
NGSS alignment: SEP6, SEP8, CCC2, CCC3

This short activity helps students make connections between lessons. Like the Synthesizing the Evolution in Garden Peas lesson (lesson 12), this is an opportunity for students to be explicit in describing how the process of evolution involves a series of interwoven processes occurring at disparate scales. Students note which lessons they have completed, discuss with peers how the content of these lesson fits together, and then write a brief summary of their discussion. This activity can be revisited throughout the implementation of the peas case, as it helps students be deliberate as they revise and expand their understanding of the evolution of garden peas.

Accessing the Curriculum

The lessons are publicly available at both https://connectedbio.org and https://learn.concord.org/cbio-peas. In addition, educators can sign up for a free teacher account on learn.concord.org, to be able to assign lessons to their students and to access a real-time dashboard that populates with student responses as they answer each question or complete each activity. From this dashboard, the teacher can also provide personalized student feedback and optionally assign a grade. Furthermore, teachers have access to additional resources to help them prepare for implementing the lessons, in which we provide an overview of the phenomenon, give summaries of each lesson, indicate NGSS alignments, and support teachers in anticipating common misconceptions. Teachers can also view a teacher edition of the lesson materials that provides teaching tips, discussion prompts, background knowledge, and exemplar answers to questions.

○ Acknowledgments

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ABT AUTHORS & PHOTOGRAPHERS

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Revised July 2022

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A note about article word count: Please recognize that tables, figures, and photographs add to the overall length of the article. One page of text has approximately 1,000 words, therefore a 1/4-page graphic will count for 250 words. More extensive graphics should be budgeted accordingly. References are also included in the final article word count.

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continued

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In order to maintain the word count for individual articles, we are pleased to facilitate publication of supplemental materials accompanying the online issue. If authors have materials (figures, examples, worksheets, appendices, multimedia files, etc.) that support but are not essential to the printed text of the article, authors can include those as separate files with their article submission.

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INQUIRY & INVESTIGATION

The Human Gut Game: An Inquiry-Based Simulation That Teaches Students How Their Diet and Life Choices Influence the Diversity of Their Gut Microbiome

NABT BioClub RECOMMENDATION

KYLEE YAM

Abstract

The human gut microbiome is an important part of the digestive system and the human body as a whole. The abundance and richness of bacterial species in your digestive tract change based on your diet and lifestyle. A diet full of fresh vegetables, meat, and whole grains will cultivate a variety of bacteria that will help you absorb nutrients, prevent disease, keep your skin hydrated, and even improve cognitive function. A diet of sugar- and fat-rich foods will cultivate less diverse bacteria and species that cause inflammation in your gut and impair memory and focus (Quercia et al., 2014). I designed a simulation that uses inquiry and game play to teach high school (grades 9–12) biology students these concepts. The game involves teamwork, decisions about diet, cause and effect of various life events, and data analysis. Educational approaches such as game play and active learning have been shown to improve student engagement, retention of concepts, and overall success of a lesson (Sengupta and Clark, 2016; Michael, 2006).

Key Words: *gut microbiome; diversity; simulation; bacteria; diet; active learning.*

○ Introduction

The human gut microbiome (GM) has been shown to rapidly evolve in one lifetime due to a change in diet. The relative abundance and richness of bacterial species in the human digestive tract is directly connected to a person's diet, exercise, lifestyle, and use of antibiotics (Rinninella et al., 2019). Higher species richness and presence of certain species lead to better digestive health and can greatly reduce the risk of many gastrointestinal disorders, mood changes, hyperactivity in children, and obesity (De Filippo et al., 2010). Eating more fiber, less sugar, and less animal fat supports a diverse gut microbiome with bacterial species that help keep the lining of the small intestine functioning effectively (Rinninella et al., 2019). Higher levels of Firmicutes, such as Ruminococcaceae, and lower levels of Bacteroidetes, such as Bacteroidaceae and *Bacteroides*, have been connected with obesity (Wexler, 2007). A study of European children and African children showed that the African diet, rich in millet, sorghum, and local vegetables and lower in lipids and animal protein, selected for microbiota with high abundance of *Prevotella* and *Xylanibacter*, and low levels of *Shigella* and *Escherichia* (De Filippo et al., 2010). A greater ratio of *Prevotella* to *Bacteroides* has been shown to increase weight loss in humans on a high-fiber diet (Hjorth et al., 2019). *Xylanibacter* extracts energy from fiber well and provides protection from chronic inflammatory colonic disorders (Walsh et al., 2014). A transition from an omnivorous to a vegetarian diet is enough to drastically change the diversity and abundance of your microbiome (Huitzil, 2018). In one study where 10 young adults were given either a plant- or animal-based diet for five days, there was significant change in the GMs of the participants. Those given a plant-based diet had a higher colonization rate of species such as *Roseburia* spp., *Eubacterium rectale*, and *Faecalibacterium prausnitzii*. The animal-based diet resulted in colonization by species such as *Bacteroides* spp., *Bilophila wadsworthia*, and *Alistipes* spp. The animal-based diet caused the most structural damage to the gut lining and increased the risk of inflammatory bowel disorder (Quercia et al., 2014; David et al., 2014).

> *This lesson offers an active-learning approach to teaching high school students about the connection between their diet and their gut microbiome.*

Digestive health is an uncommon topic in a high school introductory biology class. The biology curriculum usually includes a unit on the human body systems, which may cover the basics of the digestive system but is focused mostly on structure and function. Even a health education class might cover nutrition and the importance of healthy eating, but not many will dive into the physiological importance of maintaining a diverse gut microbiome.

The immense effect of the human gut microbiome on the function of the human body is a relatively new area of research. There is much evidence to support the idea that your diet and lifestyle directly affect what species of bacteria occupy your digestive tract (Davenport et al., 2017; Quercia et al., 2014). The

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species composition, richness, and abundance of bacteria have been shown to control mood, focus in school, emotional responses, and susceptibility to many conditions such as irritable bowel syndrome (IBS), leaky gut, and obesity (Garud & Pollard, 2020). This lesson offers an active-learning approach to teaching high school students about the connection between their diet and their gut microbiome.

Traditional pedagogical approaches where the student learns passively in a teacher-centered classroom have been shown to reduce retention of the material in students and result in a lack of general interest in the topic (Michael, 2006). I designed this activity to be active and hands-on so that students are encouraged to engage more with the problem and make more connections on their own, an approach that has shown considerable promise. Instead of having the students listen to a lecture or present a research project, I created the Human Gut Game to simulate how the diversity of your gut microbiome would change based on your diet. Creating a competitive, fun, inquiry-based game engages students and drives meaningful discussion (Sengupta & Clark, 2016).

○ Game Overview

Students work in teams to outcompete their classmates in developing a model gut microbiome indicative of digestive health. The game simulates how diet and lifestyle affect the bacterial species that live inside your gut. These concepts were simplified in order to better engage high school students and to maximize understanding of the bigger picture. Ten bacterial species are represented in this game: three red bacterial species, which thrive with high-fat and sugary foods, and seven green bacterial species, which thrive with foods high in fiber and complex carbohydrates and low in animal fats.

The game follows a basic lesson on the structures of the digestive system, which includes the different structures and their roles in breaking down food to be absorbed by the bloodstream. This lesson should take a total of 60–80 minutes, depending on how long the discussions are. This is part of a larger unit on the systems of the human body. Students will be given a brief overview of the gut microbiome, including a short video (*How the Food You Eat Affects Your Gut* by Shilpa Ravella, 2017; https://www.youtube.com/watch?v=1sISguPDlhY).

Students should be divided into teams of two to three. Each team starts with a high diversity of bacteria, but as they choose which foods to eat, their bacterial diversity changes. In the beginning, the teams are asked to pick junk food or fast food options. This causes the number of "bad" bacteria to increase and the overall species richness to decrease. Teams are asked to pick three to five food cards in order to represent the variety of food they might eat for each meal in one day. Teams play five rounds and start to make healthier food choices in order to improve the number of "good" bacteria (Figure 1). Teams are also randomly assigned different life events, such as stressful situations and antibiotic prescriptions, that affect their bacterial diversity. There are analysis questions between rounds, to help students make connections, as well as reflection questions at the end of the game.

The objective of the game is to teach students how their food choices determine the environment in their gut, which then determines the diversity of their gut bacteria. The secondary goal is to get students to understand that their gut microbiome diversity impacts their overall health and behavior and can cause disease. This activity meets the Next Generation Science Standards HS-LS1-2, HS-LS1-3, HS-LS2-2, HS-LS2-6, HS-LS2-7, and HS-LS4-6.

○ Materials

Human Gut Game slideshow (in the Supplemental Material available with the online version of this article)

- Bacteria cards (available in the Supplemental Material online)
- Food cards
- Student handout

Figure 1. A student team made healthy food choices during the Human Gut Game, and this resulted in a high diversity of good bacterial species. This lesson was conducted at Merrimack Valley High School, Concord, NH, USA.

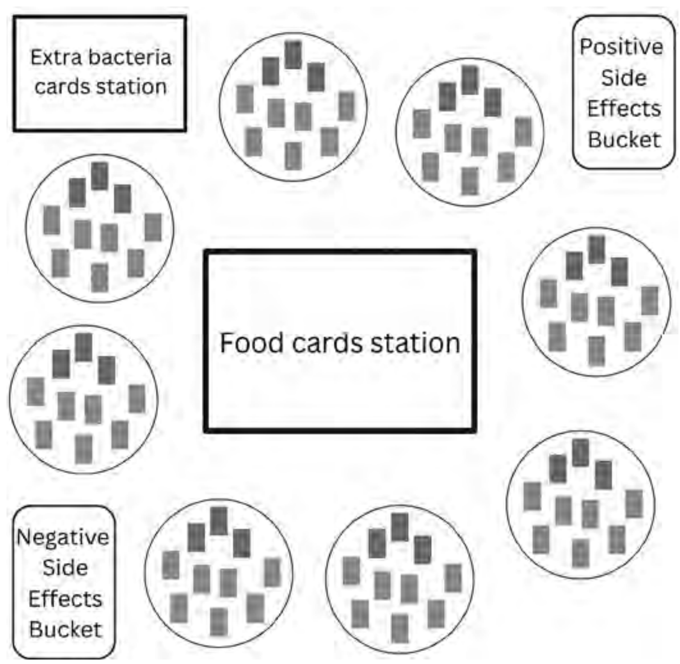

Figure 2. Classroom setup with teams of 2 or 3 students around each table, the food cards placed on the food station table in the center, and the two buckets, one filled with the positive side effects cards, one filled with the negative side effects cards. This is an overview of how to set up for a class of 24 students. Rectangles represent bacteria cards starting in round 1.

- One table for each team of students
- One larger table for the food station
- One table for the piles of bacterial species cards
- Two 5 gallon buckets
- Side effects cards
- Dice

○ Game Setup

○ Interpreting the Results

The Human Gut Game was conducted with six introductory biology classes (grade 10) and one ecology class (grades 10–12) for a total of 79 students (ages 14–18). Based on the results from my reflection questions, 98% of the students stated that they enjoyed learning about the gut microbiome through this game. They explained that it was fun and interactive. Some expressed that the visuals appealed to their learning style. The learning objectives of this game were to improve understanding of how food impacts gut bacteria and how gut bacterial diversity affects

Table 1. Teacher guide to steps in the Human Gut Game.

Introduce the lesson.	Use the slideshow (available in online Supplemental Material) to introduce the lesson to your students. I start this lesson by asking students if they knew that they have many bacterial species that live in their digestive systems. Play the video embedded in the slideshow, which introduces the idea that diet affects your gut bacteria.
Introduce the game, and set up teams of two to three.	Describe the game to your students and review the objectives. Assign students into teams, and pass out the student handout.
The game!	Teachers should familiarize themselves with the game as it is presented in the slideshow. Be sure to pause between each round for questions, to discuss side effects, and to discuss the effect of diet and life events on bacterial diversity.
Conclusion	At the end of the game, have each team share out loud the number of bacterial species they had in round 5 and some of the side effects they experienced. Give them quiet time to answer the analysis and reflection questions.
Discussion	Wrap up the lesson by having students share the answers to their reflection questions out loud. Discuss what types of foods increased the number of "good" bacteria and what foods led to more "bad" bacteria. Make sure that students understand the connection between healthy foods and higher bacterial diversity.
Homework and the next day	Start class by having students share out loud the answers to their homework assignment from the Human Gut Game handout.

Table 2. A general overview of game play for the Human Gut Game.

Start	Teams pick seven green bacteria cards and three red bacteria cards from the piles. They can be any species of bacteria. Students get familiar with the cards and record the number of each species and the total number of species on their handout.
Round 1	Junk food: Students choose food cards to represent the junk foods or fast foods that they might eat. Bad bacteria will increase and good bacteria will decrease. Species diversity will decrease.
Round 2	Life event: Students roll dice to figure out which life event happens to them. Diversity will decrease if they get a stress-related or antibiotic event. Diversity will increase if they get a stress-free or exercise-related event.
Round 3	School day food: Students choose food cards to represent foods they would eat on a typical weekday. Bacteria will change depending on what they choose.
Round 4	Life event: Students roll dice to figure out which life event happens to them. Diversity will decrease if they get a stress-related or antibiotic event. Diversity will increase if they get a stress-free or exercise-related event.
Round 5	Improve diet: Students choose foods to improve their gut bacteria diversity. They should choose healthy, unprocessed foods. Side effects will improve.

Table 3. Sample student responses from the handout after playing the Human Gut Game in a high school introductory biology course.

Analysis/Reflection Question	Sample Student Responses
How does the diversity of gut bacteria connect to your overall health?	"Well the more diversity you had the better your health was because bad bacteria had no diversity and the bad bacteria sent signals to your brain for what to eat and good bacteria fought off harmful bacteria." "The more diverse the bacteria, the healthier you are." "Different species of bacteria help different things. SO having more diversity means that there will be more bacteria helping either attack bad things in your gut or repair and strengthen your gut." "When you have a good amount with a good diversity can help bowel movement, your immune system, and can help fight against bad bacteria you ate."
Will you make different choices about food after playing the game?	"Yes, well because the bad bacteria has weird side effects but also because good bacteria looks pretty cool." "Probably not; I might change a few things, but I've been fairing pretty well with my current diet so far." "After playing this game I think my snacking choices might change. I don't eat a lot of fried food to begin with but maybe I'll try ordering something other than chicken fingers when I go out. I will also be eating more fiber and foods that are not highly processed." "I think I might just because I got to see all of the effects on your mood and test scores."
Did you enjoy learning through this game? Explain.	"Yes! That was a very fun way to learn. I will remember this lesson." "I did! It was a very fun way to learn." "Yes, I'm a very visual person." "Yes I did enjoy learning through this game because it both had information and also a fun gameplay." "Yes, because I'm a visual learner so it was a lot easier to retain information." "I thought the game was fun and really helped me learn. I liked that it was hands-on. I would not have understood/comprehended the information as much if it was in a different form of learning. I love how hands on this class is." "I did enjoy learning through this game because its very interactive and competitive in a way."

human behavior and anatomy. These objectives appear to have been met, as shown by the student answers to the analysis questions: 95% of students demonstrated understanding of the connection between diet and diversity of bacterial species. In many of the written responses and conversations during the game, students commented on how the types of gut bacterial species directly affect how they feel and behave. Most of the students who did not understand this concept were using distance learning and had to watch the game through video. At this point, there is not a good alternative for remote education. Feedback from my colleagues was also very positive. They gave me some suggestions and were pleased with the engagement from the students. Some of the suggestions included adding small pictures of the bacterial species to the student handout and some other minor changes to the slideshow, which I fixed. Sample student responses are shown in Table 3.

○ Conclusion

Engaging students in learning about complex biological systems and relationships can be difficult. Creating opportunities for students to relax, compete, and make active choices helps to improve their focus and understanding. The Human Gut Game was successful in providing a differentiated approach to learning about the human body and this complicated relationship with bacteria. I was pleased with the level of comprehension by the students following the game and hope that it gives them the courage to make better choices surrounding their health. Additionally, through the implementation of this game, I hope that other biology teachers will be inspired and encouraged to create their own inquiry-based learning opportunities for their students.

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INQUIRY & INVESTIGATION

Using RNAi to Examine the Connection between Phenotype and Genotype in *Caenorhabditis elegans* to Enhance the Undergraduate Research Experience

RECOMMENDED FOR AP Biology

KHAMEEKA N. KITT

Abstract

Gene expression plays a pivotal role in the development, differentiation, and maintenance of organisms by allowing genes to encode for an observable trait (i.e., phenotype). To understand the function of a particular gene, several approaches can be taken, ranging from removing the gene entirely to targeting the product of the gene (i.e., the protein). RNA interference (RNAi) has been shown to be a powerful approach used to silence gene activity and examine the connection between DNA and protein along with controlling gene expression. The course-based undergraduate research experience (CURE) described in this article is a hands-on molecular biology lab–based lesson that allows students to examine the impact of RNAi on Caenorhabditis elegans reproduction and development through the examination of the central dogma of biology. Through these activities, students gain practice in the scientific method of inquiry by designing experiments to observe how genotype connects to phenotype and, subsequently, organism behavior.

Key Words: *Caenorhabditis elegans (C. elegans); RNA interference (RNAi); gene expression; CURE; central dogma; genotype; phenotype.*

○ Introduction

The primary goal of any course-based undergraduate research experience (CURE) in the natural sciences is to provide an opportunity to integrate teaching and research in a traditional undergraduate lab to foster scientific inquiry and collaboration (Burmeister et al., 2021). Incorporating independent research projects into a course curriculum requires students to take the knowledge learned from lower-division courses and move up the ladder of higher-order thinking to apply it to a scientific conundrum they find interesting. Studies have shown that hands-on inquiry-based activities not only help students further develop their critical thinking and problem-solving skills, but also have a positive impact on student learning and build confidence in carrying out basic laboratory techniques (Pavlova et al., 2021). Students develop a sense of ownership around their proposed projects, which authenticates the independent research experience.

> *Students come away from the project with the understanding that many questions remain unanswered … and that the experimental process requires troubleshooting, time, and perseverance.*

The CURE described in this article is meant to provide a framework for students to explore the scientific method of inquiry using the nematode *Caenorhabditis elegans* and become proficient in fundamental molecular biology. *Caenorhabditis elegans* are soil-dwelling microscopic nematodes that feed on bacteria in the wild. Due to their transparency, size (~1 mm in length), short larval stages, and small genome size as well as the ease of culturing them, they serve as an excellent model organism with which to examine the role of genes of interest and their homologs in humans (Meneely et al., 2019). In addition, because *C. elegans* are invertebrates, research ethics approval is not required, making them ideal for an undergraduate lab curriculum and less likely to generate emotional reactions from students. For decades, *C. elegans* have been used to study neuronal growth and development, ion channel sensitivity and function, memory and plasticity, DNA structure and repair, cell contact and organ formation, and more (Apfeld & Alper, 2018).

One approach to examining gene function in *C. elegans* growth and development is the introduction of RNA-mediated interference (RNAi) (Fire et al., 1998; Maine, 2008). RNAi is a well-established biological process that works to silence gene activity through transcriptional and/or translational repression. For additional background information on the RNAi mechanism of action and its impact on biological function, see Grishok (2005) and Agrawal et al. (2003). Applications of RNAi range far and wide, from the treatment of various human diseases to genetic engineering of food and crops for human consumption (Ibrahim & Aragão, 2015; Wittenburg et al., 2000). Introduction of an inducible DNA plasmid expressing double-stranded RNA

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(dsRNA) for a gene of interest inactivates gene activity by targeting the endogenous messenger RNA (mRNA) (Conte et al., 2015). Several effective methods have been used to introduce dsRNA (i.e., RNAi induction) into *C. elegans*, and because of their ability to produce results within 72 hours after RNAi induction, they make a prime model organism to use in a classroom lab setting (Andersen et al., 2008). By introducing dsRNA plasmids containing genes of interest into *C. elegans,* one can examine the connection between phenotype (observable trait) and genotype (genetic makeup), explore the central dogma of biology to understand gene expression (DNA → mRNA → protein), and begin to appreciate the elaborate connection (and redundancy) of genes to organism development and function.

The lab activities laid out in the current article are designed to be an iterative process, where students practice established techniques on a given model system, perform experiments, tweak, and refine their lab procedures to generate data, and report out their results in an oral and written fashion (Light et al., 2019). This approach allows the instructor to provide a transformative lab experience and for students to have an authentic research experience within a structured classroom setting.

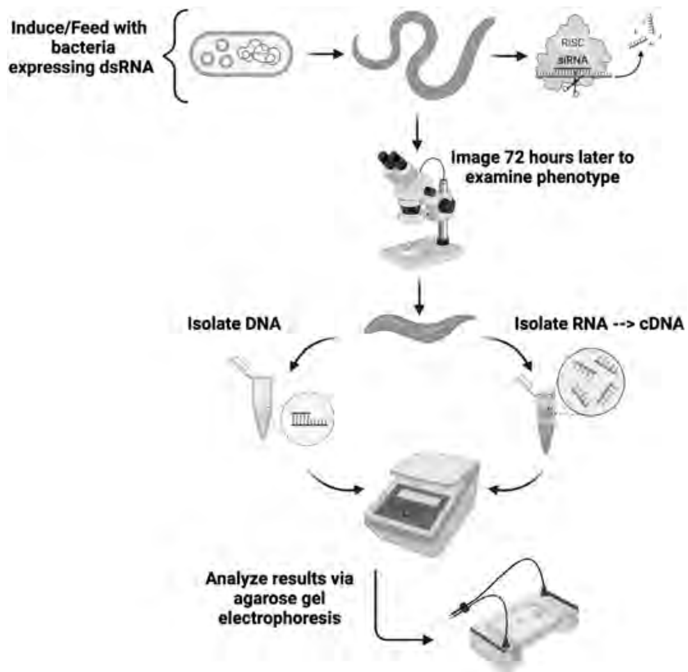

Figure 1. General *C. elegans* experimental design flow for events in Parts I and II. Created with permission from BioRender.com (paid subscription).

○ Lab Learning Outcomes

After completing the following activities, students will be able to:

1. Examine the RNAi mechanism in *C. elegans* to understand the connection between genotype and phenotype.
2. Develop a research question and hypothesis related to a gene of interest and design experiments to test the hypothesis.
3. Identify and evaluate relevant primary literature and background information related to the project.
4. Understand how to use logic and evidence to build arguments and draw conclusions from collected data.
5. Demonstrate how to effectively communicate research findings in oral and written scientific formats.

○ Materials and Methods Overview

The lab activities for this CURE take place over a 14-week semester and are divided into two parts with students working in small groups. Part I involves students examining the RNAi molecular mechanism in *C. elegans* to make the connection between phenotype and genotype, using established protocols from a Carolina Biological kit. This allows both instructor and students to explore *C. elegans* biology and development together with a known outcome. Part II involves students taking the RNAi techniques from Part I and applying those techniques to a chosen gene of interest (GOI) selected by the student group. Each group replicates what has already been published on the chosen GOI and/or discovers a different phenotype when RNA levels have been reduced. For an overview of the experimental process in *C. elegans*, see Figure 1. For additional information on materials, recipes, cost, protocols, and genes of interest to consider for the following procedures, see Appendix 1 in the Supplemental Material available with the online version of this article.

○ Part I: Introduction to the RNAi Mechanism

For students to become familiar with not only the care and maintenance of *C. elegans*, but also the major steps involved in silencing genes in *C. elegans*, all groups spend the first six weeks of the semester following the step-by-step protocol in the Examining the RNAi Mechanism kit from Carolina Biological Supply (Figure 2). The kit explores how the introduction of double-stranded RNA (dsRNA) in *C. elegans* can lead to inactivation of a particular gene through the degradation of endogenous mRNA. Students are introduced to the RNA mechanism by observing wild-type (*N2*) and mutant (e.g., *dumpy 13*, or *dpy-13*) worms to become familiar with worm development, growth, movement, and appearance. In addition, students are exposed to the basics of bioinformatics and learn how to navigate the National Center for Biotechnology Information (NCBI) and worm-based database (i.e., WormBook) sites to examine the *dpy-13* gene and resulting protein in the worm. Students start the experiment knowing the phenotype they are looking for in RNAi-treated worms and learn to describe the difference between negative, positive, and experimental treatment groups for comparison.

One advantage of the kit is that the reagents are designed for the project to work if students follow the instructions carefully. Included in the kit are worksheets for students to complete, to facilitate their understanding of the designed experiment. Students learn the basics of DNA isolation, polymerase chain reaction (PCR), and gel electrophoresis, and they observe first-hand the amount of time required to follow through with the experiment and achieve results. The worksheet and questions associated with each step of the protocol enhance the students' appreciation for the targeting effect of RNAi on the worm, especially when they can replicate the "dumpy" phenotype in the

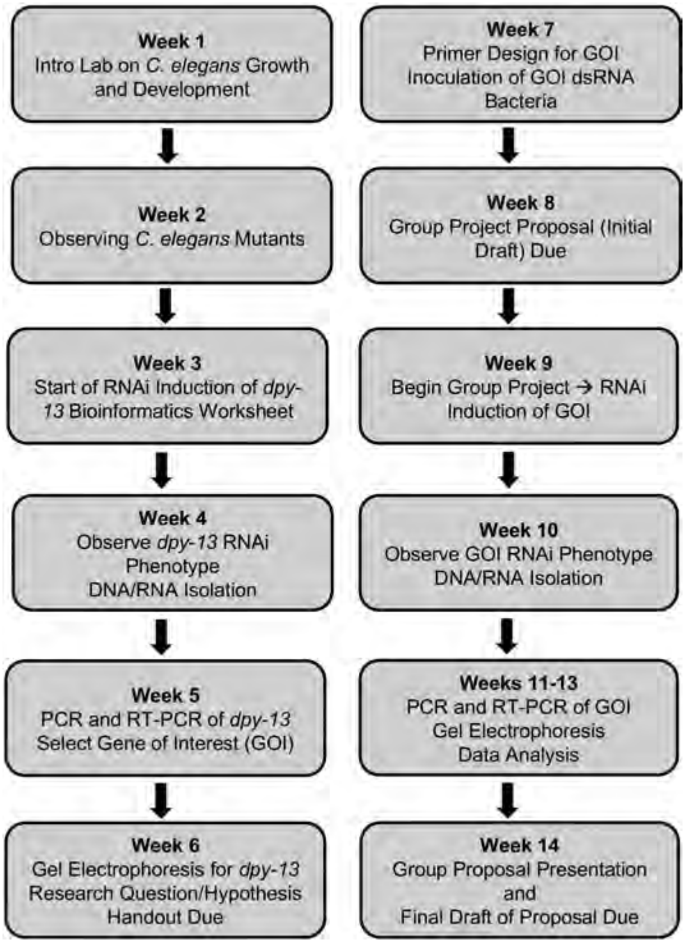

Figure 2. Flowchart of events for Parts I and II of the proposed CURE using *C.elegans.*

RNAi treated group. One disadvantage is that students usually have a difficult time grasping the molecular impact of RNAi on RNA levels, because the kit only has students examine DNA levels to draw conclusions about whether the RNAi treatment was effective in isolated worms.

To help students understand the impact of the *dpy-13* dsRNA feeding strain on mRNA levels, the Carolina Biological kit is modified by adding a couple more steps to the protocol. This includes selecting worms from the various treatment groups to isolate RNA using the Monarch Total RNA Miniprep Kit. Students work through the process of converting RNA to complementary DNA (cDNA, a double-stranded version of mRNA and a measure of how much mRNA is present) using the First Strand cDNA Synthesis Kit and reverse transcription–polymerase chain reaction (RT-PCR) protocol. Subsequently, agarose gels are loaded with similar amounts of cDNA to determine if the RNAi treatment had a significant impact on the *dpy-13* RNA product. Using this approach, students work through the differences between DNA, mRNA, and cDNA and how each contributes to the process of determining whether their particular gene product (i.e., *dpy-13)* was reduced in RNAi-treated worms (e.g., connecting genotype to phenotype). Furthermore, students learn a series of techniques that they can now apply to an independent research project on a different gene in the worm.

○ Part II: Examining the RNAi Mechanism through an Independent Group Research Project

The group-driven independent research in the second half of the semester project provides students with the chance to apply their newly acquired molecular biology techniques to a different gene in the worm. The Horizon company has a database of dsRNA bacterial feeding strains for many *C. elegans* genes cloned into a pL4440-DEST vector. The instructor picks 10 genes from the collection that may or may not have a strong observable phenotype. In a classroom setting, this approach allows students to make the connection between phenotype and genotype and/or learn how genetic redundancy can cause a no-phenotype knockdown effect. Each group works to select a gene from the list of 10 for their independent research project (see Appendix 1 in the Supplementary Material available online for a list of genes with observable phenotypes). The remaining weeks of the semester are dedicated to the selected gene of interest (GOI). Components of the flowchart in Figure 2 are outlined to provide additional context for the group independent research project.

Week 6. Students work in their established groups to do background literature searches on their GOI. Based on the knowledge they gather, they work to design a research question and hypothesis centered around their background research on the GOI. Students are required to complete a Research Question and Hypothesis worksheet (see Appendix 2 in the Supplementary Material available online). The worksheet provides a way for the instructor to (1) give initial feedback to each group, (2) help students understand the difference between question and hypothesis, and (3) ensure students have a solid question and hypothesis before moving forward with the proposal portion of the project.

Week 7. Once each group has identified a GOI, one class session is dedicated to learning how to design primers to identify their gene product at the molecular level. The primer design activity provides students with the “know-how” on how to properly design primers (see Appendix 1 in the Supplemental Material online for a Primer Design Tips link) to achieve optimal results. Students use a primer design program through the company Integrated DNA Technologies (IDT) to design the best primer pair for their GOI (see Appendix 1 for a PrimerQuest Tool link). Instructors are encouraged to have students test out the generated primers to ensure that the designed primers yield the predicted GOI PCR amplicon length/size.

Week 8. Each group designs and submits a two-to-three-page initial research proposal, using a rubric and set of guidelines (see Appendix 3 in the Supplementary Material online for details). Typically, students spend weeks 6–8 on the proposal. The instructor is encouraged to give feedback on the proposal design to ensure that students (1) select the proper experimental techniques to collect data that will inform their project hypothesis and (2) consider all the potential results that either support or do not support the project hypothesis.

Weeks 9–13. Groups work on the proposed project experiments, using techniques similar to those in Part I, but now examining a different GOI. For the independent research portion, each group is provided with a different wild-type *C. elegans* worm strain, *rrf-3*. This "wild-type" strain has been genetically engineered to be highly sensitive to RNAi treatment (Simmer et al., 2002). In Part II, students use OP50 bacteria as their negative RNAi control, as in Part I. However, an empty pL4440-DEST vector in a bacterial feeding strain would serve as a proper negative RNAi control to demonstrate that the RNAi silencing mechanism is due to the presence of the gene. Each group performs various molecular biology techniques, which include bioinformatic searches, DNA and RNA isolations, PCR and RT-PCR, and gel electrophoresis. Students are required to collect two pieces of evidence from their group project (see an example of data generated by students in Figure 3). One example of required phenotypic evidence students provide is an image of the preliminary phenotype in the *C. elegans* and/or quantification of a possible outcome from knocking down the GOI (e.g., body bends and/or spontaneous reversals to examine locomotion defects) to determine if the RNAi effect causes a disruption to *C. elegans* development, growth, and/or behavior. These types of functional assays are impactful if the GOI being examined is connected to muscle/nerve development such as the WASP (actin cytoskeleton modulator) homologous gene, *wsp-1*, involved in regulating the actin cytoskeleton in *C. elegans* (Figure 3A–B). For the experiment described in Figure 3, six individual worms from the control and RNAi-treated plates were measured for the body

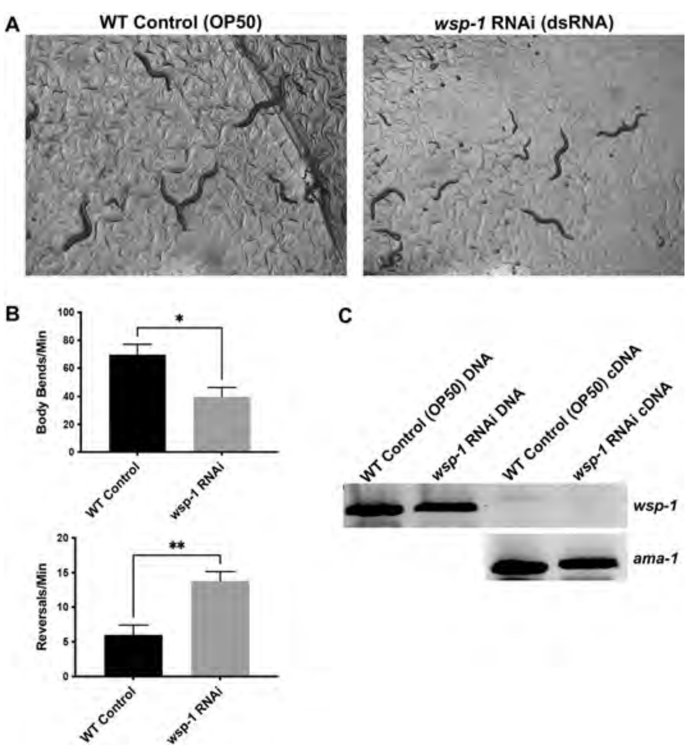

Figure 3. Example data generated during Part II of the CURE. *Caenorhabditis elegans* development and growth in control and *wsp-1* RNAi-induced worms (A). Functional assay data examining *C. elegans* behavior after *wsp-1* RNAi induction. Preliminary data suggests reduced RNA for *wsp-1* results in less body bends and an increase in erratic reversal movement compared with control worms ($n = 6$) ($^*p < 0.05$, unpaired t-test) (B). Gel electrophoresis of DNA and RNA (cDNA) isolated from *wsp-1* RNAi-induced worms (C).

bends and reversals data from one RNAi induction. Additional worms should be scored to validate the behavior phenotype (minimum 20–30 worms per treatment). For more information on how to set up and control for behavior-based assays, see the Behavior section of the WormBook site (Hart, 2006).

The genotypic piece of evidence required is an image of an agarose gel displaying DNA and cDNA expression products of the GOI to determine if the RNAi was successful. The DNA agarose gel is a critical component of the group project, as it shows whether the dsRNA for the GOI was effective in reducing the amount of mRNA (i.e., cDNA) of the targeted gene. Preliminary results in Figure 3C suggest that the RNAi (dsRNA) feeding strain for *wsp-1* successfully reduced the amount of *wsp-1* cDNA (mRNA) present in worms—compare RNAi-treated versus WT control (OP50) lanes. DNA levels were unaffected, which shows that RNAi treatment effectively targets mRNA, not DNA. To confirm that the RNAi feeding strain targeted *wsp-1*, students also examined a housekeeping gene that should not be targeted by the RNAi treatment. As indicated in Figure 3C, *ama-1* (RNA polymerase gene) expression levels were unaffected in both WT control (OP50) and RNAi-treated worms. Students may have to troubleshoot the PCR conditions for their GOI to achieve a final product; most groups achieve the correct amplicon length/size for their GOI on isolated DNA. It has proved to be somewhat difficult at times to get a detectable amount of cDNA PCR product at the correct length/size for RT-PCR experiments; however, most groups have achieved varied success (as demonstrated in Figure 3C—compare cDNA from WT (OP50) lanes and RNAi-treated lanes). The instructor should continually remind students that RNA is not stable and that proper collection and handling procedures must be followed to achieve a final product.

Week 14. In the final week of lab, students are required to present their findings in an oral presentation to the instructor and their peers, using PowerPoint or Google Slides (see Appendix 4 in the Supplementary Material online for presentation guidelines and a rubric). In addition to the presentation, each group modifies their research proposal to include their preliminary results/findings from their proposed experiments and discusses these findings in the context of their research question(s) and hypothesis along with future directions. The written products described above are submitted on Turnitin.com to monitor for originality.

○ Discussion

The inquiry-based lab activities described in this article are meant to guide instructors on how to implement an RNAi-based technique in an upper-division molecular biology, genetics, or cell biology course where students have learned the fundamentals of gene expression in a lower-division biology course for majors. Parts I and II described above are typically carried out in an upper-division undergraduate molecular biology lab course of 14 to 16 students. Students have the opportunity to not only analyze and evaluate data, but possibly generate new data on a particular gene and find ways to explain their results in the context of what is currently in the literature. Furthermore, the inclusive curriculum design provides students with some form of research experience, especially students who are unable to complete independent research projects with faculty outside of a traditional classroom lab setting (Bangera & Brownell, 2014).

From the activities described, several goals are achieved. First, students have a much better appreciation for the flow of information (DNA → RNA → protein) and the impact of RNAi on gene expression. Students appreciate firsthand how genotype is connected to phenotype and that disruption of only one gene can have a major impact on worm development and function. The iterative process students go through as they learn basic molecular biology techniques (e.g., DNA/RNA isolations, PCR) provides a critical step in their development as scientists, to refine their ideas and practice applying new skills to different questions surrounding worm development (Corwin et al., 2018).

A second goal of the inquiry-based lab activities is to help students develop oral and scientific writing skills (Brownell et al., 2013). A critical part of the project in Part II requires students to come up with a question and hypothesis based on background data on a particular gene. By developing a solid hypothesis, students learn how to anticipate possible outcomes from the experiment. It is imperative for instructors to remind students of the importance of revisiting their question and hypothesis throughout the process, to ensure their data are in alignment with what they set out to accomplish. By doing this, students further hone their critical thinking skills and learn how to connect their hypothesis to the overarching importance of the gene to *C. elegans* development and function. The proposal, research paper, and oral presentation challenge students to describe their justifications for why they have chosen a particular GOI and practice fielding questions based on their project design and discovery. These activities build self-confidence and provide the necessary scientific communication skills to help prepare students to take on other science courses in their major or higher degree programs.

A final goal of the inquiry-based lab activities is to further ingrain within students the importance of project design and time management skills. Students learn the time frame for the lab activities in Part I and translate this timeline to an independent research project where they build the design and structure of the project based on their hypothesis. In addition, by working in groups, students build leadership and effective communication skills within their peer groups to not only carry out the experiments on a weekly basis, but also be effective listeners and team players in the process (Esparza et al., 2020). Many of these skills will eventually translate to their future careers once they graduate and move on to the next phase of their educational journeys.

Overall, this lab design provides a hands-on approach to understanding how gene structure and expression connects to protein function and organism development. At the end of the semester, students come away from the project with the understanding that many questions remain unanswered when it comes to the role of genes in growth and development and that the experimental process requires troubleshooting, time, and perseverance.

○ Supplemental Material

- Appendix 1. RNAi Materials and Protocols List
- Appendix 2. Background Information and Hypothesis/Research Question
- Appendix 3. Research Proposal Guidelines and Rubric
- Appendix 4. Research Proposal Presentation Guidelines

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CLASSROOM MATERIALS & MEDIA REVIEW

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Leeuwenhoek: The First Master of Microscopes
Journey to the Microcosmos (https://youtube/y4B3kl9Rvrs)

"Among all the marvels I have discovered in nature, these are the most marvelous of all."

So goes a quote from Anton van Leeuwenhoek referring to the animalcules he observed through the earliest of microscopes. He was looking at a drop of rainwater that had collected outside his home and had been sitting there for two days. His animalcules, of course, are microbes, but he did not know that term.

A video from Journey to the Microcosmos called *Leeuwenhoek: The First Master of Microscopes* takes the viewer through the fascinating early days of Leeuwenhoek's work and discoveries. Most people credit Robert Hooke as the father of the microscope, but it was actually Leeuwenhoek who provided the basis of Hooke's work. Leeuwenhoek was known to be an avid writer, publishing many works documenting his journeys into the microcosmos.

Leeuwenhoek started his career as a draper, one who sells cloth. His work with the microscope started as just a hobby. He taught himself how to grind lenses, which he then used as tools to look at those objects that could not be seen with the naked eye. His early microscopes were far better than those of his contemporaries, having images that were better magnified and clearer.

While Leeuwenhoek wrote his material in his native Dutch, other scientists have done their best to translate it. One such interesting translation describes Leeuwenhoek's analysis of his poop. He describes its consistency, but more importantly, he talks about the microbes (or animalcules) he found within it. While he had no specific names for these organisms as we do today, from the descriptions he provided, it has been possible to determine to which organisms he was referring.

Many people tried to replicate Leeuwenhoek's work, but failed. While he published a lot, he did not leave behind any documentation of his methods of building his microscopes or observing the organisms. It was not until Robert Hooke that the microorganisms Leeuwenhoek had seen were observed again. Hooke did share his methods and even demonstrated them for others, validating the observations Leeuwenhoek had made.

The video shows footage that matches the narration, showing various microscopic images of the organisms Leeuwenhoek saw. The footage provides the viewer with upward of 200× magnification, showing the organisms in very high detail. These images, of course, are far better than anything Leeuwenhoek saw, but they give the viewer the general idea of what he was seeing. The narration is pleasant and informative.

Leeuwenhoek: The First Master of Microscopes is appropriate for all students in grades 5 and up. It would be a great way to introduce the microscope to younger students, and diversity of life to older ones. Advanced students could use the images in the video as the basis for individual research projects, trying to find similar organisms in ponds or rainwater.

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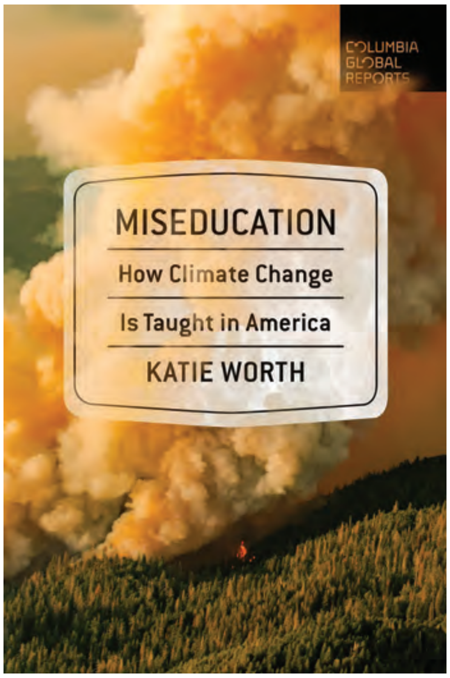
York Community High School, Marion, IL

The mission of the NABT BioClub is to recruit, support, nurture, and promote students who have an interest in biological sciences for personal reasons, academic preparation, the betterment of society, and possible career opportunities by providing guidance, resources, and activities to meet these goals.

Look for the BioClub logo to indicate recommended articles for NABT BioClub members. If you are interested in forming a chapter of the NABT BioClub, contact NABT at office@nabt.org.

KIRSTIN MILKS & FRANK BROWN CLOUD, DEPARTMENT EDITORS

Miseducation: How Climate Change Is Taught in America. By Katie Worth. 2021. Columbia Global Reports. (ISBN 9781735913643). 180 pp. Paperback, $16.00. Audiobook also available.



"Climate science literacy bills in Washington fail."

"Climate change education bills die in Rhode Island."

"Italian students will soon be required to learn about global warming. American kids? Not so much."

These are just a few of the recent headlines taken from science education organizations, such as the National Center for Science Education (NCSE), who have been "working with teachers, parents, scientists, and concerned citizens at the local, state, and national levels to ensure that topics including evolution and climate change are taught accurately, honestly, and confidently." Defending the teaching of topics such as evolution and climate change in K–12 schools is not new to the NCSE. Alongside these heroic organizations stands Katie Worth, a former investigative journalist for PBS's FRONTLINE and a stalwart defender of teaching the science of climate change. In her book *Miseducation: How Climate Change Is Taught in America*, Worth describes various groups at the local, state, and national levels who have fought hard to prevent the teaching of climate change to K–12 students in every state in the country. Although most of these initiatives have failed, unfortunately quite a few have survived the legislative process. Through meticulous research, Worth reviewed hundreds of textbooks, designed and built a 50-state database, and traveled to more than a dozen communities where she interviewed students and teachers about what is being taught about climate change in America's K–12 public schools. Her findings are both interesting and alarming.

The book reads like a suspenseful detective novel as Worth connects the pieces of the climate-change-education-thwarting puzzle by following the money and influence of oil corporations, local and state legislatures, school board officials, libertarian think tanks, conservative lobby groups, and textbook publishers—all of whom have taken their lead and marching orders from previous fights over the teaching of evolution and the advertising of tobacco products. In the first chapter, "The Science and the Doubt," Worth highlights the disconnect between the nature of science (NOS) and some science teachers' misconception that science's openness to change is its weakness, rather than one of its strengths. Her interview with one such science teacher is eye-opening to say the least. Chapter 2, "The Teachers," recounts a few other teacher interviews and focuses on how little time is spent on the topic of climate change in the science classroom and how pedagogical strategies such as classroom debate, if done poorly when climate change is considered, can lead to student misconceptions about human-induced climate change. Worth states, "No teacher would encourage a class to debate cell theory, when there is no evidence for a competing theory, and neither should students be asked to debate whether significantly raising the amount of carbon dioxide in the atmosphere does or does not heat the planet."

Chapters 3–6 ("The Evolution," "The Standards," "The Textbooks," and "Selling Kids on Fossil Fuels") highlight the history of science content, focusing on the effect of cancel culture on the teaching of evolution; the nature and development of K–12 science standards in the United States; and the influence of the multimillion-dollar "energy education" campaign by big oil companies, such as the American Petroleum Institute's decades-long, shrewd, and at times unethical messaging blatantly rejecting the science of climate change. In the closing chapter, "The Victory," Worth highlights a few of the minor climate change education victories that have been achieved by local, state, and national education boards, while at the same time stating that "the American public's perspective on global climate change remains wildly out of step with that of scientists." She goes on to say that "as of 2019, 30 percent of Americans falsely thought global warming was mostly natural." Worth ends the chapter by warning the reader that "every year, state lawmakers propose legislation that would allow teachers to miseducate their students about human-induced climate change" and that there are still "troves of misleading educational materials [that] are available online."

Miseducation: How Climate Change Is Taught in America is an informative book with richly investigated text and an impressively detailed reference section. It takes the reader on an engaging, data-laden, historical, science education journey, exposing us to the widespread ignorance about the scientific consensus, to ideological pressures,

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and to the disinformation campaigns promoted by the fossil fuel industry, textbook publishers, school board officials, and other ill-intentioned groups. As a practicing biologist and science educator, I remain hopeful that due to the heroic efforts of investigative reporters such as Katie Worth, books like this will continue to serve as platforms for further conversations about the importance of climate change education, and I think they should be essential reading for teachers of science and other subjects alike, who are concerned for the future of humanity.

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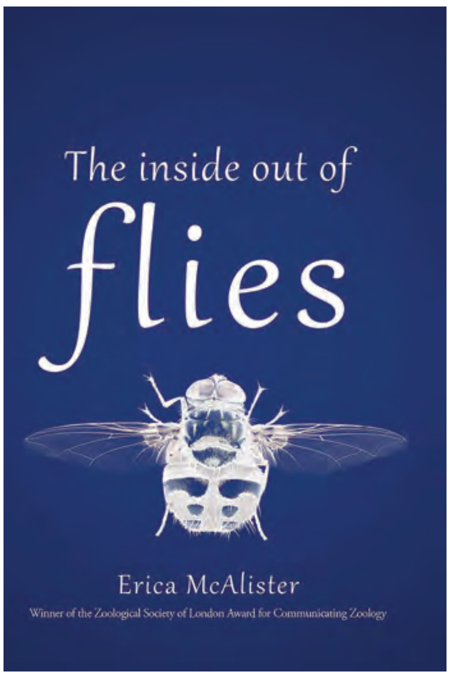
The Inside Out of Flies. By Erica McAlister. 2021. Firefly Books. (ISBN 9780565095260). 288 pp. Paperback, $14.99. Hardcover also available.



There can be absolutely no doubt that author and researcher Erica McAlister finds flies fascinating. The enthusiasm and joy with which she discusses her Diptera subjects is infectious; although readers might not end up truly loving flies, as McAlister does, they will certainly come away with appreciation for the evolutionary wonder of this ubiquitous group of insects. McAlister's previous book, *The Secret Life of Flies*, examined fly behavior. This companion volume, *The Inside Out of Flies,* examines fly anatomical adaptations. The first chapter describes "pre-adulthood" (development of flies, from egg through adulthood); the following eight chapters focus on adult anatomy, working their way from the head (with separate chapters on the antennae and the mouthparts) to the end of the abdomen (the terminalia). In each chapter the anatomical aspects and evolutionary adaptations shown in each body part are carefully presented with many unique examples. The book itself is physically lovely: great attention has been paid to layout, and visuals are clear and informative.

Open this book to any page, and you will find something astounding about flies. Flies being flies, this could be somewhat revolting (maggot extra-oral digestion and tissue debridement) or amazing ("magbot" robots that mimic maggot motion to deliver pharmaceuticals to specifically targeted locations in the body). Did you know that some flies don't even have functional mouthparts as adults? Or that flies have sensory hairs that literally "taste" bitter and sweet molecules in the atmosphere? Or that some female flies have eversible sacs (McAlister describes them as "resembling car air bags") that emit pheromones and make the female's body look bigger to entice males to mate? The almost unending examples are fascinating, though they are in a way the source of the book's biggest fault. Although McAlister undoubtedly felt she omitted many examples important to dipterists (such as the global attendees at "fly school" described in the end chapter), the more general student of biology would have benefitted from a more selective presentation. Example after example with scientific names, classifications, and adaptations lead at times to reader fatigue. Overall, however, this is a wonderful volume, of potential interest and use to a wide variety of readers interested in biology, entomology, behavior, and evolution.

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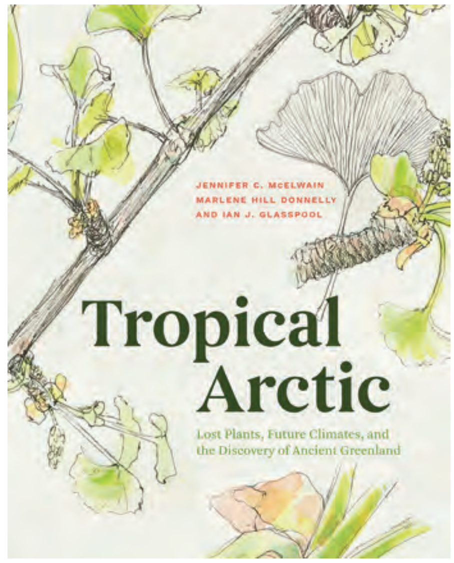
Tropical Arctic: Lost Plants, Future Climates, and the Discovery of Ancient Greenland. By Jennifer McElwain, Marlene Hill Donnelly, and Ian Glasspool. 2021. University of Chicago Press. (ISBN 9780226534435). 138 pp. Hardcover. $30.00.



Tropical Arctic asks us to imagine the Arctic as a lush, green paradise (think Miami) through the story of an expedition to Greenland and the subsequent analysis of the fossils collected there by a team including an artist, botanist, and paleobotanist. The expedition team was specifically interested in a mass extinction event that occurred during a period of catastrophic climate change 200 million years ago between the Triassic and Jurassic periods. The team targeted particular fossil beds to help them find the answers they were looking for. They collected more than a metric ton of fossils and used the data to put together a picture of what the ancient Arctic ecosystem looked like by determining which plants were dominant and which were rare both before and after the mass extinction. They then used the fossils to create three-dimensional models to increase their understanding of the plant characteristics. This provided insight into which plants were resilient in the face of change and which characteristics might have contributed to that resilience. The team coupled this evidence with laboratory evidence on extant plants that measured their response to conditions similar to ancient Earth's.

What was a dense forest canopy in the Triassic transitioned to lower-growing plants that had to survive harsh atmospheric conditions in addition to frequent fire in the early Jurassic. When conditions stabilized, those plants able to survive could thrive. The team hypothesized that the slow rate of change of Earth's conditions contributed to the plants' ability to migrate, essentially germinating where conditions were favorable. Certain species were better adapted for conditions on one side of the boundary. The book ends with lessons for the future, pinpointing the magnitude of temperature change associated with a reduction in biodiversity and loss of species and what it might mean for Earth's future.

While the photographs and illustrations are beautiful, I found the story hard to follow. At the end of the book, I wished some of the information presented in Chapter 4 had been presented earlier. I was less motivated to learn about the expedition and all of the fossils collected because I didn't know the story of the conditions on Earth that led to and followed the mass extinction. Had that context come first, it would have provided framing for the expedition, sparking curiosity about what the team found. I found myself wanting a map or a timeline to help me track Earth's history both spatially and temporally.

It may be beyond the scope of this book, but I was left wondering about the conditions in the Arctic that allowed all that plant life to grow in the first place. How does the 40,000-year cyclical change in the Earth's tilt play into the story of energy and matter in the Arctic? Does that explain why it was lush there once but cold and icy there now? And how will the anthropogenic changes we have made to Earth's system interact with that natural cycle?

Today, many high school biology teachers are tasked with integrating Earth and space science standards into their curricula. Using historical events that caused changes to Earth's system as phenomena can help students understand the flow of energy and matter and provide a context for understanding why the mechanisms of photosynthesis and cellular respiration are so important. *Tropical Arctic* is a beautiful text and illuminates a part of Earth's history that I knew little about. On its own, it is missing necessary pieces to spark the interest of someone who lacks expertise in the field, but it would be a worthwhile complement to other texts on the topic.

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THE QUIXOTIC QUEST FOR OBJECTIVITY IN OBSERVATION

DOUGLAS ALLCHIN, DEPARTMENT EDITOR

What if the legendary character Don Quixote had been a scientist? Surely his quest would have been the noble pursuit of objectivity. Scientists endeavor to transcend mere opinion or individual interpretation. They strive for publicly confirmable facts. Accordingly, scientists appeal to empirical evidence, measurements, and observations—regarded as the bedrock for factual claims.

Yet, at the same time, ordinary humans can be fallible observers. Their interpretations can be skewed by prior expectations or personal desires. Historians, philosophers, and sociologists of science thus now typically contend that observations are "theory laden"—easily reflecting the researchers' assumptions. In the past, the ideal of science was expressed in the simple motto "I'll believe it when I see it!" Now, some cynics contend, an honest scientist might admit the ironic converse: "I'll *see* it when I *believe* it."

Are we inevitable puppets to our beliefs? To what degree are observations in science trustworthy? How else would we defend scientific claims? (How else would we resolve contentious facts in our society?) Most teachers, I think, endorse the conventional view—that scientists and their observations are inherently objective. And that this makes science privileged. Here I explore this revered view (this month's "Sacred Bovine"). Ultimately, I maintain, we are not as perfect as in the quixotic image. Yet science has developed tools to accommodate our cognitive flaws and to rescue science's claim to its much-vaunted objectivity.

○ Observer Bias & Blinding

Objectivity is a hallmark principle of our justice system too. Think of the allegorical figure holding aloft the scales of justice, blindfolded and impartial. Courts need trustworthy evidence to decide whether someone is culpable or innocent. For example, they rely on witnesses.

However, cognitive research has shown that observers' perceptions can be shaped and reshaped by personal experience and prejudices. Memories are vulnerable to suggestion too. Eyewitness testimony is—counterintuitively perhaps—among the *least* reliable in a courtroom (see the provocative volume by Loftus et al., 2019). That is, witnesses are susceptible to *observer bias*. We might, therefore, turn to forensic science and physical evidence—fingerprints, blood, DNA—as more secure.

But even here, observer bias can intrude. We know this because science has turned on itself, to investigate its own objectivity. Psychologists have tested forensic experts in historical crime scenarios. Their assessment of bullet and shoeprint evidence seemed pretty consistent. But when contextual information about a case was available, it could affect how they interpreted a crime scene, how they matched fingerprints, how they identified individuals from the DNA when a sample mixes DNA from multiple persons, how they interpreted bloodstain patterns, and how they assessed skin injuries, at least. Even what dog handlers believed about possible culprits could influence the behavior of their sniffer dogs (Colloff, 2018; Cooper & Meterko, 2019). What can be done to ensure justice?

Managing observer bias is standard now in modern medical research. To prevent judgment about a patient's condition being primed, the doctors are metaphorically blindfolded. They are not informed about who is receiving a new drug or treatment and who has been given an inert placebo. Bias is not possible, even unconsciously.

Such practices emerged over a century ago. One landmark study was done by Adolf Bingel in 1912–1913 at the City General Hospital in Brunswick, Germany (Tröhler, 2011). For decades, diphtheria had been a major scourge across Europe. Serum therapy (recognized in the very first Nobel Prize in Physiology or Medicine in 1901) had certainly improved the situation. Bingel acknowledged its efficacy but questioned whether it worked because of a specific antitoxin in the serum. Might the serum itself—any serum—be equally effective? By this time, the notion of controls for experimental comparison was widely appreciated (Sacred Bovines, March 2020). So, Bingel established two groups. Some patients received the conventional "antitoxin" serum, and others ordinary horse serum. To avoid inadvertently biasing his sample, he methodically assigned every other admitted patient to the alternate group.

Bingel was aware that given the controversial nature of his idea, the physicians' preconceptions posed a special danger. He reminded his readers that it is "extraordinarily difficult to evaluate the influences of therapy on disease unless they are obvious, as for example, the success of a surgical operation or cure of syphilis with mercury or Salvarsan. The therapeutic optimist very easily sees improvement, and the skeptic sees nothing." He thus wanted "to achieve an objective overall assessment," rather than the doctor's informal, possibly biased, "impressions." So, "to make the trial as objective as possible," he explained, "I have not relied on my own judgement alone but have sought the views of the [at least six] assistant physicians of the diphtheria ward, without informing them about the nature of the serum under test (namely the ordinary horse serum). Their judgement was thus completely without prejudice. I am keen to see my observations checked independently, and most warmly recommend this 'blind' method for the purpose" (Bingel, 1918, p. 288). Here, Bingel used the term still common today: *blinding*. That method gave stronger credence to Bingel's contentious conclusion: the theoretical claims of the Nobel Prize winner were mistaken. *Any* serum was effective.

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○ Documenting Unconscious Bias

Documenting specific instances of observer bias can be difficult. However, one can gauge the magnitude of the general problem by bulk comparison of blinded and non-blinded observations. One such analysis looked at clinical studies about a range of medical treatments, from heart conditions to wounds to psychiatric disorders (Hróbjartsson et al., 2013; Hróbjartsson et al., 2014). In the non-blinded studies—the ones open to observer bias—the conclusions were (on average) more dramatic. Probabilities of benefit were 36% higher. Effect sizes increased by 68%. Similar discrepancies were found even for lab studies on animal models (Bello et al., 2014). Overall, blinded studies seemed to yield more modest results. Even among clinical trials with large, randomized samples, unwanted observer bias can intrude and yield misleading findings.

One might well imagine that observer bias would be limited to scientific studies where judgment is critical and where prior beliefs are strong. Not so. This method of comparing blinded and non-blinded studies has helped us probe that assumption (a further expression of this month's Sacred Bovine—that one may assume by default that a scientist's observations are immune to such influences).

For example, do ants recognize nestmates (their genetic kin)? According to the theory of kin selection, the behavior of an individual should tend to benefit its closest genetic relatives. So, this apparently simple question of insect behavior has significant implications for understanding evolutionary biology. A standard way to measure such kin-oriented behavior is to observe ants from the same versus different colonies meeting, and to tally the various types of encounters between them. To what degree do they exhibit aggressive behavior toward kin (nestmates) or toward "others"? Even with the relevant behaviors clearly defined, those assessments can be subtle, it turns out. Identifying instances of "mandible flaring" or "recoil" from a tactile encounter, for example, requires some experimenter judgment. In one recent meta-analysis, investigators found 156 experiments of nestmate versus non-kin behavior (van Wilgenburg & Elgar, 2013). Of those, 53 met the criteria for analysis of observer bias. Fifteen of those used blinded behavioral analysis. As was the case in the clinical studies, the results of the non-blinded studies tended to provide stronger evidence for the predominant theory. First, "aggression among nestmates was three times more likely to be reported in blinded than non-blinded experiments." Second, "the effect size—the differences between the level of aggression among nestmates and that among non-nestmates—in non-blind experiments was twice that of blind experiments." Here, blinded experiments seem to have escaped bias from theoretical expectations.

Another unlikely topic for observational error might be plant herbivory: namely, how much tree foliage do insects consume? One might envision a fairly straightforward task of sampling leaves and measuring the amount of loss—scan their surface area, weigh them, or count the proportion of leaves with damage. Or estimate defoliation visually, from photos of whole trees (and cross-check this method with some direct sampling). Simple measurements—manageable even by introductory students?

This topic, too, has been examined for evidence of observer bias—based on 42 publications of insect herbivory in Brazil (Kozlov et al., 2014). Again, blinded and non-blinded studies were compared. The plant damage differed by a factor of *five to ten*, depending on the methods used. Non-blinded studies reported significantly more damage than blinded studies. That is, they matched the widespread assumption that such rates are very high in the tropics. In addition, studies that focused on only one or a few species (1–3) found twice as much damage as those studying 10 or more species. Thus, the researcher's choice of individual species seems to have been a biasing factor. Perhaps one chooses a species because the damage is more noticeable (or "typical") to the observer who is seeking to measure it? Or the species is more prevalent, enabling easy sampling. But the selected species apparently did not fairly represent all species, and this error has led to misleading claims about insect herbivory in the tropics in general.

In a follow-up analysis (based on 125 publications), the same team identified other ways apparently insignificant choices seem to unconsciously bias such research: selection of study site; selection of timing (season and duration); and selection of individual branches or leaves to be sampled (Zvereva & Kozlov, 2019). Casual (technically, "haphazard") sampling can open the way to observer bias. In addition, primary authors who participated in the sampling or measurement, or others who knew where the samples had originated, inevitably inflated the magnitude of the results. The reviewers concluded sadly, "Our ecological and environmental knowledge is considerably biased due to an unconscious tendency of researchers to lend support for their hypotheses and expectations, which generally leads to overestimation of the effects under study." Blinding matters.

○ Pragmatic Horizons

These studies—of serum therapy, forensic analysis, clinical trials, ant behavior, and insect herbivory—document the widespread occurrence of unconscious observer bias in biology. Ironically, they equally indicate how blinding is effective in reducing its effects. Objectivity in science may be threatened by the infelicities of human observation, but it can also be salvaged by appropriate countermeasures. Accordingly, the custom of blinding—familiar to medical and psychological researchers for over a century now—is gradually informing more fields of science. (Note, too, its relevance to NGSS's third Scientific and Engineering Practice: Planning and Carrying Out Investigations.)

Observer bias is insidious, surely. Unconscious and easily hidden. It can severely threaten the quixotic ideal of objectivity in science. Yet turning a "blind eye" to such flaws only compounds the problem, allowing bias to fester at a yet deeper level. Fortunately, perhaps, while observer bias is unintentional, it can nonetheless be managed intentionally—through the strategy of blinding. In a society where facts are disputed, and allegations of prejudiced observations are rampant, such tools for reclaiming objectivity might well be more widely known—and perhaps fruitfully applied even by nonscientists.

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Nominate yourself or a colleague for one or more of the following NABT awards by visiting **nabt.org/Awards-About**. The nominee will be sent the necessary information, materials, and instructions to submit their application for consideration.

BIOLOGY EDUCATOR LEADERSHIP SCHOLARSHIP (BELS) ... The Biology Educator Leadership Scholarship (BELS) supports teachers who are furthering their education in the life sciences or life science education. The recipient is required to be a practicing educator who is also enrolled (or anticipates enrolling) in a graduate program at Masters or Doctoral level. NABT members with less than or equal to ten years of teaching experience are eligible. The BELS program is sponsored by NABT Members and includes a $5000 tuition assistance award, a plaque to be presented at the NABT Professional Development Conference, and one-year complimentary membership to NABT. The nomination deadline is **March 15, 2023.**

DISTINGUISHED SERVICE AWARD ... NABT members and friends are invited to nominate outstanding scientists, science communicators, and educators to receive the NABT Distinguished Service Award, which was established in 1988 to commemorate the 50th anniversary of the Association. Nominees should be nationally recognized for major contributions to biology education through their research, writing, and/or teaching. Recipients are honored at the NABT Professional Development Conference.

PROF. CHAN TWO YEAR COLLEGE AWARD FOR ENGAGED TEACHING OF BIOLOGY ... Sponsored by Sarah McBride and John Melville, the Prof. Chan Two-Year College Award for the Engaged Teaching of Biology is given to a two-year college faculty member who has successfully developed and demonstrated an innovative, hands-on approach in the teaching of biology and has carried their commitment to the community. This award includes $500 toward travel to the NABT Professional Development Conference, a plaque to be presented at the NABT Professional Development Conference, and one-year complimentary membership to NABT. The nomination deadline is **May 1, 2023.**

ECOLOGY/ENVIRONMENTAL SCIENCE TEACHING AWARD ... Sponsored by Vernier Software & Technology, the Ecology/Environmental Teaching Award is given to a secondary school teacher who has successfully demonstrated an innovative approach in the teaching of ecology/environmental science and has carried that commitment to the environment into the broader community. Vernier's sponsorship of this award includes up to $500 toward travel to the NABT Professional Development Conference, and Vernier equipment. The recipient also receives a plaque to be presented at the NABT Professional Development Conference and a one-year complimentary membership to NABT. The nomination deadline is **March 15, 2023.**

EXCELLENCE IN ENCOURAGING JEDI AWARD ... The NABT Excellence in Encouraging Justice, Equity, Diversity, and Inclusion (JEDI) Award recognizes efforts to promote equity in life science education. The recipient/recipients demonstrate a passion and commitment for JEDI through their teaching and outreach while also identifying successful strategies that increase enthusiasm for biology. Sponsored by NABT, this award includes a $500 honorarium, a recognition plaque to be presented at the NABT Professional Development Conference, and a one-year complimentary NABT membership. The nomination deadline is **March 15, 2023.**

EVOLUTION EDUCATION AWARD ... The Evolution Education Award, sponsored by BSCS Science Learning and NCSE recognizes innovative classroom teaching and community education efforts to promote the accurate understanding of biological evolution. The award is presented to K-12 and higher education faculty on alternating years. Undergraduate faculty are eligible in 2023. The award includes a combined $1,000 honorarium, a recognition plaque to be presented at the NABT Professional Development Conference, and a one-year complimentary membership to NABT. The nomination deadline is **March 15, 2023.**

FOUR-YEAR COLLEGE & UNIVERSITY SECTION BIOLOGY TEACHING AWARD ... This award, sponsored by NABT's Four-Year College & University Section, recognizes creativity and innovation in undergraduate biology teaching. These innovations may include curriculum design, teaching strategies, and laboratory utilization. Additionally, award winners will agree to present their work during the NABT Conference. The award is open to NABT members and includes $500, a recognition plaque to be presented at the NABT Professional Development Conference, and a one-year complimentary membership to NABT. The nomination deadline is **May 1, 2023.**

FOUR-YEAR COLLEGE & UNIVERSITY SECTION RESEARCH IN BIOLOGY EDUCATION AWARD ... This award, sponsored by NABT's Four-Year College & University Section, recognizes creativity and innovation in research that furthers our understanding of biology teaching and education. These innovations may include scholarship and research in biology education. Additionally, award winners will agree to present their work at the NABT Conference. The award is open to NABT members, and includes $500, a recognition plaque to be presented at the NABT Professional Development Conference, and a one-year complementary membership to NABT. The nomination deadline is **May 1, 2023.**

GENETICS EDUCATION AWARD ... Sponsored by the American Society of Human Genetics (ASHG) and the Genetics Society of America (GSA), the Genetics Education Award recognizes innovative, student-centered classroom instruction to promote the understanding of genetics and its impact on inheritance, health, and biological research. The award includes a $1000 honorarium, a recognition plaque to be presented at the NABT Professional Development Conference, and one year of complimentary membership to NABT. The nomination deadline is **March 15, 2023.**

HONORARY MEMBERSHIP ... The highest honor bestowed by NABT, this award recognizes individuals who have "achieved distinction in teaching, research, or service in the biological sciences" as Honorary Members. Those selected become lifetime members of the Association and receive recognition in NABT publications and at the NABT Professional Development Conference. Nominations may be made by any NABT member and must include (1) a description of the candidate's qualifications, (2) a detailed biographical summary, and (3) supporting letters from at least nine NABT members. The nomination deadline is **March 15, 2023.**

JENNIFER PFANNERSTILL TRAVEL AWARD ... Established to honor the memory of Jennifer Pfannerstill, this need-based scholarship provides support for a teacher who has successfully demonstrated a commitment to developing as a professional by attending the NABT Conference for the first time. Sponsored by private contributions and NABT, the recipient will receive registration to the NABT Professional Development Conference, hotel accommodations for the duration, travel reimbursement, and a one-year complimentary membership to NABT. The scholarship is open to teachers at all levels, but nominees must be current NABT members. The nomination deadline is **March 15, 2023.**

KIM FOGLIA AP BIOLOGY SERVICE AWARD ... The Kim Foglia AP Biology Service Award recognizes an AP Biology teacher who displays a willingness to share materials, serves as a mentor to both students and professional colleagues, creates an innovative and student centered classroom environment, and exemplifies a personal philosophy that encourages professional growth as an AP biology teacher. Sponsored by Pearson and the Neil A. Campbell Educational Trust, the Kim Foglia AP Biology Service Award includes a $1000 honorarium, a recognition plaque to be presented at the NABT Professional Development Conference, and a one-year complimentary membership to NABT. The nomination deadline is **March 15, 2023.**

OUTSTANDING BIOLOGY TEACHER AWARD ... Every year, the Outstanding Biology Teacher Award (OBTA) program attempts to recognize an outstanding biology educator (grades 7-12) in each of the 50 states; Washington, DC; Canada; Puerto Rico; and overseas territories. Candidates for this award must have at least three years public, private, or parochial school teaching experience. A major portion of the nominee's career must be devoted to the teaching of biology/life science, and candidates are judged on their teaching ability and experience, cooperativeness in the school and community, and student-teacher relationships. OBTA recipients are special guests at the Honors Luncheon during the NABT Professional Development Conference; receive gift certificates from Carolina Biological Supply Company, materials from other sponsors, and award certificates and complimentary one-year membership to NABT. The nomination deadline is **February 1, 2023**

OUTSTANDING NEW BIOLOGY TEACHER ACHIEVEMENT AWARD ... This award, sponsored by Pearson and the Neil A. Campbell Educational Trust, recognizes outstanding teaching (grades 7-12) by a "new" biology/life science instructor within their first three years of teaching (when nominated) who has developed an original and outstanding program or technique and made a contribution to the profession at the start of their career. The award includes a $1,000 honorarium, recognition plaque to be presented at the NABT Professional Development Conference, and a one-year complimentary membership to NABT. The nomination deadline is **March 15, 2023.**

THE RON MARDIGIAN BIOTECHNOLOGY TEACHING AWARD ... The Ron Mardigian Biotechnology Teaching Award, sponsored by Bio-Rad Laboratories, recognizes a teacher who demonstrates outstanding and creative teaching of biotechnology in the classroom. The award is given to secondary school teachers in even numbered years, college/university instructors in odd numbered years. The award may be given for either a short-term series of activities or a long integration of biotechnology into the curriculum. The award is presented at NABT's Professional Development Conference and includes a recognition plaque, a one-year complimentary membership to NABT and up to $500 toward travel to the NABT Professional Development Conference and Bio-Rad materials. The nomination deadline is **March 15, 2023.**

TWO-YEAR COLLEGE BIOLOGY TEACHING AWARD ... Sponsored by NABT's Two-Year College Section and Cell Zone, Inc., this award recognizes a two-year college biology educator who employs new and creative techniques in their classroom teaching. The primary criterion for the award is skill in teaching, although serious consideration is given to scholarship, curriculum design, or laboratory utilization. Nominees must be current members of NABT. The award includes $500 toward travel to the NABT Conference, a recognition plaque to be presented at the NABT Professional Development Conference, and a one-year complimentary membership to NABT. The nomination deadline is **May 1, 2023.**

VISIT nabt.org/Awards-About for more information.

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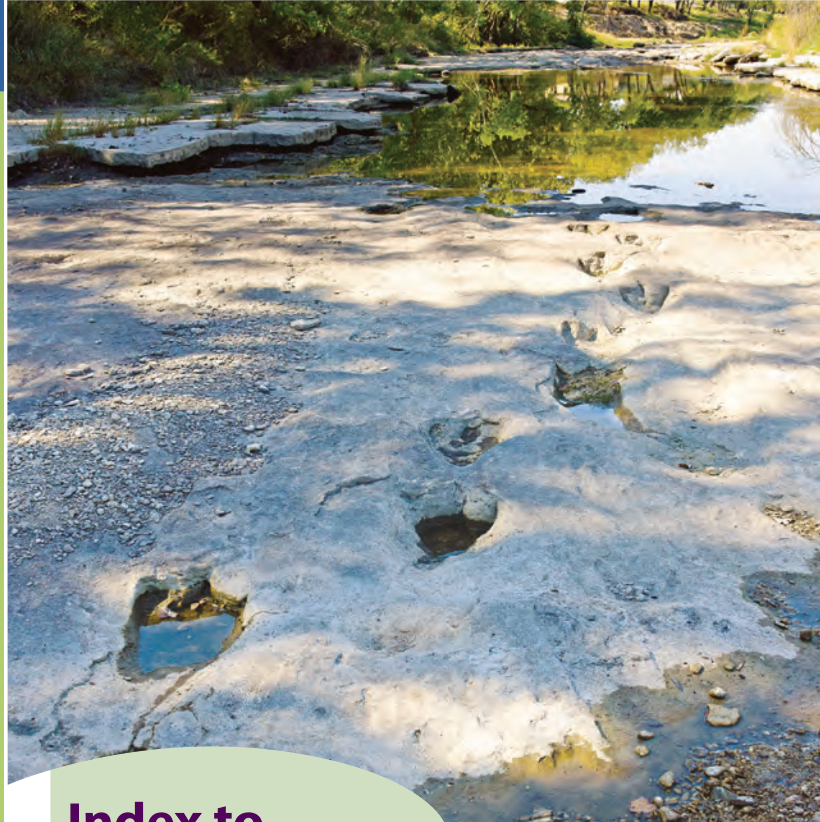

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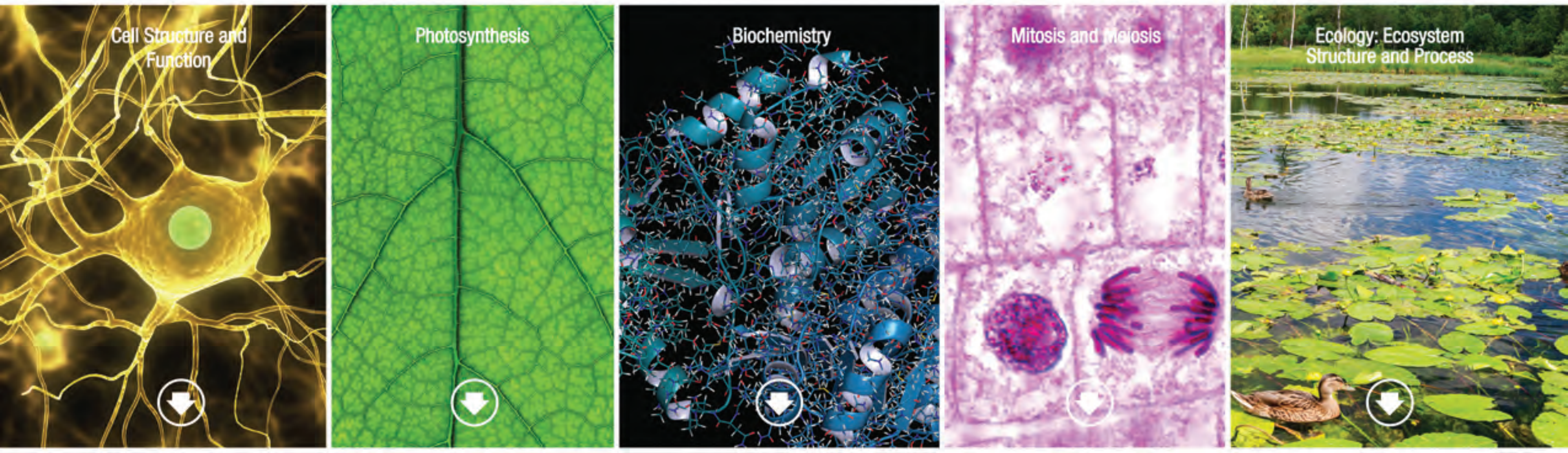

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