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Theme Issue: Darwin and Evolution

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About Our Cover

***Monotropa uniflora* (The Ghost Plant)**

Our cover photo for the annual evolution issue is the remarkable herbaceous plant *Monotropa uniflora*. Often called the Indian pipe, ghost, or corpse plant, it is seen here growing in the Big Thicket National Preserve, northeast of Houston, Texas. This image appeared previously in *The American Biology Teacher* as a BioMystery in August 2020, but we are sharing it with you again to highlight an interesting evolutionary change in these plants, a lack of chlorophyl.

In the various names for this plant, we see the characteristics reflected: the single flower ("unifora") on a downturned pipe-like stem ("monotropa"), and characteristic translucent pinkish or ghostly white color. From early summer to autumn, ghost plants can grow to their full height (5–30 cm) in just a few days when moisture conditions are right. They will produce a single flower surrounded by up to eight translucent petals.

Ghost plants come from a very large family, Ericaceae, which contains heathers, blueberries, rhododendrons, azaleas, and cranberries. All members of this genus are native to temperate regions of the Northern Hemisphere. However, it is virtually alone in its genus, which has only three species, all lacking chlorophyl, a characteristic most often associated with plants. There is still debate about the natural selection mechanisms that resulted in this lack of photosynthetic ability and fascinating lifestyle.

These plants are called *mycoheterotrophs*—to acquire the sugars needed for growth and development, they rely on a parasitic relationship with fungi. In turn, those fungi are associated with the roots of beech trees. So, in a kind of hand-me-down fashion, pipestems do benefit from photosynthetic processes, but only indirectly. As Stephen Jay Gould often pointed out in his wonderful essays, sometimes the exceptions to the norm can be quite instructive when thinking about how organisms change through time—the process of evolution.

This digital image was recorded with a Nikon D810 camera using a 28-300 mm 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

Ross H. Nehm

Considering Explanatory Diversity and Holistic Understanding of Biological Phenomena

A half century of research in biology education confirms what biology teachers likely know: students have significant difficulty generating robust scientific explanations for many aspects of the living world. This challenge becomes particularly salient given that the *Next Generation Science Standards* (NGSS) and the report *Vision and Change in Undergraduate Biology* emphasize explanation as a central tenet of science and a practice essential for learning science. Although biology teachers often provide scientific explanations to students, and students often generate explanations in labs and on assessments, remarkably little attention has focused on the various ways that life scientists approach the practice of explanation and the extent to which these approaches are integrated into the biology curriculum.

What we *do* know is that many studies of students' biological explanations have identified common challenges: the reliance on inappropriate teleological (need- and goal-driven) explanations grounded in design-based reasoning; the inclination toward function-based accounts when explaining evolutionary origins; the tendency to produce descriptions rather than causal and mechanistic narratives; and the formulation of numerous phenomenon-specific explanations rooted in observable features rather than generalizable causal abstractions. Given the lack of explicit attention to explanatory practices in biology instruction, is it possible that students' struggles in formulating accurate explanations are due to a lack of comprehension of, and practice with, biological explanation? If true, how should explanation be taught?

Understanding the variety of explanatory practices in the life sciences necessitates confronting the fragmented nature of life science research and education. Contrary to conventional wisdom, there isn't a singular "biology" discipline. The life sciences can be conceptualized as a federation of distinct disciplines (such as cell and molecular biology), each residing in separate academic departments, aligned with distinct academic societies, focused on specific biological scales, pursuing distinct epistemic aims, publishing in different journals, and competing with other "biologists" for funding resources. This organizational complexity extends to the diverse approaches various fields adopt when studying (and explaining) life, leading to the conclusion that a single explanatory practice might not suffice for all biological phenomena.

To illustrate the diverse vantage points or explanatory stances affiliated with individual biological disciplines, consider the observable phenomenon of a cone snail harpooning, paralyzing, and killing a fish. Biochemists, for example, may seek to explain this phenomenon by focusing on the venom and its chemical compounds. After isolating different macromolecules, their explanatory aim may be to account for how venom is able to paralyze fish. Their explanatory practice often involves investigation at a deeper level (e.g., documenting how the 3-D structure of peptides blocks cell surface receptors in the fish). Here, the explanatory practice focuses on moving to lower scales to explain how something works at a higher level.

In contrast, evolutionary biologists' explanatory practices do not generally look to a lower level or scale to explain, but rather tend to focus on where poison came from initially (not how it works, but when and how it originated). Here, explaining involves identifying the origin of the poison in time through analyses of gene families and mutation patterns in a phylogeny calibrated with the fossil record. Ecologists, on the other hand, may use yet another explanatory approach and seek to causally account for how the poison impacts predator-prey (snail-fish) dynamics in the snails' niche. Here the aim is not looking downward or backward but looking upward to the scale of the organism. That is, poison is explained by the role it plays in promoting successful functioning of the organism as a unit in its niche. These instances exemplify the diverse vantage points and explanatory practices that different biological subdisciplines employ. However, together they provide an integrative framework to comprehend and explain an observable phenomenon; a single type of explanation usually proves inadequate when deciphering real-world phenomena, just as one life science discipline falls short when studying life.

The above examples illustrate how distinct disciplines break down facets of a phenomenon in ways that align with their epistemic aims (How does it work? Where did it come from?) to render the explanatory process manageable; after all, life is complex. The ultimate aim, however, remains explaining the overarching phenomenon. Take Huntington disease as an example. Downward-looking explanatory approaches elucidate neuronal cell death by investigating lower-level molecular-genetic mechanisms stemming from CAG repeats. Upward-looking explanatory approaches investigate how CAG repeats account for the persistence of the illness in human populations, revealing organismal-level late onset and hypersexuality. Yet, neither of these approaches explains the disease's origin within a family; for that, a backward-looking approach is required. One explanatory practice (downward-looking investigations) can't comprehensively elucidate all aspects of disease.

Biology educators have devoted considerable effort to documenting student challenges related to explanation, but they've allocated insufficient attention to developing theoretical frameworks and educational tools for fostering effective, practical, and varied explanatory practices. The phenomenon-centered approach of NGSS lessons presents a promising avenue to deploy diverse explanatory practices when grappling with the same phenomenon. Guiding students in comprehending explanatory aims (Are we seeking to explain a trait's function or origin?) and engaging in explanatory practices (the downward-, backward-, and upward-looking framework) represent a potential strategy to fill a void in our instructional approaches.

Could the difficulties students face in formulating normative explanations—relying on function-based explanations for origins, and succumbing to inappropriate teleological explanations—be rooted in misunderstanding or conflating forms of biological explanation? As a field, we are far from having all the answers. It's evident that there is much more explaining to be done.

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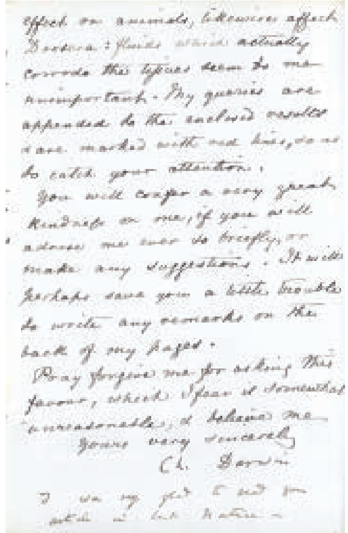
FEATURE ARTICLE

Using Darwin's Pangenesis Correspondences to Examine Science as a Human Endeavor

ANTHONY LORSBACH,
ALLISON ANTINK MEYER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Drosera; fluids which actually
corrode the tissues seem to me
unimportant. My queries are
appended to the enclosed results
& are marked with red line, so as
to catch your attention.
You will confer a very great
kindness on me, if you will
answer me ever so briefly, or
make any suggestions. It will
perhaps save you a little trouble
to write any remarks on the
back of my pages.
Pray forgive me for asking this
favour, which I fear is somewhat
unreasonable, & believe me
Yours very sincerely
Ch. Darwin

Abstract

This lesson used the correspondence of Charles Darwin as an exploration of nature of science (NOS) in a historical context. Specifically, we used his original correspondence about his "provisional hypothesis" of pangenesis as a novel way to explore a scientist's social community. Darwin's community of friends and colleagues in the natural sciences at the time of his writing of his 1868 book Variations formed the basis of this lesson. One basic descriptor of NOS, science as a human endeavor, was used to drive explicit reflection. These letters were rich in detail regarding the idea of science as a community of practice. Our elementary education students' responses indicate the letters surprised them in how personal the correspondents were with one another and how reliant Darwin was on his friends and colleagues for input on his work. Darwin became human as students imagined Darwin's mental state and how he wrestled with his idea and made it public. Students learned that despite Darwin's fame, his idea of pangenesis lacked empirical evidence and thus received little support. They discovered an eminent scientist who was insecure and nervous and who worked hard to develop, study, and publicize his novel idea. This contrasts with popular views of major scientific figures as natural geniuses rather than their success resulting from labor and perseverance.

Key Words: *Nature of science, Science as a human endeavor, History of science, Charles Darwin, Pangenesis, Primary sources, Preservice teacher preparation.*

○ Using Darwin's Pangenesis Correspondence to Examine Science as a Human Endeavor

Along with a science methods course, our elementary education program requires a science inquiry course that emphasizes science and engineering practices (*Next Generation Science Standards* (*NGSS*); NGSS Lead States, 2013) and nature of science (NOS). The course is designed to provide students with opportunities to engage in science inquiry and better understand what science is and how it works. Historically, teachers have been asked to develop lessons and teach science without engaging in scientific inquiry outside of the classroom. Instead, most elementary teachers' inquiry experiences are confirmatory labs in introductory science courses.

NOS instruction accompanies science inquiry experiences. The lesson described here relates to NOS as a human endeavor (SHE) and is based on the correspondence of Charles Darwin, available online at the Darwin Correspondence Project (DCP; https://darwinproject.ac.uk). The letters focus on Darwin's challenge of providing a mechanism to explain natural selection, without which his grand idea was incomplete. His solution was pangenesis.

"Darwin used letters as a way both of discussing his ideas and gathering the 'great quantities of facts' that he used in developing and supporting his theories" (https://www.darwinproject.ac.uk/letters/darwins-life-letters). As such, his letters provide insight into the community of practice with whom Darwin communicated and provide glimpses into Darwin's personal life, feelings, and the thoughts of others surrounding his work.

> *[Darwin's] letters provide insight into the community of practice with whom Darwin communicated and provide glimpses into Darwin's personal life, feelings, and the thoughts of others surrounding his work.*

Creating historical cases is not new to the teaching of NOS. Conant created the Harvard case studies, Histories in Experimental Science, in the 1950s and Klopfer created the History of Science Case Studies in the 1960s (McComas, 2008). NGSS recommends the use of case studies to "broaden and deepen" understanding about NOS to "bridge tactics and strategies with practice" (Appendix H, pp 7–8). However, Hodson (2009) warns that it is not enough for teachers to read case studies. They also need to develop pedagogical content knowledge about NOS using case studies to "enable teachers

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to talk comfortably about NOS issues" (p. 74). Abd-El-Khalick and Lederman (2000) argued that teachers should be able to substantiate NOS claims in their teaching using examples and cases. We believe using actual correspondence between scientists can be used to provide rich examples of NOS attributes that students can use to compare with their own experiences to better enable them to understand NOS and substantiate NOS claims. While we used this lesson to enhance elementary education students' understanding of science as a human endeavor in a science inquiry course, we feel this lesson could be a useful addition to NOS units in high school and college biology classes.

Our pangenesis lesson used details in the actual words of scientists to illustrate how they communicated with one another as they discussed a new idea and whether an experiment disproved that idea. Previously, we created a supplemental lesson on critical thinking to highlight NOS characteristics by using DCP letters (Author Citation, 2019a, 2019b). Here, we used Darwin's correspondence about his "provisional hypothesis" of pangenesis to explore the scientist's social community within which he operated; specifically, his community of friends and colleagues in the natural sciences at the time he was writing his 1868 book, *Variations of Animals and Plants under Domestication* (*Variations*). Darwin shared his ideas about pangenesis with this community to provide an explanation for ideas associated with his theory of natural selection. Additionally, we thought it might be provocative to use an example from a well-known scientist whose idea was not embraced by the science community to serve as an introduction to the idea that even historical geniuses can have ideas rejected in the light of evidence.

○ NOS and Science as a Human Endeavor

NOS "refers to the characteristics of scientific knowledge that necessarily result from the conventional approaches (e.g., scientific inquiry) that scientists use to develop knowledge" (p. 296, Lederman et al., 2020). Decades of NOS research (for example Lederman & Lederman, 2014; McComas & Clough, 2020) demonstrate that teachers and students typically lack an "adequate" understanding of NOS. Regardless of teachers' views about NOS, translation into classroom practice should not be assumed and they do not typically view NOS learning outcomes for their students as of equal importance to other science learning outcomes. Research clarifies that NOS learning is most effective through explicit, reflective instruction about NOS attributes in the context of science examples and experiences. This can take many forms: historical case studies (Irwin, 2000), inquiry experiences (Akerson et al. (2000); Khishfe & Abd-El-Khalick, 2002), general activities (Lederman et al., 2020), part of blended science content instruction (Neumann et al., 2020), digital technologies (DeCoito, 2020), among many others (see Erduran et al., 2020).

This lesson drew on the historical case study strategy to promote growth in pre-service teachers' understanding of one of the eight NOS attributes from Appendix H of NGSS: *science is a human endeavor*. SHE can mean personal habits of mind such as intellectual honesty, tolerance of ambiguity, skepticism, and openness to new ideas as described by NGSS, but is also used to describe broad sociocultural influences in science. In this sense, Sammel (2014) describes SHE, in part, as "placing the discipline of science within socio-cultural frames and allowing students to view [the] current and historical nature of science" (p. 850). While our lesson included habits of mind described by NGSS, we also emphasized science as a community of practice. A community of practice (COP) was described by Lave and Wenger (1991) and more explicitly explored by Wenger (1998). Defined by Wenger-Traynor and Wenger-Traynor (2015), a COP is "groups of people who share a concern or a passion for something they do and learn how to do it better as they interact regularly." Like other communities of practice, Darwin's COP was characterized by a shared domain of interest (evolution by natural selection), engaged in joint activities and discussions (such as letter writing), and a shared repertoire of experiences, stories, and tools to address recurring problems that Wenger calls practices (https://wenger-trayner.com/introduction-to-communities-of-practice/).

Hodson (2009) believed science as a COP is the area of NOS that is the most poorly understood by teachers and students—and the least likely to be taught. Hodson (2009) goes on to say that

> because students are studying the characteristics of a community, social issues are center stage….[T]extbook accounts of theoretical developments that pay scant attention to the personal and social dimensions of scientific practice, neglect to consider ways in which the decisions and actions of scientists are influenced by worldview, feelings, attitudes and prejudices, and fail to acknowledge how science is subject to a wide range of sociocultural and economic influences. (p.86)

Context of the Lesson

The lesson was part of a NOS unit at the beginning of the course (see the Supplemental Materials provided with the online version of this article). Other components of the unit included course readings and a group investigation in which students, in small groups, developed an explanation for a natural phenomenon. Groups presented their findings to the class, developed tests for competing hypotheses, and developed new models to explain their understandings. This investigation was the first "hands-on" experience in the class. These experiences were discussed considering SHE attributes discussed above. Students then completed the Darwin letters assignment described below, followed by readings and discussion of McIntyre's (2019) idea of the scientific attitude, which he frames as a commitment to empirical evidence and a willingness to change our ideas in light of new evidence. This was followed by a discussion of Oreskes's (2019) five pillars of science (consensus, method, evidence, diversity, and values), which includes the idea that science is trusted, not because it has a reliable method but because the trustworthiness of science claims is due to the social processes by which claims are vetted.

Darwin's Correspondence and Pangenesis

Not all students were familiar with Charles Darwin, so a brief video (*Detecting Darwin*) from DCP was used as an introduction (https://vimeo.com/user10337388). The video was followed by a question-and-answer discussion. While the letters illustrated other aspects of NOS, for example that science is based on empirical evidence, most questions were designed to emphasize SHE and science as a community of practice. Student comments included other NOS aspects, however.

Letters were chosen by initially entering the keyword *pangenesis* in the DCP search engine—239 letters resulted from this keyword search. We narrowed the letters to the 13 used in the lesson that specifically pertained to Darwin's solicitation of opinions about the pangenesis chapter of *Variations*, Galton's rabbit experiments to test Darwin's idea, and were suitable to our NOS instructional purposes (i.e., readability and sufficient context). An additional letter from Galton to the journal *Nature* from Galton's collected works was included to show both Galton's public and private response to Darwin's criticism of his experiment. The chosen letters were listed chronologically to better tell the story of the reception of pangenesis by Darwin's colleagues and friends.

Peer review as we know it was developed in 1731 by the Royal Society of Edinburgh but was not widely implemented until the middle of the 20th century (*The Lancet* did not adopt peer review until 1976). During Darwin's time, science journals were rapidly proliferating, and it was common for the editor to be the sole reviewer. But as it is today, it was common practice to ask friends and colleagues to critique a manuscript (Spier, 2002). Darwin began to share his idea of pangenesis with a circle of friends and colleagues—a community of practice. We put together a series of letters to and from Darwin that we feel highlighted the responses of his colleagues and friends in the science community to his idea, and his reaction to those responses. We wanted to highlight what scientists do and how they react to new ideas in science—particularly from one of its most esteemed members at the time.

Darwin's Problems

After years of collecting and processing data, Darwin was compelled to publish *The Origin of Species* outlining his theory of natural selection in 1859 when Alfred Russel Wallace independently developed the same idea in 1858 and wrote to Darwin outlining his concept. Eventually, Charles Lyell and Joseph Hooker, Darwin's friends and fellow scientists, presented papers for both Darwin and Wallace publicly to the Linnaean Society. Since Darwin's work preceded that of Wallace, and Darwin quickly followed up the papers with his book, *The Origin of Species*, natural selection is now seen as primarily his idea.

However, Darwin's theory of natural selection needed an explanation for inheritance. Thomas H. Huxley asserted that without it, his theory of natural selection was incomplete (Browne, 2002). Critics and Darwin himself agreed, natural selection was dependent on a model of inheritance.

Natural selection needed a complimentary model of inheritance and Darwin spent time after publishing *The Origin of Species* developing such a model. In 1868 he published another book called *The Variation of Animals and Plants under Domestication*. In *Variation* he included a chapter describing his "provisional hypothesis of pangenesis."

Pangenesis

Darwin introduced pangenesis as an explanation for, not only natural selection, but other phenomena found in animals and plants, such as the ability of some animals to regrow limbs, or graft hybrids "in which the offspring of a branch from one species of plant when growing on another occasionally had characteristics of both the graft and the stock" (McComas, 2012, p. 87).

> I am aware that my view is merely a provisional hypothesis or speculation; but until a better one is advanced, it will serve to bring together a multitude of facts which are at present left disconnected by any efficient cause. As Whewell, the historian of the inductive sciences, remarks: "Hypotheses may often be of service to science, when they involve a certain portion of incompleteness, and even of error." Under this point of view, I venture to advance the hypothesis of Pangenesis, which implies that every separate part of the whole organization [i.e., organism] reproduces itself. So that ovules, spermatozoa, and pollen-grains—the fertilized egg or seed, as well as buds—include and consist of a multitude of germs thrown off from each separate part or unit [of the organism]. (Darwin, *Variations*, 1896, p .350)

Darwin called these pieces thrown off from each part of an organism "gemmules." In Darwin's view each part of an organism produce gemmules that circulate in the body; gemmules from any body part can be found throughout the body. The gemmules collected in reproductive organs are then mixed with the other parental gemmules and passed to the next generation. To Darwin, pangenesis explained why offspring can look like parents, or more like one parent than another. Darwin explained that some gemmules can pass from generation to generation lying dormant. When later generations express a trait from a long-ago ancestor, it is merely the gemmules coming out of this dormancy. This mixing of gemmules in plants could also explain graft hybrids. It can also explain an organism's ability to regrow parts: gemmules concentrate at the wound of a lost limb and begin to grow.

Though we often think of Darwin as disputing Lamarck's theory of acquired characteristics, "Darwin fully accepted the Lamarckian principle of 'use and disuse' as contributing to new variants" (McComas, 2012, p. 87). Pangenesis explained the inheritance of traits, which were selected by processes of natural selection.

> [T]he direct action of changed conditions on the organisation [i.e., organism], and of the increased use or disuse of parts; and in this case the gemmules from the modified units [i.e., body parts] will be themselves modified, and, when sufficiently multiplied, will supplant the old gemmules and be developed into new structures. (Darwin, *Variations*, 1896, p. 390)

Pangenesis alleviated Darwin's concern about inbreeding among first cousins, as were Darwin and his wife Emma. Browne (2002) explained using the pangenesis explanation for use/disuse:

> Darwin proposed that some limited effects from the environment might become embedded in an individual's constitution and thus be liable to be transmitted, via the gemmules, to the offspring. If two very closely related individuals, who had grown up under rather different external circumstances, were paired, these small differences would make each sufficiently distinct from each other to bear normal offspring. (p. 281–282)

In fact, Browne (2002) believed that Darwin's theory of pangenesis clearly reflected his own family concerns and the implications for his children's health resulting from marriage between close relatives.

The objectives for this lesson were to introduce concepts of SHE (e.g., habits of mind, skepticism, sociocultural influences) and the idea of a community of practice for students to compare with their own experiences, especially their experiences in our class. The pangenesis lesson also provided a basis to discuss the scientific attitude and the five pillars of science that followed this lesson.

○ Student Responses

In the lesson, letters were grouped into four sections. Each section included questions students answered in writing. Written responses from 59 students, totaling over 300 pages, across three sections of a science inquiry course for elementary teachers taught by the authors were examined. The goal here was to provide a glimpse of the type of connections students made between the letters, the controversy, and SHE, rather than a full analysis of each question.

The letters surprised students with how personal the correspondents were with one another and how reliant Darwin was on his friends and colleagues for input on his work. Predominant responses to Question 1, What were your first impressions of the letters?, emphasize the affable nature of the letters describing them using words such as *cordial* and *friendly*. For example, one student wrote, "some of the letters were more humorous despite being used as a form of peer review, such as when Huxley joked about people finding Darwin's manuscript in the future and blaming Huxley for the fact that they were never published."

Students noted the personal nature of the relationships within Darwin's correspondence.

One student commented on

> how much each scientist knew and cared about the other's life. I often think that scientists were just in the same field of study but didn't really communicate…[but]they used their friends to help them with their ideas because a lot of the time, I always think scientists figure everything out on their own without consulting others.

Students also observed that the letters provided insight into the thoughts of the participants. For students, the letters demonstrated *"how heavily the scientists rely on each other for their opinions and critiques on their own work. It really shows how collaborative scientists can, and need to, be in order to put out their best work."*

Students also noted how informal the letters were regarding how science is discussed between colleagues and friends in answer to Question 3, What might this say about how science is discussed among a scientist and his colleagues and friends? One student, for example, observed that Huxley expressed that he was

> more than willing to read what Darwin sent him and he is happy to read any insights he might have…. This shows how science and important questions are talked about causally among scientists. In the letter, they talk about personal details and that shows that while their life might revolve around science, they know more about each other than just what the other has discovered.

Furthermore, students emphasized how collaborative the correspondents were. For example, "Huxley seems to be excited to engage in Darwin's work and provide any feedback in which he can help him with. [A]mong scientists… I can tell it is very collaborative work where you cannot get stuck being narrow minded." Another student connected these two themes, that the scientists were friendly and that was a factor in their collaboration.

> Science seems to be discussed in a friendly manner and even though the science that is being discussed is very intense and complicated, the scientists are very relaxed and are willing to help a friend out. They are not in competition with each other. They are looking for opportunities to help each other and are glad to do so.

Students also discovered that scientists are not always immune to how they are perceived by others. For example, one student interpreted the first two letters as reflective of Darwin's "[concern] with what Huxley had to say about his ideas. He seems to place a lot of weight on Huxley's opinion [as] outside opinions meant a lot to Darwin and he wants his thoughts to be acceptable to others."

These letters humanized the work of Darwin by intimating Darwin's feelings when criticized. For Question 7—How does Darwin portray pangenesis to his friends, and how does Darwin anticipate his friends' impressions of pangenesis?—students interpreted some correspondence as intended jokingly. "He is criticizing the speculative nature of his work by comparing it to another scientist's work (whom neither of the two scientists seems to support). I think poking fun at himself was Darwin's way to handle the criticism." Students empathized with these letters and perceived "that Darwin is afraid of what the scientific community will think of his pangenesis."

Yet, despite this fear, Darwin persisted in asking for feedback, knowing his friends would be critical of his idea. As one student wrote,

> Darwin anticipates his friends' thoughts to be a little skeptical and confused. Darwin seems to be nervous about the responses he may get and is ready for questions and criticism. In the third letter written to Hooker, Darwin says that he "shall not be at all surprised if [he] attacked it and [him]." This quote show that Darwin is prepared for the criticism and would expect nothing less from such a chapter.

Students also perceived the mental toll *Variations* had on Darwin and many were empathetic. They perceived Darwin feeling stressed and used words such as anxious, overwhelmed, *and* disheartened *to describe his writing where "his words kind of portray a worried tone at times" (Question 8).*

The letters concerning Galton's rabbit experiments also demonstrated to students Darwin's all-too-human response to Galton's results. One response to Question 10—Why do you think Darwin disagrees with Galton's methods?—illustrates the sentiments of most students.

> I think the reason that Darwin disagrees with Galton's method is because Galton's findings did not confirm Darwin's theory. Darwin was initially pleased to hear that someone was trying to prove his theory, and he probably assumed Galton's research would confirm his theory…. [Galton] apologized for misunderstanding (even though Darwin previously supported his understanding), then pointed out specific parts of Darwin's Pangenesis chapter to support his experiment.

Following the lesson, we discussed students' perceptions that Galton was very deferential to Darwin. As one student wrote, there was "clearly a hierarchy of power when it comes to scientists." As mentioned in the lesson epilogue, Darwin's prestige as a world-renown scientist very likely affected how everyone, even friends and cousins, treated his ideas, but, in the end, the lack of empirical evidence doomed the idea.

Finally, when asked in Question 17 How do these letters and the story of pangenesis inform your understanding of science and scientists?, students made connections to a COP. For example, one student wrote that “science requires input from others who are supportive of the new idea and they all work together to challenge the work to create more credible explanations to present to others.”

○ Conclusions

This lesson provides opportunities for teacher candidates to see Darwin as a feeling and fallible individual and a member of a COP. The lesson promotes reflection on how Darwin’s community communicated among itself. Students described Darwin’s community of practice as cordial, personal, casual, and collaborative. Doing so highlighted the aspects of a community of practice described by Wenger (1998). Students saw that Darwin and his colleagues had a *shared domain of interest* in evolution by natural selection and a quest to find a mechanism for it. The letters were evidence of the *joint activities and discussions* relating to evolution and natural selection engaged in by the members. And finally, the letters were among the *practices* members used to address problems. Follow-up discussions highlighted Darwin’s community of practice by comparing it to Oreskes’s (2019) assertion that, since there is no one method that is scientific, the question of why one should trust science must look beyond the methods used to understand it and to the broader social practices in science, specifically that science is consensual.

McIntyre (2019) provides another useful idea for teachers in this context, the scientific attitude. McIntyre argues that scientists, philosophers of science, and sociologists of science have not satisfactorily agreed upon any foolproof definition that can demark science from non-science, yet they know when something is scientific and when it is not. Instead of focusing on the demarcation of science, McIntyre asserts it is better to adopt the idea of the scientific attitude. Simply put, to adopt a scientific attitude we must care about empirical evidence and be willing to change our theories in light of new evidence. Students agreed that Darwin’s community of practice also exhibited a scientific attitude.

Darwin also became human; students were imagining Darwin’s mental state and how he wrestled with his idea and made it public. They found a well-known scientist who seemed insecure and nervous and who worked hard to develop, study, and publicize new ideas. This contrasts with popular views of major figures in science as natural geniuses rather than their success resulting from hard work and oftentimes failure. Perhaps more such examples can help change public views of science to one that is more casual, informal, and both personal and social. How might this make science more attractive as a career? Teacher candidates also describe the development of ideas in science (hypothesizing/theorizing) in a way not discussed before: personal, emotional, and invested in the story. They were also able to conclude that empirical data wins over fame and speculation, which supports the idea that science is based on empirical evidence and that scientific knowledge is open to revision based on new evidence.

Using Darwin’s pangenesis correspondence early in the semester provided a common experience that enriched many discussions throughout the semester and helped establish the idea of a community of practice in the class guided by a scientific attitude. We believe students were more comfortable talking about SHE as they made comparisons between their own community of practice and Darwin’s.

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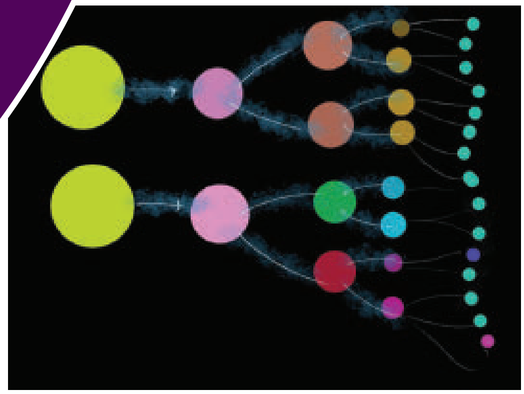
FEATURE ARTICLE

Of Phylogenies and Tumors: Cancer as a Model System to Teach Evolution



CARYN BABAIAN, SUDHIR KUMAR

Abstract

When students think of evolution, they might imagine T. rex, or perhaps an abiotic scene of sizzling electrical storms and harsh reducing atmospheres, an Earth that looks like a lunar landscape. Natural selection automatically elicits responses that include "survival of the fittest," and "descent with modification," and with these historical biological catch phrases, one conjures up images of large animals battling it out on the Mesozoic plane. Rarely do teachers or students apply these same ideas to cancer and the evolution of somatic cells, which have accrued mutations and epigenetic imprinting and relentlessly survive and proliferate. Our questions in this paper include the following: Can cancer become an important teaching model for students to explore fundamental hypotheses about evolutionary process? Can the multistep somatic cancer model encourage visualizations that enable students to revisit and reenter previous primary concepts in general biology such as the cell, mitosis, chromosomes, genetic diversity, ecological diversity, immune function, and of course evolution, continually integrating their biology knowledge into process and pattern knowledge? Can the somatic cancer model expose similar patterns and protagonists, linking Darwinian observations of the natural world to our body? And, can the cancer clone model excite critical thinking and student hypotheses about what cancer is as a biological process? Does this visually simple model assist students in recognizing patterns, connecting their biological curriculum dots into a more coherent learning experience? These biological dynamics and intercepting aptitudes of cells are amplified through the cancer model and can help shape the way biology students begin to appreciate the interrelatedness of all biological systems while they continue to explore pivotal points of biological fuzziness, such as the microbiome, limitations of models, and the complex coordination of genomic networks required for the function of even a single cell and the realization of phenotypes.

In this paper we use clonal evolution of cancer as a model experience for students to recreate how a single, non-germline cell appears to shadow the classic pattern of natural selection in body cells that have gone awry. With authentic STEAM activities students can easily crossover and revisit previous biological topics and the ubiquitous nature of natural selection as seen in the example of somatic cells that result in a metastasizing tumor, giving students insight into natural selection's accommodating and tractable patterns throughout the planet.

By illustrating this simple model, students can compare and contrast cellular processes and mechanisms that may become derailed in the progression of cancer and come to appreciate that all living systems are complex, variable, and changing.

Key Words: *Cancer, Clones, Evolution, Somatic cells*

○ Background

Cancer will touch everyone at some point in their lifetime either personally or through a dear relationship.

Despite advances in treatments and increased knowledge about cancer, cancer rates continue to rise globally. Childhood cancers have been steadily increasing (Zahm, 1995). Some cancers such as thyroid carcinomas have risen sharply over the last 30 years (Miranda-Filho, 2021). In 2022, a Harvard report revealed a dramatic increase in cancer in people under 50 with risk increasing in every generation (Brigham & Women's Hospital Communications, 2022). The progression and outcomes of this broad and often fatal disease are largely unknown with growth and metastasis becoming difficult barriers to a cure. One significant reason for cancer's tenacity is the variable, diverse nature of cells themselves, environments, and individual genomes and the cellular response to new mutations and epigenetic changes (Boland, 2005). Cancer as a topic in biology can offer students opportunities to explore the cell cycle in more detail, the effect of environment on cell dysregulation, all possible causes, and the effect of mutations on regulatory genes, and of course insight into evolutionary process. For

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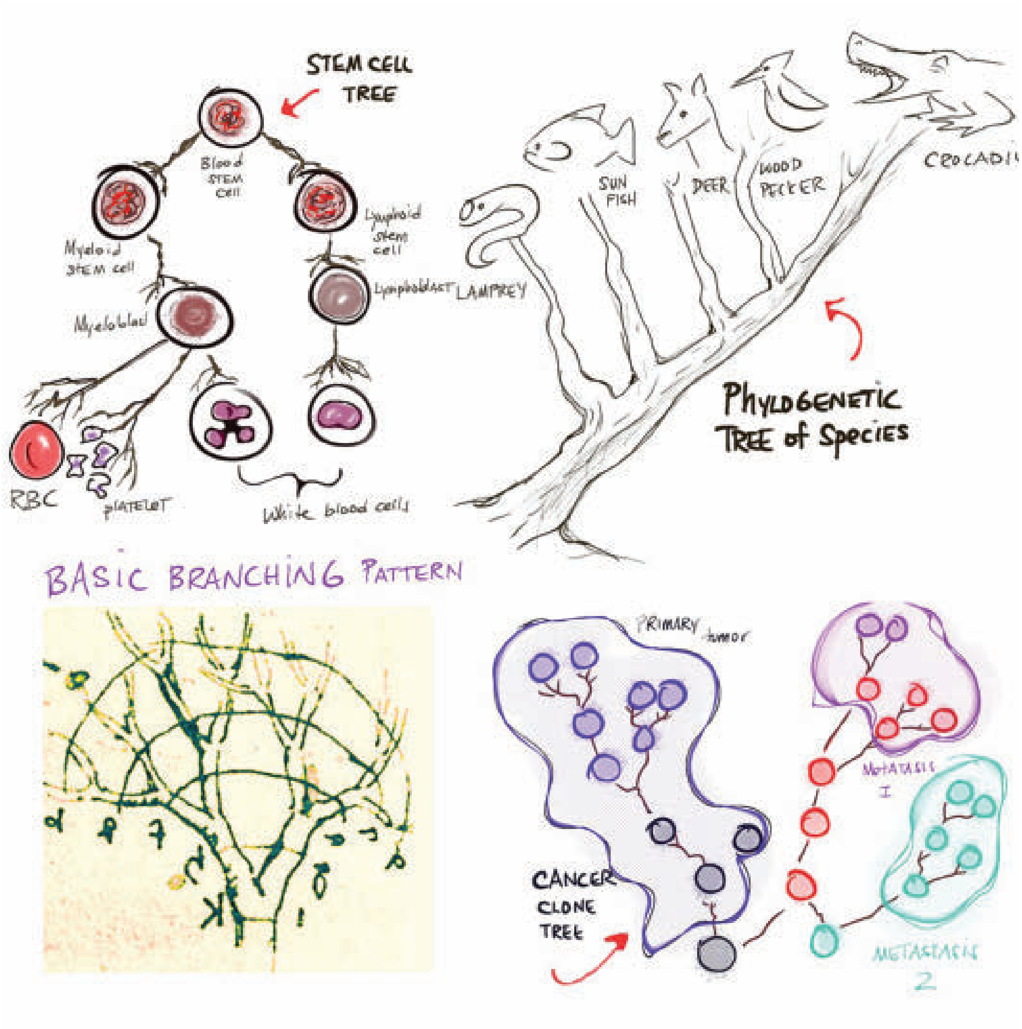

Figure 1. Comparison of different types of branching trees used for three different processes: stem cells, evolution of species, and cancer. To the farthest left, Leonardo DaVinci's sketch of branching patterns in trees.

students, cancer would be an example of somatic cell evolution as opposed to the germline evolution of sexually reproducing species, or stem cell differentiation. Students can see the differences in the evolution of single cells that make up tissue communities by comparing different simplified tree diagrams (see Figure 1). Somatic mutations in cancer models provide three very important teaching points: (a) a simplified example of eco-evolutionary relationships, one that students themselves can visualize at the cell level; (b) cancer as a sub-interdisciplinary activity, drawing in such topics as cell function, genetics, mitosis, evolution, ecology, and immune function; and (c) encouraging students to recognize similar biological patterns throughout the natural world (genetic diversity, community interactions, community diversity, selective pressures, convergent evolution, interdependency within all living systems, inter and intraspecific competition). Together these allow students to evaluate the model itself. Understanding the conceptual model of somatic cancer spread can act as a scaffold for other biological inputs to the process.

Somatic evolution plays out in everyone, making evolution visible and experienceable! This is unlike the tree of life in which the whole process has run once to produce the tapestry of life around us. The repeatability of cancer makes it possible to learn general evolutionary rules (Townsend and evolutionary tape is rerun all the time; https://www.yalescientific.org/2016/08/replaying-the-tape-of-cancer-development/).

○ Cancer across the Tree of Life

Almost everyone knows someone with cancer, but in biology we also know that some species seem almost impenetrable to the disease, and others seem more susceptible. We know that in somatic cells that do not typically divide, cancer is sparse or nonexistent, such as in striated muscles or neurons of our own bodies. We also marvel at species such as naked mole rats who never seem to develop cancer or rotifers who defy aging. Even water bears (Tardigrades) can teach us about evolutionary resiliency and resistance to cancer-causing agents such as radiation. Models that explore the cost-benefit ratio of tumor suppressor genes posit the drawbacks of dedicating significant genomic energy to staving off cancer such as reduced fertility. This is an opportunity to explore biological, cellular, and genomic diversity across the tree of life, introducing students to organisms such as the naked mole rat and maintaining the theme of diversity in living systems.

Here we can introduce students to the animals that are less susceptible to cancer such as elephants and bowhead whales, and we can also explore animals such as clams that transmit cancer through the horizontal transmission of cancer cells. Again, even with bivalves, fatal leukemia that has appeared in marine bivalves across the world could be traced back to a clonal transmissible cell derived from a single original clam (Metzger, 2015). This is somewhat like the viral cancers of Tasmanian devils. And, still other animals such as Beluga whales have been experiencing extremely high rates of cancer while close relatives such as bowhead whales do not (Nair, 2022). The connection between cancer and environmental toxins cannot be denied, as a plethora of new synthetic chemicals and their unknown combinations have been and continue to be introduced to the environment. Many substances that never existed in the billions of years of cellular evolution have the potential to induce mutations leading to genomic instability, and this too can be introduced in the cancer discussion for students as they explore species. Searching across the tree of life for diverse organisms that can get cancer, finding those that do not can stir up some inquiry and hypothesizing by students in important dialogues that showcase what students perceive, know, and understand about the biology they have acquired. To add to discussions such as comparing cancer rates in one whale species with those rates in another, we suggest exploring the TimeTree: The Timescale of Life website (http://www.timetree.org/) for students to explore divergence times between cancer-resistant species and cancer-susceptible species.

○ Biological Diversity and Cancer Clones

"*Cancer cells have defects in regulatory circuits that govern normal cell proliferation and homeostasis. There are more than 100 distinct types of cancer, and subtypes of tumors can be found within specific organs*" (Hanahan, 2000). Distinct cancer types are a mirror of the complexity of normal functioning cells and, therefore, offer an excellent contrast. But what about other kingdoms, they also have complex cellular systems. Students sometimes wonder whether plants get cancer, as they are multicellular. Saguaro cactuses have cancer-like protrusions on their surfaces as these cacti can develop mutations in their meristem cells leading to over proliferation (Nedelcu, 2022). And that cute goldfish with the lumps on its head (Oranda goldfish), those are an excess proliferation of cells from a genetic mutation that creates the morphological variation. In the case of the Oranda goldfish, the tumor on its head is benign and won't grow or spread, unlike metastasizing cells of cancer clones. The Saguaro cactus doesn't circulate cells within its vascular system, and if part of the cactus dies, it can grow another part elsewhere. What about other kingdoms such as fungi—could they also develop a type of cancer too? What limits the growth of some cancers and not of others? We can ask students, is a tree gall like a tumor, and what is unique about the animal kingdom regarding cancer? This is an opportunity to contrast benign versus metastatic, to take another look at the cell cycle, not only in animals but in plants and fungi as well, and to explore the idea of genomic repeats of regulatory regions that control cell proliferation in the genome. We might contrast what is different among these kingdoms.

Mutational fingerprints and variation are also focuses of cancer and tumors that contain inter and intra-heterogenicity, which in effect means that each tumor is made of unique, albeit uncontrolled, rogue cells. Studying cancer will help students conceptualize ideas such as convergent evolution, which would be happening within the human body such as the exchanges that happen between gut microbiota and their own cells and cell lines. In this classroom activity, with the genomic medicine perspective on cancer and tumorigenesis, we can reveal fundamental ideas about evolution and mutations, exploring multiple concepts simultaneously or focusing on just one while exploring questions about why cancer would evolve in the first place. Through the paradigm of cancer clones, students can simulate the process of evolution using paper and pencil tools, storyboarding, and flipbooks. We reexamine terms from biology such as a "clone," and we revisit the idea of why every cell is unique. Through a microscopic and histological backdrop, evolution is played out through familiar protagonists in the intimate geography of a human body.

○ Cancer Genes across the Tree of Life

Our cells comprising the tissues of organs live in a complex ecological matrix, just as we do in our individual form, consisting of diverse cells even among the same cell types. It is this variation that aids in the trajectory upon which a tumor may or may not metastasize within its microenvironment. Experiences in cells vary, genomes vary, and that produces different outcomes for progeny. The location and type of mutation also play a pivotal role while the multistage carcinogenesis model suggests that "individual cells become cancerous after accumulating a specific number of mutational hits" (Mishra, 2013). "On the basis of this model, larger (and longer-living) animals are expected to have higher cancer incidence as they have more stem cell divisions overall, resulting in a higher likelihood of producing and propagating carcinogenic mutations" (Nair, 2022). A comparative genomics approach can demonstrate to students how potential cancer genes can be identified across vertebrate species to help illuminate which species are more cancer prone or cancer resistant and demonstrate how diversity (including diversity of pathways of resistance to cancer) spans the tree of life and may or may not be related to character traits such as size or lifespan.

This brings us back to the basics of the cell cycle of mitosis—genes associated with cancer resistance appear to be enriched in the cell cycle, DNA repair, immune response, and different metabolic pathways. Students can then make the connection between robust repair and immune response in some species versus others and the breakdown of these conserved biochemical pathways that may lead to cancer.

The cell cycle is often just illustrated as a flat pizza pie diagram, but its molecular dynamic is enhanced when the cancer model is integrated with it. Protein TP53 is a cancer suppressor gene that codes for the proteins pr53 that regulate cell division. P53 has been studied extensively and is considered a keystone protein as it appears to have many regulatory functions such as halting cell cycles, repairing DNA, and triggering apoptosis (Amaral, 2010). Diverse functions and concepts showcase the diversity of gene functionality and intensify the dimensionality of that pizza pie diagram into a three-dimensional, time-expansive landscape (see Figure 2). Some genes wear many hats and have principal roles, while others have supporting roles. Mutations in genes such as the BRCA gene can demonstrate to students where and why some people are more predisposed to certain cancers than others and demonstrate that gene's existence among diverse phyla. Simultaneously, with the many metabolic events and variables of evolution and development in cells, students can see that genome integrity and stability are evolutionarily very important

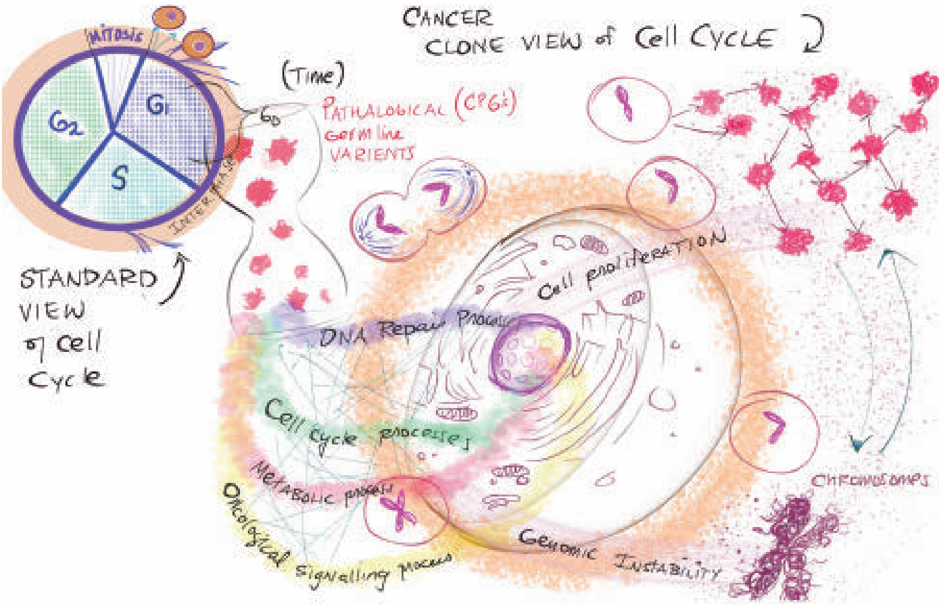

Figure 2. Comparison of the standard cell cycle diagram with a cell cycle that would include a cancer clone model. Students will get a greater sense of the complexity of a cell's life and its genome by using both kinds of visuals. In the cell cycle/cancer clone we see overlapping sub systems within the cell being affected by CpG variants, which further destabilize the cell's normal functions and repair mechanisms.

and evolved very early in animals, with an ancient creature such as the sea anemone having core genes such as TP53. This gene/protein perhaps conferred a survival advantage to early cells in times of strong UV radiation. Showing students a phylogeny of animals along with a discussion of cancer's origins in disrupted protective systems unites us across the tree of life and through evolutionary time with many of these regulatory systems evolving before multicellularity itself. The use of trees for both evolution and cancer assists in conveying multiple visual perspectives on biological processes. Teachers may want to briefly mention Peto's paradox to discuss body size and cancer; "*The evolution of multicellularity required the suppression of cancer. If every cell has some chance of becoming cancerous, large, long-lived organisms should have an increased risk of developing cancer compared to small, short-lived organisms. The lack of correlation between body size and cancer risk is known as Peto's Paradox*" (Caulin, 2011). Another research paper showed that elephants have multiple copies of P53 and are likely to avoid cancer! In another paper, cancer is correlated with a carnivorous lifestyle (Samraj, 2015).

○ Rethinking the "Clone"

Star Wars had its clones, and Dolly the sheep had hers. Clone is a word often used to describe a duplicate, which appears indistinguishable from the original, but we all know there is no such thing as an exact duplicate of anything, especially of anything living. What is surprising to most students of biology and people, in general, is that tumors are diverse populations of cells, not just all the rouge cells. If mutations and epigenetic changes are happening all the time, how is something identical possible? When we talk about cheek cells dividing and producing a new cheek cell in our mouth, we probably envision an identical cell being formed. This is true that the cells are the same cell line, perform the same functions, and are essentially equivalent in their phenotypes, but they are not the same, they are not identical. This is true of everything as it is impossible to replicate identical circumstances, and every single variable that happened along the road of mitosis to that new daughter cell has imparted a change. Every cell and the individual organism is unique as its past imprints on its present, continually. And so, a clone is not an identical cell. Along its short journey, stuff happens—a generation ago, 28 days ago, a minute ago—and is happening all the time. The more students appreciate this idea of continual change, the more evolutionary and biological processes will make sense and we can start to accept that biology will always be a little out of focus. So, for this activity, we will redefine a clone to be a similar cell with a similar genome and fate.

○ What Questions Cancer Can Raise about Evolution

No one wants cancer, just like no one wants to get sick with a cold, but we know that if we get swollen lymph nodes or sneeze or cough that our body is trying to destroy invading pathogens and expel them. The symptoms are a byproduct of a system actively protecting the whole organism. Could cancer be doing the same? The cancer puzzle is far from solved and a handful of hypotheses mingle in the literature, proffering perspectives on cancer and why it occurs. This is an important caveat to the cancer discussion for students. There are multiple models, new models, canceled old models, and revisited models, and students may start to appreciate what a model is by examining multiple templates and prototypes of scientific models. As an example, some have suggested that cancer could be an ancient pathway conservatively operating on a "safe mode," this model is called the "atavistic model." In this model,

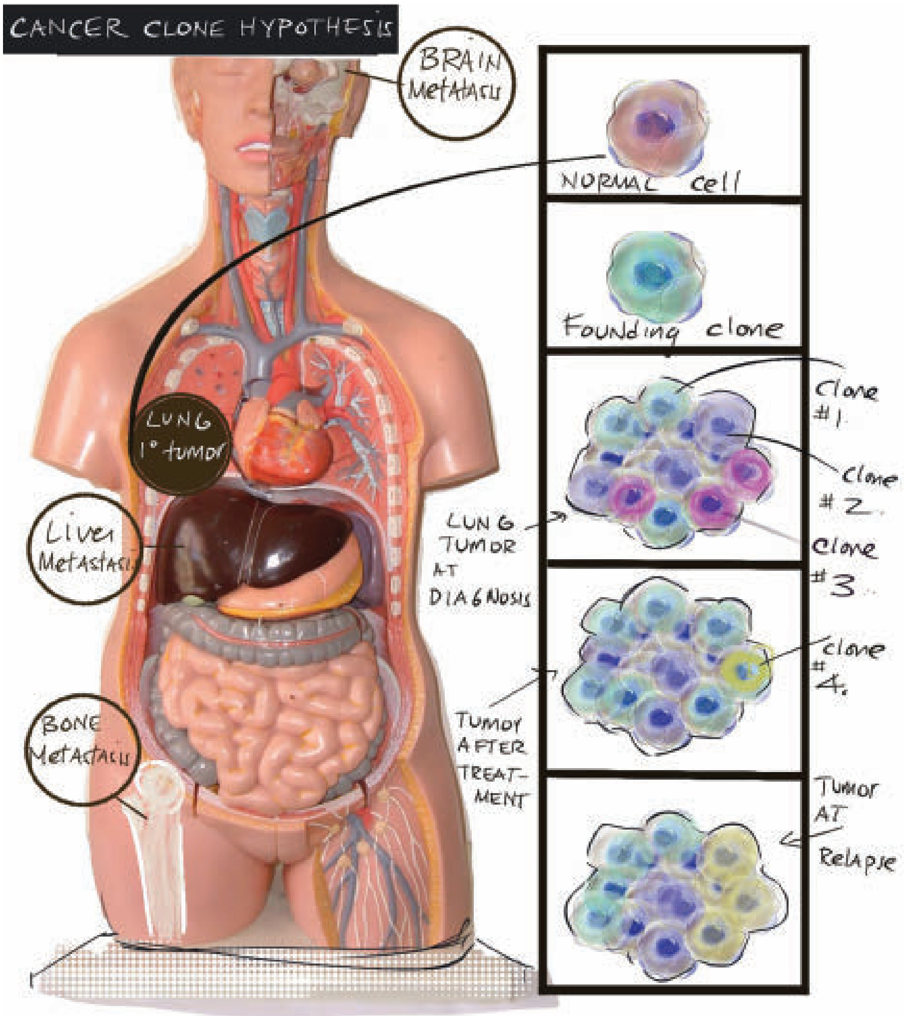

Figure 3. "Activity-in-figure" In this picture, teachers can develop an easy in-class experience using the cancer clone hypothesis, anatomical models, and storyboarding. Here we see the "founder cell," which has accrued mutations developing into metastasizing tumors. Students can cut out colored paper dots to represent the different clones and stick them to the anatomical model while they storyboard the hypothesis. Ask students to explain, in evolutionary terms what is going on, such as what is a founder cell? Or why are the cells changing into cancer clones? Build your discussion with groups around the anatomical model or draw the anatomy on the board.

the more primitive mitotic state becomes activated as genes for the more complex regulatory state become dormant. In other words, ancient genes become more active, and more evolved genes diminish their function (Lineweaver, 2021). This concept is an interesting one to explore with students as it takes students back to the primordial Earth and the first cells and propels them to take another perspective on the cell cycle and the disease itself from a grander evolutionary standpoint. From the more common perspective, with multicellular life we experience cooperation among cells and mechanisms that evolve cooperative biochemical pathways. Are cancer cells capable of cooperation? In a cellular civilization, cancer cells appear to be rule breakers. Do normal cells cooperate to curb cancerous cells from proliferating? This provides insight into the interplay and cooperative nature of the genome in health and disease. Abnormal cell growth has been around a long time simply because the proliferation of new cells is essential for the continuation and expansion of multicellularity—but why? This question is an interesting one to start with in our cancer introduction.

○ Drivers, Passengers, and Shape Shifting Mutations

Some of the most identifiable terms associated with somatic cancer cell models of increasing mutations are the terms "driver mutation" and "passenger mutation." It appears that all cancers are due to changes to the DNA sequences that constitute the genome. Genes that acquire mutations that facilitate tumor growth are called "driver genes." It is the accumulation of somatic mutations and various genetic alterations that impair the important conserved repair and immune functions in cell division/cell cycle check points. This leads to the formation of a tumor, and the mutations that promote and thrust a normal cell into a cancer cell are driving it to that state of instability. Drivers are under positive selection (see Figure 3). Cancer driver genes can be of two types: (a) proto-oncogenes or (b) tumor suppressor genes, such as TP53 (Salk, 2010). Driver mutations confer a proliferative advantage to the cancer cell by increasing the fitness of the cancer cell while passenger mutations are those which are accumulated along the way through cell division, and just appear to ride along in and through the clonal expansion of cancer. This is the model of cancer that students will be illustrating a storyboard to flipbook activity later. It turns out, however, that identifying driver and passenger mutations is not that simple. In silico simulations and "virtual" tumors, environmental conditions can shift altering the fecundity and survivability of the cancer cell and altering whether a mutation remains a driver. In other words, just like any ecological state, our cells are in constant fluctuation and change, met with new variables and conditions continually, shifting outcomes one way or another (Wala, 2017). This alters the spatial variations and molecular properties of a developing tumor along with the accumulation of mutations and epigenetic imprints. This also confers an evolutionary "history" to the tumor and moves us to a discussion on ecology—the ecology or microenvironment of the tumor. Even with the complexities of driver and passenger mutations, with epigenetic imprinting students start to see a simplified evolutionarily process in multistage carcinogenesis. "Species evolve by mutation and selection acting on individuals in a population; tumors evolve by mutation and selection acting on cells in a tissue" (Muir, 2016). This demonstrates that cancer biology can be an across-the-board teaching tool for connecting the dots of fundamental biological concepts and the fuzziness of biology in general.

○ The Concept of the Niche

Each visceral space within a human body is a niche and biogeography is one of the major evidences of evolution. Tumors have been described as "evolutionary, biogeographic islands" (Chroni, 2021), complete with migrations, new colonization, and the same mathematical models as traditional biogeographical studies in evolution. We often find tumors growing into areas where there is space, such as the lumen of the intestine, the bladder, or the uterus (Li, 2006). Tumor cells may be sensing out new landscapes and niches where other cells are not occupying that space. This is an opportunity to discuss what an ecosystem is if it has not been encountered and that an ecosystem can be anywhere on this planet, in armpits, intestines, and oceans, but that some important differences exist even though the terminology is similar. The primeval nature of the cell is expansion, and tumor cells, as the atavistic model implied, may be reverting to a baseline function of proliferation without constraint into any area free of other cells. The niche a cell or tissue occupies is very similar to the ecological niche concept. For students, the two comparisons, that is, the ecological niche of the outside environment and the inside niche of the cell, may be beneficial to developing an understanding of the niche concept as applied to living systems. For cancer cells, there may be the realized niche and the real niche, the competition for resources, and the evolution of a specialized "role" within the system. Most people would ask, "Do cancer cells have a specialized role?" Cancer seems counterintuitive to an interdependent system. However, perhaps there is more to cancer's evolutionary function in evolving our immune systems, and this might get students thinking about how a niche functions in similar and different ways throughout living systems.

○ Competition for Resources

When cancer starts, the drive is to reproduce and often outstrip the environment by hoarding resources. Cancer cells do this very effectively, they break boundaries and they exploit the vascular system by siphoning nutrients into growing cancer cells through angiogenesis. Angiogenesis along with unlimited replicative adaptations and dysregulation of apoptotic mechanisms enhance cancer cell nutrient procurement (Allen, 2011). Cancer cells have an adaptive advantage and to achieve these advantages specific tumor suppressor proteins must be disrupted, but even when cells continue to divide uncontrollably the disruption is halted as the system enters a "crisis" state, which stops continued growth with massive cell death. Karyotypes of fibroblast cells reveal this intervention, which results in fused and deformed chromosomes, however, out of this massive die-off, an occasional variant emerges, one that has resisted the systems senescence shut down (Allen, 2011) and a reason why telomere maintenance is extremely important. Even one hundred years ago, messy-looking, tangled chromosomes were indicators of cancer or tumorigenesis (Holland, 2009). Again, this gross morphological view of the chromosome is a great teaching point and prelude to cancer clones. Students can contrast and compare tangled, distorted chromosomes against healthy-looking ones (see Figure 4) in a sort of chromosome "line up."

The ecological and evolutionary perspective views cancer as a sort of "species" that is operating outside of healthy ecological parameters, goes with the idea of the chromosome as an individual, and encourages the student to think about the dynamic ecological space of cell as it relates to competition among cancer and normal cell lines. The competition concept between cells ushers in all sorts of questions about the breakdown of regulatory systems in a cell and mutations in regulatory regions of the genome. Students see that ecosystems, where uncontrolled consumption have taken place (cancer) become "unhealthy" and if regulating proteins just like apex and meso-predators have been compromised then cooperation too becomes compromised. The comparison of ecological niches and cell niches can evoke an understanding of how populations in systems run astray if the dynamics of the system change. The Zion National Park study where predators were eliminated caused overgrowth of herbivores and collapse of the forest ecosystem. This is a great example to use and compare alongside rouge cancer cells. The outcomes in both the cellular and the forest systems share many similarities, and this creates a great comparison for the idea of competition for resources.

○ Modes of Selection

"Evolution by natural selection is the conceptual foundation for nearly every branch of biology and increasingly also for biomedicine and medical research. In cancer biology, evolution explains how populations of cells in tumors change over time (Fortuno, 2017)." While the prime directive of cancer cells is quite unlike healthy cells, they still follow the patterns of natural selection. This creates cell competition in the tissue and the selection for the most robust of the cancer clones to survive and proliferate. Cell competition boosts clonal evolution with certain micro and macro environments selecting for greater survivability of the cancer cell. In other words, fitness between cells of a tissue or within an organ leads to the elimination of less competent fellow cells (Greaves, 2012). Students can easily model this and draw this, embodying an understanding of natural selection through the somatic cancer model. Stem cells, and all cells for that matter, are going through natural selection all the time so mitosis is not just a replacement of cells but an evolutionary fixture of mitosis, which can also be compared to the cancer clone hypothesis. From this perceptive we can see that

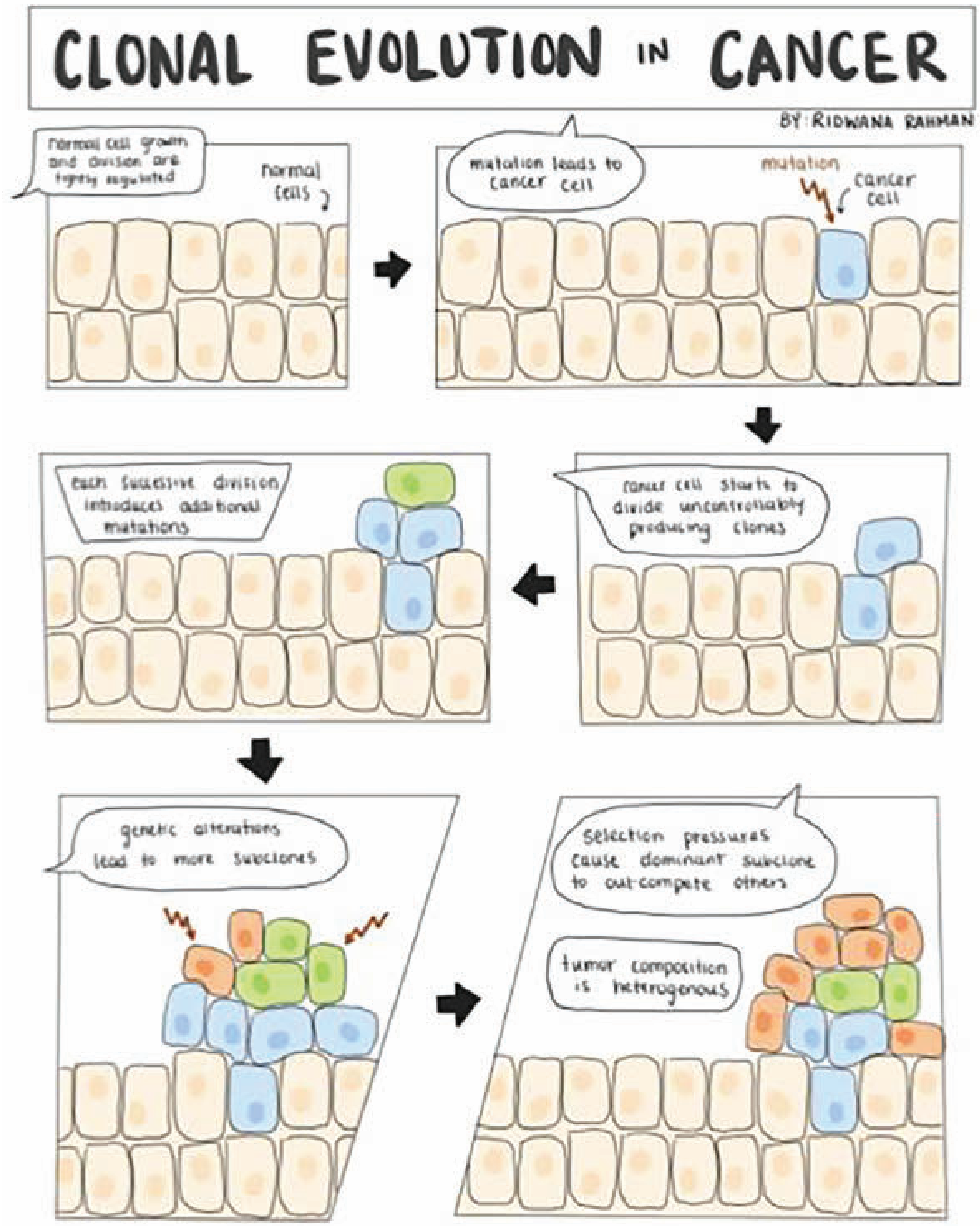

Figure 4. A student's storyboard of the cancer clone hypothesis.

no two cells are ever alike as conditions fix or imprint biochemical signatures on each cell with a plethora of one-time variables and variable interactions, translating the experience of the cell and the genome into unique phenotypes. This binds an understanding of mitosis and evolution together and presents a cross sub-disciplinary teaching point. Like antibiotic resistance, persistent cancer clones become resistant to treatments and students can gain appreciation of the processes of nature, where pushing against something sometimes makes it "stronger."

○ Founder Cells, Cancer, and Cellular Fitness

Tumor growth is an evolutionary process (Boland, 2005), so tethering students' first major conceptual topic, the cell back to and through mitosis and into evolution through the cancer clone hypothesis, is a great way for students to keep the theme of the cell contiguous. It also maintains the cell as a salient feature of their biology course. Using a pertinent, personal health topic helps to bridge and retain the beginning concept in general biology of the cell with the ending topic of a course, which is typically evolution. In between the cell and evolution are ecological archetypes of change governed by somatic mutations, clonal selection, and random genetic drift. Together, these concepts also link sequential genetic events that pop up through processes, further connecting a student's genetics unit to evolutionary process and fitness. The genome is the conduit by which genes interpret the nuanced experiences of the cell's life including selective pressures, which can be the main takeaway message from the cancer clone model as general cellular fitness is reduced as cells age, as mitochondrial dysfunction grows or cell exposure to radiation and carcinogens increases. Aging, carcinogens, and changes in the histological niche all impart varied selective pressures on cells. Giving students an example of lung cancer and talking about the ciliated endothelial niches of the lung helps students visualize that space. Students can think about changes in that specific microenvironment from toxic intrusions or disruptions such as pollution and smoking where cells are destroyed, as in emphysema. This leaves new niches to be filled by potentially cancerous cells (Satcher, 2022). To make ecological comparisons, the term "landscape" of the lungs or the respiratory membrane can be used to help students visualize this smaller ecosystem evolving inside their own lungs and the lungs as an ecosystem in direct contact with the planet's atmosphere. If the instructor has time, photosynthesis and climate regulation through forests can also be factored into the discussion. This multilayered dynamic can be easily illustrated on the board or through composite images in PowerPoint. Instructors can also cut out different colored dots and place them on anatomical models to demonstrate the metastasis of cancer clones.

○ Activities

There are many ways to visualize multistep processes such as cancer. Most students could watch an animation of cells becoming cancer, and admittedly this would be beneficial, but it is always more engaging and more advantageous for students to create something that demonstrates to themselves that they have mastered the terms and the concepts in a personal and unique way. For this experience, the cancer clone simulation can be provided to help students gain more visual insight into the process by showing healthy chromosomes next to unstable ones and healthy histology next to pathological images. We suggest students do this through the flip book. Students work in pairs and one student creates a storyboard (to layout the flip book) for healthy or normal mitosis and the other student creates the cancer clone storyboard and flip book. Students can draw these structures easily as most of them are just circles and oblong shapes or they can use crafting paper and materials to represent variations of the cell throughout the process.

○ Materials

- Somatic cancer clone model to teach the concept
- Construction paper of different colors/scrapbooking materials
- Example of a phylogenetic tree
- The human body with organs map (to show spread)
- Sample photographs of actual cancer cells, chromosomes, and histology slides
- Regular card stock paper from storyboards and flip books

○ Conclusion

The somatic cancer clone model provides many inputs and reconnections for basic biology concepts that can associate back and forth with each other and to the cancer model. Students can achieve a bigger-picture perspective on cells and the genome through this model and become acquainted with the crossover of ecological terms into a cellular and evolutionary vocabulary. By illustrating this simple model, students can compare and contrast cellular processes and mechanisms that may become derailed in the progression of cancer and come to appreciate that all living systems are complex, variable, and changing. Many interesting questions can continue to be posed regarding the cancer clone model, events such as HGT and regulatory roles of cancer genes and switches, transposons, the role of epigenetics and the microbiome in cancer, and cancer gene behavior are all expandable topics. With arts and crafts, storyboarding, and flipbooks, students can delve into complex topics and enjoy constructing their own models.

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RESEARCH ON LEARNING

A Comparison of Measured Outcomes across Tree-Thinking Interventions

NABT BioClub RECOMMENDATION

KRISTY L. DANIEL, DANIEL FERGUSON, E. AUSTIN LEONE, CARRIE J. BUCKLIN

Abstract

Phylogenetic tree diagrams are commonly found in introductory biology curricula and represent the evolutionary relationships of organisms. Tree-thinking, or the ability to accurately interpret, use, and generate these phylogenetic representations, involves a challenging set of skills for students to learn. Although many introductory biology courses incorporate tree-thinking instruction, few studies have identified which instructional methods provide the best learning gains for students. We gathered data from 884 introductory biology students using the Basic Evolutionary Tree-Thinking Skills Inventory (BET-TSI) to measure tree-thinking learning gains. We measured tree-thinking differences across five sections of introductory biology, each offering a different instructional intervention, and compared differences among STEM majors and non-STEM majors. After calculating paired differences, we performed a two-way repeated measure analysis of variance (ANOVA) and Scheffe's post hoc test to identify significant differences among and between the different interventions. We found that students who engaged in active tree-thinking instruction had significantly higher tree-thinking learning gains than students who participated in passive or no instruction. Furthermore, these learning gains became even more significant as active-learning became more multifaceted. These active-learning approaches also removed knowledge gaps between STEM majors and non-majors. Instructors must select explicit and active pedagogical approaches to support student tree-thinking to accomplish positive learning gains for all students.

Key Words: *Evolution, Active-learning, Biology education, Phylogenetic trees*

○ Introduction

Scientists often use diagrams to visually represent their understanding of concepts in multiple fields, including physics (Fredlund et al., 2012), chemistry (Kozma & Russell, 2005), genetics (Patrick et al., 2005), and evolutionary biology (Baum et al., 2005; Baum & Smith, 2013). Evolutionary biologists use a diagram called the "tree of life," or, specifically, they use phylogenetic trees to represent hypothesized evolutionary relationships. One's ability to accurately interpret and construct these diagrams is called "tree-thinking" (Halverson et al., 2011; Halverson, 2011) and proves cognitively tricky for students (Catley et al., 2010; Halverson et al., 2011). Although phylogenetic trees are difficult for students to understand, there are benefits to increasing their tree-thinking skills, such as improved scientific literacy and a greater understanding of climate change, health, agriculture, forensics, and biotechnology (Davis et al., 2010; Futuyma, 2004; Novick & Catley, 2016; Thomas et al., 2004; Yates et al., 2004). The problem is that students apply numerous alternative conceptions when tree-thinking, including exclusively reading the tips, thinking that longer lines on the tree represent a lack of change, and ladder thinking (Gregory, 2008; Kummer et al., 2016) that can make tree-thinking a complex topic to teach. In addition to students holding alternative conceptions, students also struggle with tree-thinking when different styles of phylogenies are presented (Catley et al., 2010; Dees et al., 2018). This combination of holding numerous alternative conceptions and possible exposure to different representation styles in lectures or labs presents a significant challenge to student learning about phylogenetic trees and identifies a need for successful and effective tree instruction.

Although phylogenetic trees are difficult for students to understand, there are benefits to increasing their tree-thinking skills, such as improved scientific literacy and a greater understanding of climate change, health, agriculture, forensics, and biotechnology

Teaching students about phylogenetic trees may even be challenging for educators as there is debate about the best ways to teach tree-thinking in the classroom. Research shows benefits in tree-thinking skills when students are required to build a tree based on a given dataset (Eddy et al., 2013). Some studies have even shown that mythical creatures such as dragons can be great tools for teachers as they can tailor the information to their needs. It may even help

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students build trees without previous assumptions about the organisms (Schramm et al., 2022). It has also been argued that having students build trees is a different skill from reading and understanding trees (Halverson, 2011). Building trees adds a layer of complexity to an already complex problem. Teaching students to interpret and understand phylogenetic trees has also been shown to increase tree-thinking skills; sometimes, tools as simple as pipe cleaners or even instructional booklets have been shown to be beneficial (Halverson, 2010; Novick & Catley, 2016).

There are multiple ways to help improve student tree-thinking skills. Walter et al. (2013) observed student tree-thinking in a biology course for nonmajors and found that students showed significant learning gains from a tree-intensive approach where explicit instruction about trees occurred. Another study identified significant learning gains in an introductory biology course for biology majors using an approach highly integrated with tree instruction (Gibson & Hoefnagels, 2015). However, no studies provide definitive evidence as to which instructional intervention is most effective over others, and no studies look at differences in whether a student is a STEM major.

Some research shows that nonmajors are less motivated by science, less interested in science, and generally have lower positive attitudes toward science compared with majors (Cotner et al., 2017; Hebert & Cotner, 2019; Knight & Smith, 2010). However, there seems to be no difference in students' abilities to learn scientific skills (Hebert & Cotner, 2019; Kummer et al., 2016). Thus, our study aimed to examine the differences in introductory biology students' (STEM majors and nonmajors) learning and understanding of phylogenies over a semester when provided with different instructional interventions.

Our study was motivated by the lack of studies investigating tree instruction interventions beyond explicit/tree-intensive pedagogy and their effect on university undergraduate learning. We hypothesized that the type of instruction intervention used would affect students' tree-thinking skills. We also hypothesized that there would be a difference in documented tree-thinking skills based on whether a student was a STEM major or non-STEM major. Our study looked at the potential interaction between instruction type and primary tree-thinking skills.

○ Methods

We used a quasi-experimental design to explore our research purpose using the Basic Evolutionary Tree-Thinking Skills Inventory (BETTSI), a valid and reliable measure of tree-thinking (Jenkins et al., 2021; Jenkins et al., 2022). We worked with four biology faculty at a large, Southwestern university in the United States across ten introductory biology sections (five non-STEM majors and five STEM majors) to assign different tree-thinking instructional interventions. Among the sections based on student major, we assigned four sections a unique instructional intervention. One was our control and did not include any tree-thinking instruction (see Table 1). Each instructional intervention lasted one week, about three hours of class time, with equal instructional time in class, with the documented treatments assigned as follows: *None*, *Implicit*, *Video*, *Model*, and *Extensive*.

Treatment

Students experiencing no instruction (*None*) served as our comparison control group, as no phylogenetic trees were used or discussed during the semester. The *Implicit* instructional intervention did not have the instructor directly teach students about phylogenetic trees, but rather the instructor exposed students to trees via slides presented during class lectures and through assigned textbook figures while covering lessons on biodiversity and evolution. The *Video* intervention required students to watch a video from treeroom.org (Understanding Evolution, 2021) reviewing the different parts and styles of phylogenetic trees. After watching the video, the instructor asked the students to solve questions about phylogenetic trees. During the *Model* intervention, students created manipulative models of phylogenetic trees using colored pipe cleaners (Halverson, 2010). Then, they worked through an explanatory worksheet for a tactile, hands-on experience supplementing the lecture. Finally, the *Extensive* intervention was heavily tree-intensive. For this treatment, students first watched a brief overview of the treeroom.org video. Then, they were provided with an introductory demonstration using the manipulative model.

Additionally, the instructor explicitly explained how to interpret each phylogenetic tree on the lecture slides shown to the students during the instructional treatment period. The *Video*, *Model*, and *Extensive* treatments each included active tree-thinking instruction techniques, whereas the *Implicit* and *None* treatments did not include any active instruction. We ensured the fidelity of treatments by providing individual workshops with each instructor before instruction and observing classes during the one-week intervention period. Aside from the control, each instructional intervention dedicated the same amount of class time to tree-thinking.

Data Collection

The BETTSI (Jenkins et al., 2021, 2022) served as our dependent variable, measuring student tree-thinking outcomes at the beginning (pre) and the end of the semester (post). The BETTSI consists of 11 multiple-choice items that target common tree-thinking misconceptions with confirmed reliability (ρKR20 = 0.75). We administered a pre-questionnaire to students in each biology course section within the first two weeks of the semester before the

Table 1. Sample size per instructional intervention across nonscience and science majors.

Instructional Intervention						
Nonmajors			Active Instruction			
	None	**Implicit**	**Video**	**Model**	**Extensive**	**Total**
	224	101	112	115	38	590
Majors			Active Instruction			
	None	**Implicit**	**Video**	**Model**	**Extensive**	**Total**
	113	55	45	70	11	294

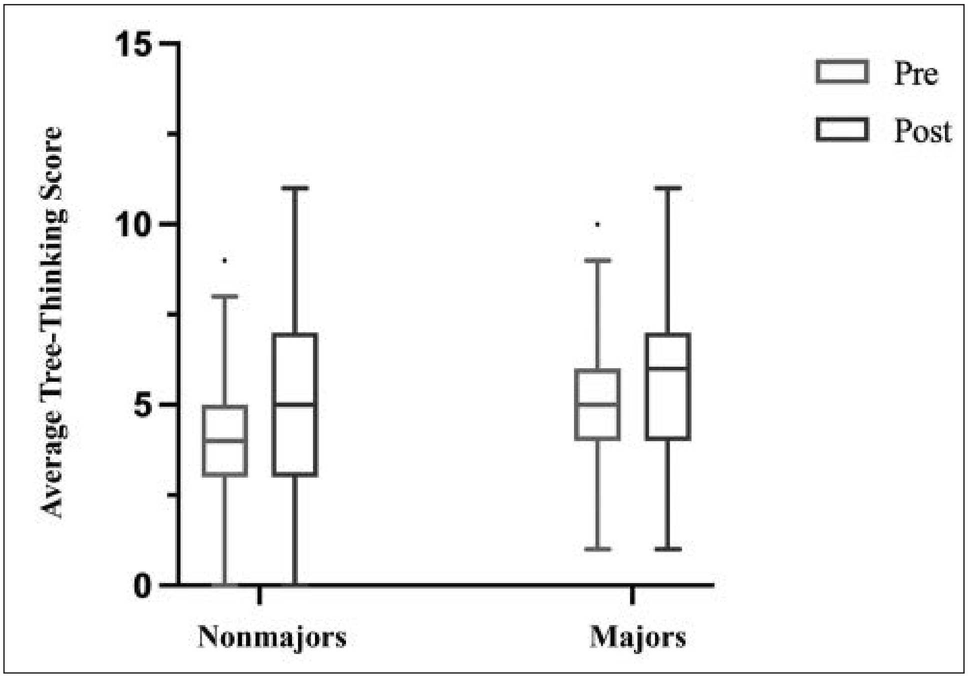

Figure 1. Average tree-thinking score pre/post instruction by major.

instructor taught the evolution unit using the assigned instructional treatment. Each intervention period ended with an exam including phylogenetic trees, during which we administered the BETTSI again as a posttest. Of the students recruited, 884 completed both questionnaires for analysis (590 non-STEM majors and 294 STEM majors).

Analysis

To determine the effect of teaching methods and STEM majors on students' tree-thinking, we ran a two-way repeated measures analysis of variance (ANOVA). The two-way repeated ANOVA calculates paired differences in pre-and post-BETTSI scores from each student to determine outcomes among the different instructional treatments and differences among STEM majors. The two-way repeated measure ANOVA also measures any potential interactions within the data. Our data was slightly left-skewed but was still normally distributed as assessed by visual inspection of their histograms and Q-Q plots. We anticipated a slight skew of the data as we did not expect students to be knowledgeable in tree-thinking when they initially completed the pretest (similar results have been documented in other studies, e.g., Novick & Catley, 2016). Due to having unequal sample sizes across instructional treatments, at least in terms of the *Extensive* instruction method, these data cannot be inferred from the general student population. We met all assumptions for a two-way repeated measures ANOVA, *p*-values were assessed using the Tukey's honestly significant difference (HSD) post-hoc test.

○ Results

The two-way repeated measure ANOVA measured each variable's pre- and post-tree-thinking scores (Major and Instruction method). Our data showed a statistically significant interaction between pre- and post-tree-thinking scores, the major variable $F(1, 874) = 11.10$, $p = 0.001$, and the type of instruction variable $F(4, 874) = 69.12$, $p < 0.001$. There was no significant interaction between major and instructional type on students' tree-thinking scores $F(4, 874) = 2.29$, $p = 0.058$.

Differences in Tree-Thinking Scores in STEM Majors

Our data showed a statistically significant difference in average tree-thinking scores between nonmajors ($M = 4.13$, s.e. = 0.86) and STEM majors ($M = 5.10$, s.e. = 0.14) at the beginning of the semester ($p < 0.0001$) but no statistically significant differences between nonmajors and STEM majors at the end of the semester ($M = 5.62$, 5.97; s.e. = 0.10; 0.16; $p = 0.065$). Suggesting that STEM majors had a greater understanding of phylogenetic trees than nonmajors at the beginning of the semester, but between these two groups, there was no significant change in their tree-thinking skills at the end of the semester. Interestingly, there was also a statistically significant interaction between students' majors and their pre/post-tree-thinking scores, $F(1, 882) = 8.239$, $p = 0.004$ (see Figure 1).

Differences in Tree-Thinking Scores in Instruction Type

Our data showed no statistically significant difference in average tree-thinking scores between instruction types entering at the beginning of the semester ($p = 0.194$). Still, there were statistically significant differences at the end of the semester based on the type of instruction. Those students in the *None* and *Implicit* intervention sections saw no significant changes in their tree-thinking scores (see Table 2 for details). In contrast, students participating in the *Video*, *Model*, and *Extensive* interventions all had statistically significant differences in their tree-thinking scores over the semester (see Table 2 for details). We also wanted to know whether there was an interaction between instruction type and tree-thinking. Our results showed a statistically significant interaction between the type of instruction students received and their pre/post-tree-thinking scores, $F(4, 879) = 33.44$, $p < 0.0001$ (see Figure 2).

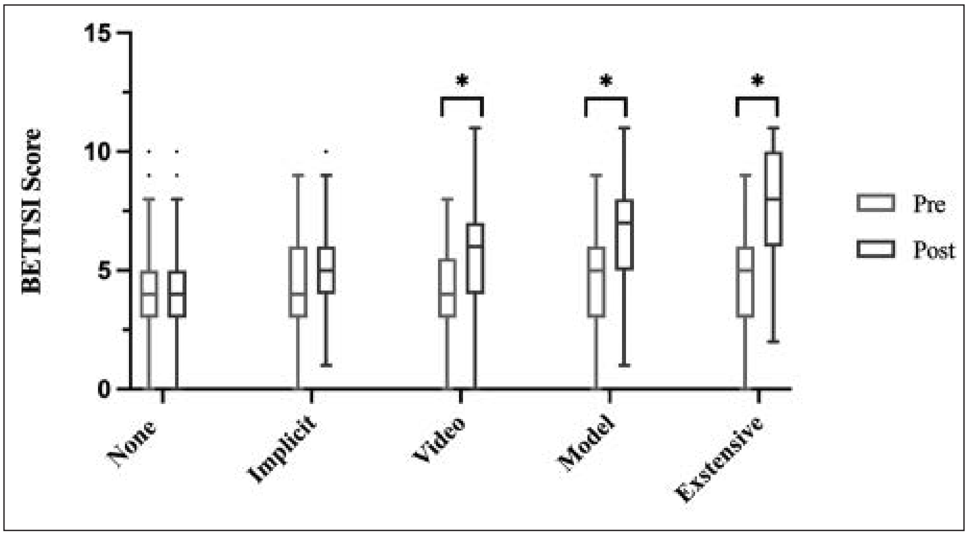

Figure 2. Average tree-thinking score (BETTSI) pre/post instruction by instructional intervention.
Note: ** indicates the significance of* p *< 0.05*.

Table 2. Mean differences of tree-thinking changes across interventions from a Tukey's HSD post-hoc test.

	Intervention				
	None	**Implicit**	**Video**	**Model**	**Extensive**
None	–				
Implicit	–0.49*	–			
Video	–0.67*	–0.18	–		
Model	–1.23*	–0.74*	–0.56*	–	
Extensive	–1.74*	–1.25*	–1.07*	–0.52*	–

Note. * indicates significance at $p = 0.05$ or below.

Table 3. Mean pre- and post-BETTSI scores within different instruction types.

Instruction type	Mean Pre-BETTSI score	Mean Post-BETTSI score	*p*-value
None	4.23	4.28	0.67
Implicit	4.61	4.89	0.079
Video	4.27	5.59	< 0.0001
Model	4.51	6.45	< 0.0001
Extensive	4.49	7.51	< 0.0001

Students who experienced the *Extensive* instruction intervention had higher tree-thinking gains than any other intervention ($p < 0.001$, see Table 3). Students participating in the *None* or *Implicit* interventions exhibited minimal gains, with a slightly significant difference between the two instruction types ($p = 0.019$, see Table 2). Students who experienced the *Video* intervention from treeroom.org had smaller tree-thinking gains than those who used the manipulative *Model*; these gains are significantly different ($p < 0.0001$, see Table 2). Students who participated in the *Extensive* intervention showed the significantly highest tree-thinking gains ($p < 0.0001$, see Table 2).

○ Discussion

Our research investigated the effect of five tree-thinking teaching interventions and their impacts on undergraduate introductory biology student learning outcomes. We surveyed 884 students, each exposed to one of the interventions, to assess their tree-thinking scores and used a two-way repeated measure ANOVA to identify significant differences across interventions. Students from the *Extensive* intervention demonstrated the most significant improvements in understanding phylogenetic trees than any other teaching intervention. However, the other active teaching interventions, *Video* and *Model*, were also significantly beneficial. STEM majors had higher tree-thinking scores at the beginning of the semester but did not perform better than nonmajors at the end.

Active-Learning Impacts Students' Learning Outcomes

Active-learning can help improve students' capability to learn complex concepts in biology, such as phylogenetics (Freeman et al., 2007). Furthermore, active approaches also support students in thinking like a scientist as they learn to understand scientific representations of models, hypotheses, and theories and grow their expertise (Gilbert, 2005; Halverson, 2011). The more multifaceted active-learning approaches are designed to provide more opportunities for students to think like scientists (Gibson & Hoefnagels, 2015; Southerland et al., 2001). Thus, we posited that how students are taught tree-thinking will impact how students understand and make sense of phylogenetic tree diagrams. Our study supports findings from previous studies, providing evidence that active tree-thinking instruction leads to subsequent positive effects on students' demonstrated tree-thinking skills (Gibson & Hoefnagels, 2015; Halverson, 2011; Walter et al., 2013). We found that even minimally faceted active-learning approaches (e.g., *Video* or *Model*) lead to significant tree-thinking learning gains. Moreover, we present evidence that as active tree-thinking instructional approaches become more multifaceted, they result in more positive tree-thinking learning outcomes, with the *Extensive* intervention magnifying the highest impacts. Such learning gains are likely due to students having more opportunities to explore tree diagrams from multiple perspectives.

One could argue that instruction of any type should lead to student learning gains. However, we found evidence that there is no significant difference in student performance comparing no instruction and passive tree-thinking instruction. In both cases, students did not show significant learning gains in tree-thinking. Thus, we argue that including any type of instruction is insufficient; active-learning approaches are critical when teaching tree-thinking. Students cannot simply be exposed to trees and expected to accurately interpret and compare represented hypotheses. Instead, instruction must be explicit, and students must actively engage with a tree diagram to learn dynamic tree-thinking. Instructors are essential in developing scientifically literate students (Archer-Bradshaw, 2017), and pedagogical choices will impact this role and teaching effectiveness. We suggest introductory biology instructors recognize that it is ineffective to rely on implicit practices when covering problematic concepts such as phylogenetics. Instead, we encourage instructors should employ a more active tree-thinking approach in the classroom. Active instructional options such as letting students interact with manipulative models (Halverson, 2010), work through an instructional booklet (Novick & Catley, 2016), or explore a website with phylogenetic tree activities (PBS, 2022) will likely facilitate improved tree-thinking skills.

Research has also shown active-learning approaches may improve minority student retention in STEM majors (Maton et al., 2016; Sto Domingo et al., 2019). Although we did not specifically investigate the effects of active tree-thinking approaches on student retention in STEM, there is reason to consider that the multifaceted degree of active-learning on retention should be further explored. Thus far, evidence suggests that any degree of active approaches will significantly impact students' learning, which in turn should improve academic grades and the likelihood of completing a university degree.

There is No Difference in Post-Scores Between Nonmajors and Majors

There is ongoing debate as to whether STEM majors and nonmajors need different instructional approaches to produce significant learning outcomes (e.g., Cotner et al., 2017; Hebert & Cotner, 2019; Knight & Smith, 2010; Kummer et al., 2016; Tamari et al., 2020). While STEM majors began with significantly higher tree-thinking scores than nonmajors, we found no significant differences between STEM majors' and nonmajors' tree-thinking scores after engaging in active tree-thinking instruction. Thus, using an active-learning pedagogical approach to teach tree-thinking closed the initial knowledge gap between the student groups, regardless of major. Still, other studies found significant differences in specific tree-thinking misconceptions after instruction between upper and lower-level students (Kummer et al., 2016; Meir et al., 2007). Specific tree-thinking misconceptions may be more resilient within select groups of students, but these groups may be different given the year in school rather than the selected major. Thus, we suggest further exploring how varying instructional approaches impact student learning across levels of their academic experience.

Changes in Tree-Thinking

Our study used the BETTSI to determine changes in students' tree-thinking skills over the semester. Of the 11 questions from the BETTSI, there were increases in students' tree-thinking skills based on nine questions over the semester. Questions six, nine, and ten of the BETTSI saw no changes or had more than 80% get these questions wrong on students' ability to understand phylogenetic trees. Question six explicitly asks students to determine the traits of an organism based on a phylogeny, which maps specific traits gained or lost over time. Question nine asks students to identify the correct lineage of a specific organism. According to Jenkins et al. (2022), students who missed questions six and nine usually have difficulty tracing a lineage through a diagram and recognizing the meaning of the trait mark on the branch. Question ten, missed by over 80% of the students in both pre- and post-surveys, specifically asks students to identify the accurate statement based on a phylogeny. Students who missed this question usually have difficulty reading across tips, tracing a lineage, counting their nodes, and understanding what a tree represents. Helping students understand the importance of picking their nodes, tracing lineage, and understanding the representations of trees, may be necessary for research in the future.

We did see changes in the tree-thinking of our students in some areas. Still, our data does not allow us to understand how this change in their understanding of phylogenetic trees affects their views of science or evolution. However, our lack of data in this area may give way to future research questions.

Limitations

Although our study suggests that *Explicit* tree-thinking instruction was most beneficial for students' tree-thinking skills, our sample size in *Explicit* intervention was very small (especially in the majors class) compared with the number of students in the other interventions. More data is needed to draw more robust conclusions on the type of instruction that may work best for increasing tree-thinking.

○ Conclusion

Evolutionary biologists typically depict evolutionary relationships as phylogenetic trees. Our study looked at students' ability to learn and understand trees and found that active instructional approaches can significantly impact nonmajors' and STEM majors' ability to use tree-thinking. How educators teach trees in classrooms seems to impact the extent to which students learn complex concepts. Helping nonscience major students, in addition to STEM majors, grasp the cornerstone of biology is a first step to increasing the publics scientific literacy. Ultimately, our goal is to continue working with scientists and teachers to ensure that introductory biology students leave their courses confident and readily prepared with scientific tools to contribute positively to society.

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RESEARCH ON LEARNING

Online Professional Development Course Helps Secondary Educators Increase Their Confidence in Teaching Evolution to Religious Audiences

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Abstract

Students with religious beliefs often find it difficult to accept the theory of evolution. It is important that educators feel comfortable addressing student questions on the compatibility of evolution and religion. We designed an online professional development course that taught the foundational principles of both evolution and religion in order to assist secular and non-secular educators in helping their students overcome religious barriers to evolution. This course increased the confidence of science educators to help students resolve perceived conflict between evolution and religion. Utilizing a reconciliation model will help religious educators drive science acceptance.

Key Words: *Secondary education, Evolution, Professional development, Reconciliation, Religion*

○ Introduction

Evolution in America

The theory of evolution undergirds our understanding of life on Earth (Dobzhansky, 1973). From the perspective of some faith traditions, however, there is perhaps no greater source of tension between faith and science than the theory of evolution. In a recent survey, nearly 40% (Brenan, 2019) of Americans responded that they still believe God created humans in their current state within the last ten-thousand years (i.e., young-earth creationism). Many religious individuals in particular still perceive the theory of evolution as something that conflicts with faith claims (Lamoureux, 2008). Many studies over the years predicted a link between understanding of evolution and acceptance, but the results are conflicting. Some indicate that a better knowledge of evolution leads to increased acceptance (Johnson & Peeples, 1987; Rutledge & Warden, 2000), yet others conclude there is no such correlation (Hasan & Donnelly, 2011; Mead et al., 2017). However, more recent studies do suggest a positive correlation between knowledge of evolution and acceptance (Dunk et al., 2017; Weisberg et al., 2018).

There is a potential disconnect between educators and the religious beliefs of their audiences. In 2019, around 65% of the American public identified as Christian (Pew Research Center, 2019) whereas in 2009 only 48% of scientists reported having any religious beliefs at all (Pew Research Center, 2009). Research with undergraduates and biology educators suggests that this gap is due to a stigma against Christians in particular in science (Ecklund et al., 2011; Scheitle & Ecklund, 2018; Rios et al., 2015). Christianity has been shown to be a Concealable Stigmatized Identity within biology graduate students (Barnes et al., 2021). One study showed that many undergraduate students assumed most biology educators were not religious (Barnes et al., 2017). It has also been demonstrated that students who are learning about evolution feel left out when instructors do not know or acknowledge their religious beliefs (Hermann, 2012). This potential disconnect is also manifested in recent studies that show the religiosity of an individual—or their strong religious feeling or belief—can predict their evolution acceptance (Dagher & BouJaoude, 1997; Rissler et al., 2014). In response, researchers have called for a model of evolution education built upon mutual understanding of the nature of science and religion between biology educators and their students, referred to as Religious Cultural Competence in Evolution Education (ReCCEE) (Barnes & Brownell, 2016). Researchers have developed a style of teaching known as a "Reconciliatory" model that focuses on teaching evolution to religious audiences (e.g., Lindsay et al., 2019; Manwaring et al., 2015; Tolman et al., 2020). The following section explains what this reconciliation model looks like in the classroom.

A Reconciliation Model

A perceived conflict between religion and the theory of evolution is the biggest predictor of whether a student will accept evolution when it is presented to them (Barnes, Supriya, et al., 2021). These perceived conflicts often come from students who find specific teachings within their religion that could be interpreted as anti-evolution, such as various accounts of how Earth and all organisms were created (Ladine, 2009) or from other sources claiming that religion and science must be in conflict (Dawkins, 2009;

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Coyne, 2015; Kopplin et al., 2016). For this reason, a reconciliatory teaching model has been developed by researchers to increase student acceptance of evolution without decreasing their religious beliefs (Manwaring et al., 2015). When using this model, educators first explain the nature of science as well as the nature of religion. Science is described as "agnostic" toward religion meaning that science does not seek to answer questions related to the existence or nature of God. Science is limited to natural explanations. Scientific questions require falsifiable hypotheses and are answered with empirical evidence gained through observations, measurements, and tests of the natural world. Investigations into topics that do not fall within those parameters require different tools from the ones employed by science. In contrast, existential questions about meaning and purpose are investigated through religion. The reconciliatory model emphasizes that science and religion are different, both in the ways they acquire and evaluate knowledge, and in the kinds of questions they are equipped to consider. This model is similar to Gould's Nonoverlapping Magisteria, which argues that science and religion both operate within two separate and non-overlapping domains of inquiry, and that conflicts between the two subjects are a result of an incorrect understanding of the two (Gould, 1997). However, the reconciliatory model described in this paper emphasizes compatibility between the two (Barnes & Brownell, 2017) and actively discourages parallel collateral learning (Aikenhead & Jegede, 1999). With this introductory information, students are able to learn about and accept evolution more readily as they understand that the theory does not seek to disprove or discredit their faith. Among college students, the reconciliation model has been shown to increase student acceptance of evolution (Ferguson & Jensen, 2021; Lindsay et al., 2019; Tolman et al., 2020). The reconciliation model has been used in undergraduate settings at both private religious universities (Lindsay et al., 2019; Manwaring et al., 2015) and public institutions (Truong et al., 2018). (To view examples of the model as curricular materials see RecoEvo.byu.edu.) Additionally, this reconciliatory model has been used successfully in a less traditional setting (Tolman et al., 2021). In all situations, no reduction in religiosity was detected.

Current State of Teaching Evolution in Secondary Education

While the reconciliatory model increases acceptance of evolution among college students, it remains untested in secondary education, likely due to religion being a complicated topic in public schools. The Supreme Court ruled that it is illegal to teach creationism on its own, or to require teaching creationism along with evolution (*Edwards v. Aguillard*, 1987). Individual teachers are also not allowed to advocate for creationism (*Webster v. New Lenox School District*, 1990). As a result of these rulings, many educators are not willing to explore the topic of religion and evolution in their classrooms for fear that students or administrators may misunderstand their intentions. Evolution is included in the Next Generation Science Standards (NGSS) (NSTA, 2014), which is utilized by over 35% of students in the United States. However, one study suggests that "more than half the teachers do not know if…it is still a crime to teach evolution anywhere in the United States today, that…the court determined that creation science has no scientific merit, or…whether the Supreme Court has endorsed the teaching of 'evidence against evolution'" (Hermann et al., 2020, p. 88). In Utah, where this study was conducted, evolution is a required part of the Science Standards (Dickson, n.d.).

While 97% of the scientific community affirm that evolution has occurred in both humans and animals (Pew Research Center, 2009), percentages of science teachers who accept evolution are less consistent. It is estimated that only 28% of biology teachers consistently teach evolution and 13% openly teach creationism or intelligent design, even in public schools where it has been ruled illegal (Berkman & Plutzer, 2011). This accounts for roughly 40% of the total number of biology teachers, leaving 60% of biology teachers who do not strongly support or teach evolution. This 60% are likely unprepared to teach evolution in a way that minimizes the conflict they are trying to avoid (Berkman & Plutzer, 2011).

Many novice teachers hold incorrect evolutionary ideas, such as Lamarckism (Yesilyurt et al., 2021). When high school science teachers in Oklahoma were asked questions regarding evolutionary concepts, they responded with an incorrect idea of evolution 23% of the time (Yates, 2011). Secondary science teachers also pointed out that their students have a low understanding of evolution (Chi, 2013; Deniz & Sahin, 2016; McLure et al., 2020). Secondary educators largely agreed that the major barrier to understanding evolution was student religious beliefs, but did not mention a desire to change their teaching methods to address this problem. (Hermann, 2013). Our study specifically targeted secondary educators in an effort to better prepare them to teach evolution using a reconciliation model.

Study Rationale

More work is needed to prepare secondary educators to teach evolution, especially when their students have high religiosity. We hypothesized that education on what evolution is and methods by which it can be taught without creating conflict—i.e., the reconciliatory model—would improve instructor confidence and willingness to teach it. We predicted that using a professional online development course designed to help educators answer student questions regarding evolution and religious beliefs would enable educators to teach evolutionary concepts to religious students, without fear of generating backlash and controversy.

Our Specific Approach

This study involved both secondary instructors of science and religious educators at the secondary level. This study defined religious educators as those actively involved in teaching secondary-level students in a seminary or church setting. We developed an online course with instructional modules and interactive discussion boards to introduce teachers to a reconciliation model of evolution instruction. Participants then attended a culminating event in which instruction was provided by a prominent scientist as well as a religious scholar. We measured changes in evolution acceptance among participants as well as their confidence to teach evolution to their students. Results show that this model is a promising way to increase teacher confidence in teaching evolution to religious students.

○ Methods

Participants

This research study is a mixed methods study. Both science and religion teacher Listservs and social media platforms were used to recruit participants. Study participants were currently teaching in a secondary setting (middle school, junior high, or high school science classes or religious teaching aimed at secondary-aged youth), and all participants voluntarily registered for the event. Our priority was to provide secondary science educators the tools to teach evolution in a "faith-friendly" way and religion teachers the tools to teach religion in a "science-friendly" way. Sixty-six educators registered for the course and participated. Five religious educators and 25 science educators completed both surveys. Only science educators were included in the analysis due to the small sample size of the religion educators.

Intervention

Participants accessed an asynchronous online course utilizing the free Canvas platform (https://canvas.instructure.com). They had three weeks to complete the experience and receive a $150 Amazon gift card and certificate of completion. Completion depended on full participation in the following: (a) the Canvas course, to be completed online asynchronously, but prior to attending the culminating event; (b) an interactive discussion board to be completed in conjunction with the course (after each activity and prior to the culminating event); and (c) a culminating synchronous Zoom conference. The Canvas course can be accessed at the following URL: https://canvas.instructure.com/enroll/NTDEA3.

Outline of the Canvas Course

We created an online Canvas Course with four main objectives: (a) to address any incorrect ideas and preconceptions of evolutionary theory, (b) to explain the reconciliatory model, (c) to help the educators personally reconcile any conflict they might feel between their own religious faith and evolution, and (d) to help educators feel more confident in teaching evolution to religious students. To accomplish these objectives, the online Canvas course was split into two modules, each containing several activities and accompanying interactive discussion boards. The various discussion boards within the two modules encouraged participants to contribute their thoughts and ideas to the collective conversation. A more complete description of the two modules is found in Supplementary Table S1.

Module 1: Unpacking religious objections to evolutionary theory & learning. This first module consisted of four activities mainly focusing on religion. First, we corrected the myth about an ongoing war between science and religion; we emphasized the model of Ian Barbour on integrating religion and science (Barbour, 1997). Second, we addressed potential challenges that religious students face when learning about evolution, and helped the educators identify ways to overcome student resistance to evolution. Third, we helped the educators better understand the literary history and purpose of Genesis 1 (i.e., the Creation story). Fourth, we offered guidance and resources to help the educators teach religion in a "science-friendly" way.

Module 2: Understanding evolution and the nature of scientific inquiry. The purpose of the second module was three-fold, mainly focusing on the nature of science. First, we clarified the strengths and limitations of science. Second, we explained the process of evolution and the evidence we have for it. Third, we offered guidance and resources to help educators teach evolution in a "faith-friendly" way.

Culminating Event

The course concluded with participants attending a two-hour synchronous Zoom event. The event began with two keynote speakers—a professional paleoanthropologist (who specializes in human evolution) and a bible scholar (who specializes in evolution-religious conflicts)—who spoke for roughly 15 to 20 minutes each on a topic focused on bridging the gap between science and religion. After both presentations, a 30-minute Q&A session allowed participants to engage with the speakers. Following the Q&A session, the participants were separated into facilitated break-out rooms for more discussion. Facilitators were members of the team who created the content along with colleagues engaged in similar work. Each break-out room was created to form groups balancing religion and science educators.

Assessment

Upon joining the course, participants completed a pre-survey asking for their opinions regarding evolution, religion, possible contentions, approaches to overcoming conflict, and potential curricular materials. After the course was completed, the participants received a post-survey, asking questions complementary to those in the pre-survey. The surveys included both multiple-choice and short-response questions.

The pre- and post-surveys assessed changes in the following: personal feelings of compatibility between religion and evolution; personal acceptance of evolutionary theory (i.e., microevolution, macroevolution, and human evolution); perceptions of student compatibility and acceptance; confidence in their ability to teach the concepts involved in both evolutionary science and religious belief; and confidence in their ability to help students find compatibility. The questions about views on evolution were patterned after items used in a previous study (Tolman et al., 2021), which heavily drew on the 100-point scale developed in another comparison study of evolution acceptance instruments (Barnes et al., 2019). The confidence questions were written specifically for this study. Items were reviewed by experts within the primary author's institution who actively work on this topic, one of which is a secondary educator. We believe the questions included in the surveys adequately measured the changes in the listed objectives as well as the effectiveness of the course (complete surveys are available in the Supplemental Materials). Table 1 shows the main question prompts for each latent variable.

Because the participants had varying personal backgrounds and professional settings in which they work, it is understood that each participant would face different challenges and pressures in accepting and teaching evolution. Included in the surveys were questions that could help shed light on areas of conflict that, perhaps, had not been considered by researchers prior to the creation of the study. These questions focused on how much pushback they get from their schools/church, how they are currently navigating the pushback, and any remaining concerns they had.

○ Data Analysis

All statistical tests were performed using SPSS Statistical Package ver. 28. Descriptive statistics were run on each type of educator separately (i.e., science and religious). Due to our small sample size, we did not meet the assumptions for parametric tests so the nonparametric Wilcoxon signed rank tests were used as the alternative. Qualitative quotes were taken from the Canvas discussion boards.

Table 1. Survey Instrument.

Personal feelings of compatibility between religion and evolution*	
	Evolution is compatible with a belief in God
Personal acceptance of evolutionary theory	
	Over time, a species can adapt to better survive in its environment (e.g., Microevolution)
	New species can evolve over time (e.g., Macroevolution)
	Humans evolved from primitive life forms
Perceived student feelings of compatibility between religion and evolution*	
	Evolution is compatible with a belief in God
Perceived student feelings of acceptance of evolutionary theory	
	Over time, a species can adapt to better survive in its environment (e.g., Microevolution)
	New species can evolve over time (e.g., Macroevolution)
	Humans evolved from primitive life forms
Confidence in their ability to teach the concepts involved in both evolutionary science and religious belief (Prompt began with "Please rate your confidence in…")	
	My ability to teach the concepts of evolution
	My ability to teach the religious aspects of creation (e.g., Genesis)
Confidence in their ability to help students find compatibility (Prompt began with "Please rate your confidence in…")	
	My ability to help students overcome perceived conflicts between religion and evolution

*All items were measured on the following scale: Scale: 0 (none at all) - 25 (a little) - 50 (a moderate amount) - 75 (a lot) - 100 (a great deal); participants could drag the slider to any number in between.

○ Results

Personal Feelings of Compatibility Between Religion and Evolution

In order to assess the effectiveness of this experience in prompting changes in the beliefs of the educator concerning how religion and evolution can be reconciled in the classroom, we analyzed both a pre- and post-survey. Science educators indicated high levels of compatibility at the start of the course (averaging 83.97 on a 100-point scale). Overall, we saw a significant change in science educators in their perceptions of compatibility of evolution and religion, averaging 89.52 in the post-survey (see Figure 1). Of the 23 science educators who responded, eight made positive shifts in the idea that evolution is compatible with a belief in God ($z = 2.09$, $p = .04$).

Personal Acceptance of Evolutionary Theory (i.e., microevolution, macroevolution, and human evolution)

We retrieved data from both the pre- and post-survey, allowing us to assess how personal acceptance of evolutionary theory changed among science educators during this course. We found that science educators did not significantly change their views of microevolution, scoring a 98 on a 100-point scale both pre and post ($z = 0.00$, $p = 1.00$). Science educators also did not significantly change their opinion on macroevolution, averaging 97.57 and 98.35 pre to post, respectively ($z = 0.95$, $p = .34$), or human evolution, averaging 91 and 93.38 pre to post, respectively ($z = 1.21$, $p = .23$) (see Figure 1).

Educators' Perceptions of Their Student's Perception of Compatibility Between Evolution and a Belief in God

By looking at both the pre- and post-survey data given to science educators, we were able to determine how educators felt about what their students think of possible compatibility between evolution and religion. The perceptions of science educators concerning what their students think of compatibility of evolution and religion began with very low compatibility (averaging 40.30 on a 100-point scale) and did not change significantly (51.13 on the post-survey; $z = 1.75$, $p = .08$).

Educators' Confidence in Their Ability to Teach the Concepts Involved in Both Evolutionary Science and Religious Belief

Pre- and post-surveys allowed us to analyze how science educators felt about their abilities to teach students concepts in evolutionary science while being sensitive to religious beliefs. On average,

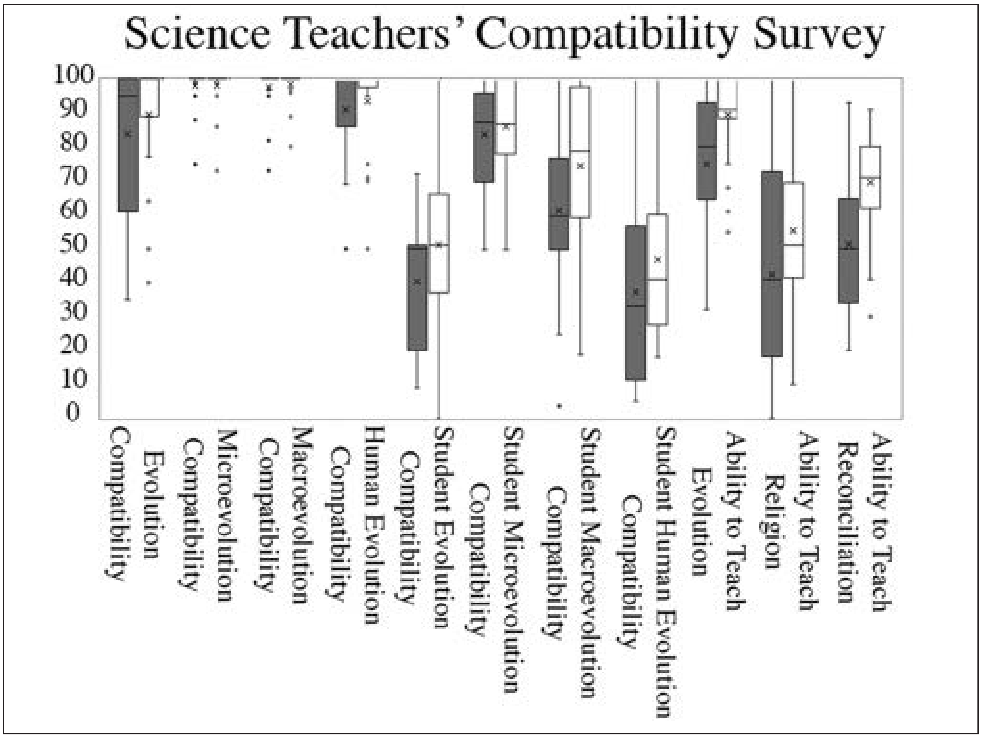

Figure 1. A graphical representation of our pre- and post-survey data for participating science educators; displaying level of confidence in addressing different topics mentioned in our survey.

educators indicated a confidence of 74.92 on a 100-point scale prior to participation. Out of 25 science educators, 19 made positive shifts in their perception of their own ability to teach the concepts of evolution, increasing the average rating to 89.56 ($z = 3.70$, $p = < .001$). Science educators indicated low confidence in their ability to address religious aspects of creation, averaging 42.55 on a 100-point scale. After participation, that average rose to 55.40; 13 out of 20 science educators made positive shifts in their perception of their ability to address the religious aspects of creation, as in being able to explain the potential compatibility or at least recognize compatibility issues ($z = 2.28$, $p = .02$). We want to make it clear that we did *not* advocate for the teaching of creationism or any religious topics or beliefs in public schools, as this is not the job of a public school teacher. Instead we advocated for an increased use of the reconciliatory teaching model and teaching evolution within that context. When asked in the survey about their ability to help students overcome perceived conflicts between religion and evolution, science educators averaged 51.29 on a 100-point scale. This increased to 69.58 on the post-survey. Nineteen out of 24 science educators made positive shifts, indicating more confidence ($z = 3.27$, $p = .001$).

Findings Within Religious Educators

This study would define religious educators as those actively involved in teaching secondary-level students in a seminary or church setting. Very few religious educators took both pre- and post-surveys, but based on the raw data we can offer a descriptive overview of the data. Religious educators appear to have equivalent acceptance of compatibility (82 on a 100-point scale) and microevolution (93.6 on a 100-point scale) as science educators. However, they showed lower macroevolution acceptance (83.60 compared with 97.57) and much lower human evolution acceptance (60 vs. 91) than science teachers. After participating in this online course, religious educators appear to have increased their acceptance of evolution and its compatibility with a belief in God ($n = 5$).

Religious educators had extremely low confidence in their ability to teach the concepts of evolution before participation (8.33 on a 100-point scale), which increased to 50.33 after participation. They appear to have much higher confidence to teach the religious aspects of creation (92.75 compared to 42.55) than science teachers, but much lower confidence to help students overcome conflict (23 vs. 51.29). This confidence appears to have improved (65.75 on the post-survey). While only a small portion of the religious educators took both surveys, even the small sample ($n = 5$) of data is encouraging that this approach could be successful for religious educators.

○ Discussion

Significant Findings

Our study showed that this online course helped science and religious educators reconcile religious beliefs with the theory of evolution. A significant number of science educators now felt that evolution and a belief in God were compatible. Perhaps more importantly, both groups of educators felt substantially more confident in their ability to help students overcome conflict whether teaching the concepts of evolution in a religious setting or reconciling religion in a scientific setting, which supported our original hypothesis. The following excerpt from a discussion board in the module highlights some current ideologies illustrated through a conversation between participants (Lee and Beth) and a moderator (Johnson). This exchange shows the change that can occur as educators learn more about the reconciliatory model. (The names have been changed to conceal the identity of the participants.)

Lee: An agnostic approach to science is appropriate due to the nature of the process of conducting scientific investigations. Religion is not necessary to conduct science, so it need not be included in a scientific explanation. For instance we can take an example of students whose religions assert that Thor is responsible for lightning being taught a lesson on science. They may experience apprehension in learning about electric charge being built up in clouds (i.e., a scientific explanation of what was previously ascribed to the realm of the gods). We can show the evidence and the ability to replicate similar phenomenon [*sic*]… and our approach (agnostically) doesn't raise the offense of the student.

Beth: We can also say, these observations, evidences, tests, might explain the way Thor is able to command lighting [*sic*].

Lee: I don't think I would say that. There is no evidence that Thor exists and in a science class I wouldn't want to give validity to that. In my own home when my kids ask about the tooth fairy I'm more likely to try and answer in this way and reconcile my kids beliefs without taking the magic of childhood away, but in the classroom, as an educator I need to take a different approach.

Johnson: [Beth], I agree with the sentiment of your statement, and [Lee], I agree with your role as a scientist. There is no scientific evidence of Thor and therefore, using Thor as an explanation is certainly not within the realms of science. But, in a personal conversation with a student who believes in Thor as a deity, [Beth's] approach can be a way for this student to reconcile. Certainly, you would make it clear that no scientific evidence supports this, but the agnostic nature of science also means that we have no evidence to refute this, and I think that is what is important for students to understand IF they are having a struggle with the potential conflict.

As you can see from this conversation, Lee brought in "Thor" as an example of a religious belief that may directly conflict with science. Because science is agnostic, Beth suggested a way that reconciliation could allow for a student's religious beliefs to coexist with accurate science, while Johnson helped Lee to understand that allowing for a religious belief did not have to diminish the accuracy of the science being taught. This approach allows for the student to progress in their scientific understanding by finding potential compatibility with their religious beliefs. With the information and tools presented in this module, a significant number of science educators felt they could now better help students understand that their religious beliefs do not have to interfere with the scientific findings. The following quote from the culminating event summarizes this finding: "I used to think my role as a science teacher was to just present the facts and say this just makes sense! That's not the case, I have an opportunity to help the students reconcile!"

We have also found that using a reconciliatory model helps individuals better accept evolution by emphasizing potential compatibility with religious beliefs (Lindsay et al., 2019). Students' religious beliefs can be the main predictor of whether they accept evolution, and for this reason, the reconciliatory model aims to help students understand how religious beliefs fit in with the theory of evolution by explaining what can and cannot be explained by both science and religion. This can be categorized within the ReCCEE framework, specifically emphasizing compatibility. Others have also found success with this approach (e.g., Barnes & Brownell, 2017; Tolman et al., 2020; Tolman et al., 2021).

It is also interesting to note we also found among the science educators a large gap between how they perceived compatibility between evolution and religion and how they think their students perceive the same relationship. As seen in Table 1, the educators at large find evolution and religion to be compatible, but they perceive their students hold fewer compatible views. One possible explanation for this large gap in perceived compatibility may relate to the large gap in belief in God between scientists and students as discussed in the introduction. Non-religious instructors may have an exaggerated perception of conflict in students because of an underlying stigma about religious individuals (Barnes et al., 2021).

In addition, an equal number of science educators made positive shifts in their perception of their abilities to teach evolution as they did in their ability to support students in reconciliation. This highlights the potential of professional development courses such as this to help educators feel more confident in teaching the concepts of evolution as well as their confidence in helping students reconcile evolution and religion.

Limitations

While we found that the data is suggestive of an improvement in reconciliation among educators and their confidence in teaching evolution to their students, there are some limitations worth mentioning. The sample size of the study was relatively small. The data from religious educators was even more so. Due to this lack of survey response from our sample of religious educators, it is difficult to make decisive conclusions. However, the trends are promising that this course is indeed beneficial to the educators and their students. Another limitation to note is the regionally specific nature of the sample. Most participants came from the intermountain west region. With this in mind it is difficult to apply this study generally to all educators across the country, although we expect the trends would be similar. Finally, self-selection bias must also be considered when evaluating the data mentioned in the results section of this paper as all participants volunteered to take part in the study.

Educational Implications

Biology teachers often have students who come from a wide range of religious beliefs. According to a Pew Research Center survey (2018), eight-in-ten young adults ages 18–29 claim they believe in some type of spiritual force. This reconciliation module equips biology educators with tools to help religious students understand and reconcile evolution with their beliefs. Educators who complete this module may be better equipped to act as role models for their students (i.e., guide them through the reconciliation process). Role models have been shown to be important in helping change religious students' minds about evolution and decrease perceived conflict between religious beliefs and evolution (Ferguson & Jensen, 2021; Holt et al., 2018). In addition, this module helps educators feel more confident in talking about perceived conflict between religious beliefs and evolution, allowing their students to focus on learning biological concepts rather than struggling with feelings of incompatibility, and allowing them to feel more comfortable covering evolution as part of their curriculum.

Our module may also benefit new religious and biology teachers as a training tool to help them foresee future student concerns that could come up as they discuss evolution. By acknowledging potential conflict and perceived incompatibility at the beginning of the evolutionary unit, students will likely face less anxiety while learning the concepts of evolution and understanding the scientific method in general (Bertka et al., 2019; Lindsay et al., 2019; Truong et al., 2018).

This module could also be expanded to help others outside of academia, such as museum patrons, parents, church leaders, etc. By modifying the trainings in our module, it can easily be applied to a more general population without any education or scientific background. This online workshop could be helpful for those who want to understand the mechanisms of evolution, but also want to protect their religious beliefs.

○ Conclusion

As students begin their journey into a STEM degree, many may feel uncomfortable due to the religious beliefs they hold. Christianity has been shown to be a concealable stigmatized identity impacting students' decision whether to choose biology as a major (Barnes et al., 2021). This module focused on helping educators teach students about the nature of science. We originally predicted that this module would increase an educator's confidence in teaching evolution and religion. We observed that their confidence significantly increased. We conclude that future professional development courses should be structured similarly to ours to give educators the appropriate reconciliation tools for their classroom. The effectiveness of the tools provided in this online course apply only to how educators' opinions were changed. Additional research should be done to understand how students may increase their acceptance and understanding of evolution when these tools are applied.

Through continued exploration of this professional development method, there is great potential to create reconciliatory opportunities for divided topics such as climate change, vaccine uptake, conservation efforts, sexual orientation, genetic engineering, and science denialism in general. The promising trends found in this reconciliatory model between evolution and religion could have great benefits in improving acceptance of science in our society.

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RESEARCH ON LEARNING

Public vs. Private: High School Biology Teachers' Acceptance and Teaching of Evolutionary Theory in Arkansas

BRITTENY BERUMEN, MISTY BOATMAN, MARK W. BLAND

Abstract

Evolutionary theory is fundamental to biology, yet evolution instruction in high schools has often been unsatisfactory. How or whether high school biology teachers teach evolution is influenced by their own acceptance or rejection of evolutionary theory, parents' and community members' views, and in the case of some private schools, their religious affiliations. Studies documenting how evolution is taught in public high schools have been conducted, yet private schools remain underresearched.

Arkansas high school biology teachers employed by public and private schools were invited to complete a survey composed of the Measure of Acceptance of the Theory of Evolution (MATE) and other items designed to allow comparison of their treatment of topics within evolutionary theory. Specifically, we sought to compare public and private teachers' acceptance of evolution, how they teach it in their classrooms, and how their acceptance of the validity of evolution compares with four other widely accepted scientific theories (cell, gene, germ, and atomic). Results suggest that public school teachers have higher levels of acceptance of evolution than private school teachers. However, teachers in both public and private schools reported lower acceptance of the validity of evolutionary theory compared with the other four scientific theories. Across topics within evolution, natural selection was given the most treatment while human evolution was given the least.

Key Words: *Evolution education, Nature of science*

○ Introduction

Dozens of scientific, educational, and even some religious organizations have published position statements verifying evolution as a central and unifying scientific theme (Voices for Evolution, 2016). Though recent evidence suggests that acceptance rates have increased in recent years, and biology teachers are now teaching more evolution to their students (Plutzer et al., 2020), national polls have reported consistently low public acceptance for several decades, and the percentage of Americans who reject evolution is high compared with other developed countries (Miller et al., 2021). Evolution education has been especially contentious in southern states, with legislation aimed at weakening the status of evolutionary theory introduced as recently as February 2023 in West Virginia (Senate Bill 619, 2023).

Public acceptance or rejection of evolution in the United States is culturally complex, stemming from "a myriad of often interwoven reasons" (Pobiner, 2016) including identity-protective cognition (Walker et al., 2017) and creationists' views (Wingert et al., 2023), and correlates with factors such as GDP and educational attainment (Heddy & Nadelson, 2013). Notably, religiosity has been found to be more predictive of low scientific literacy than income, race, or gender (Sherkat, 2011), and Rissler et al. (2014) found that religiosity was a better predictor of evolution acceptance than education. Jensen et al. (2019) suggested that religious affiliation and religiosity (e.g., how one's religion affects behavior) both affect acceptance of creationist claims (e.g., the six-day creation story), which, in turn, affect evolution acceptance.

Studies suggest teachers have been pressured to modify their teaching of evolution or to present nonscientific explanations for the diversity of life, such as intelligent design (Berkman et al., 2008; Griffith & Brem, 2004; Pobiner, 2016). While teaching standards generally include evolution, its teaching has historically been avoided, de-emphasized, or replaced with nonscientific alternatives in high schools (Bland & Moore, 2011; Moore, 2008; Rutledge & Warden, 2000). Moreover, some studies suggest science standards may not matter where teaching evolution is concerned (Bandoli, 2008; Moore, 2002; Moore & Kraemer, 2005).

Science provides insights into natural phenomena through rigorous testing, formulating conclusions based on empirical evidence, and the development of theories. Because science is limited to naturalistic explanations, science teachers have no basis for introducing nonscientific explanations for natural phenomena because science cannot test or reject these. Moreover, scientific theories are "well-substantiated *explanation[s]* [emphasis added] of some aspect of the natural world that can incorporate facts, laws, inferences, and tested hypotheses" (National Academy of Sciences, 1998). Several scientific theories help us understand the nature of living things, such as germ theory, gene theory, and the overarching evolutionary theory (NSTA, 2013). However, acceptance of evolution's validity has been found to be significantly lower than for germ, cell, gene, and atomic theory (Rutledge & Sadler, 2011).

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Views on evolution have been studied for a variety of demographics, yet there is a lack of information from private schools. Schulteis (2010) examined evolution instruction in Lutheran schools across the United States, reporting that all respondents taught at least one of seven fundamental concepts of evolution (speciation, diversity, descent with modification, evidence for evolution, natural selection, pace and rate of evolution, and human evolution) in their classes. Unsurprisingly, natural selection was emphasized more than human evolution. However, 75% of Lutheran high school teachers disagreed with the statement "Evolution is a central and unifying theme in biology," and 59% disagreed with "Evolutionary topics are supported by scientific evidence" (Schulteis, 2010).

Approximately 4.7 million students are enrolled in private schools in the United States (National Center for Education Statistics, 2022). Many states, including Arkansas, have or are considering school voucher programs enabling the use of taxpayer dollars to enroll students in private schools, which will surely bolster private school enrollment. Because scientific literacy in general and evolutionary literacy in particular are important as students become citizens (Kampourakis, 2022), an understanding of private school science curricula can help inform interested parties about trends in scientific literacy of United States citizens. This information also would be valuable to college-level instructors in meeting the needs of a significant number of their students.

Opponents of evolution have worked for decades to influence high school science curricula in the United States. In southern states, the anti-evolution movement can be traced to the publication of pamphlets entitled *The Fundamentals: A Testimony* between 1905 and 1915 (Halliburton, 1964; Le Beau, 2007). Fundamentalists "took violent exception to the advocacy and teaching of evolutionary theories" and worked to make teaching evolution unlawful, especially in southern states: "Each and every Southern state experienced a vitriolic anti-evolution controversy" (Halliburton, 1964). Between 1921 and 1929, 37 anti-evolution legislation articles were introduced in Arkansas and other states (Halliburton, 1964), and anti-evolution petitions were circulated in Arkansas newspapers before the 46th Arkansas General Assembly (Bush, 1926).

In 1927, the "Rotenberry Bill" was introduced by A. L. Rotenberry, of Little Rock. This bill passed the Arkansas House but was defeated in the Senate by a vote of 17–14. With the aim of adding a referendum to the subsequent general election ballot, required signatures on circulated petitions were quickly obtained, and a referendum essentially identical to the Rotenberry Bill was passed into law (Halliburton, 1964; Ledbetter, 1979). This made it unlawful for any teacher employed by a publicly funded institution to teach "the theory or doctrine that mankind ascended or descended from a lower order of animal" (Ledbetter, 1979).

The Arkansas statute was in place from 1929 to 1968, when the U.S. Supreme Court found that it violated the First Amendment's Establishment Clause (Epperson v. Arkansas, 1968). Creationists in Arkansas later worked to undermine the status of evolution with legislation granting "equal time" for creationist-based science teaching. The Arkansas Federal District Court ruled that "creation science" is not science, and teaching creation-based "science" in public schools violates the Establishment Clause (McLean v. Arkansas, 1982). The U.S. Supreme Court agreed in Edwards v. Aguillard (1987) by overturning a Louisiana law requiring public school science teachers to teach creation science.

The anti-evolution movement in Arkansas is far from over. Introduced in 2017, Arkansas House Bill 2050 read, "To allow public school teachers to teach creationism and intelligent design" (HB2050, 2017). While this bill died in the House, House Bill 1701, introduced in 2021, passed by a vote of 72–21, but then died in the Senate Education Committee (HB1701, 2021):

> TO ALLOW CREATIONISM AS A THEORY OF HOW THE EARTH CAME TO EXIST TO BE TAUGHT IN KINDERGARTEN THROUGH GRADE TWELVE CLASSES IN PUBLIC SCHOOLS AND OPEN-ENROLLMENT PUBLIC CHARTER SCHOOLS.

Though neither of these bills were passed into law, their filing—and that 72 state representatives voted in favor of HB1701—reveals much about the current climate in Arkansas.

On the national level, research suggests teachers were teaching more evolution and less creationism in 2019 than a decade prior (Plutzer et al., 2020), perhaps due to implementation of the Next Generation Science Standards (NGSS) and improvements in teacher education programs. Because of ongoing resistance to evolution education, we sought to assess the status in both public and private schools in Arkansas. Because (a) teaching licenses are not required for private school teachers, (b) private school science teachers are therefore not required to complete a state-recognized teacher licensure program including a sufficient number of science courses, and (c) teachers who accept jobs at private schools may be doing so for religious reasons, we hypothesized that there would be differences in how private and public school teachers treat evolution in their classrooms. Specifically, we sought to answer these questions: (a) How do Arkansas public and private high school biology teachers compare in their levels of acceptance of evolution? (b) Are there differences in acceptance of evolution's scientific validity compared with other widely accepted scientific theories (cell, germ, gene, and atomic)? and (c) To what extent do public and private high school teachers treat the topics within evolution?

○ Methods

The Survey Instrument

Rutledge & Warden (1999) designed the Measure of Acceptance of the Theory of Evolution (MATE) to measure high school biology teachers' acceptance of evolution, though it has since been used for other demographics such as high school students (Wiles & Alters, 2011), undergraduate college students (Moore & Cotner, 2009; Nadelson & Southerland, 2010), and university faculty (Rice et al., 2015). The MATE has been validated through classical test theory (Rutledge & Warden, 1999), Rasch analysis (Romine et al., 2017), and factor analysis (Rissler, 2014), and high reliability values have also been reported (Romine et al., 2017; Rissler, 2014; Rutledge & Sadler, 2007). Romine et al. (2017) conducted a thorough evaluation of the MATE, and while their analysis points to some limitations, they found it to be psychometrically sound.

Additionally, Romine et al. (2018) compared the MATE with more recently developed survey instruments and concluded all three—the MATE (Rutledge & Warden, 1999), GAENE (Smith et al., 2016), and I-SEA (Nadelson & Southerland, 2012)—produce measures with very similar quantitative interpretations.

Items from a survey designed by Rutledge & Sadler (2011) also were used to assess teachers' acceptance of the validity of five scientific theories—gene, atomic, evolution, germ, and cell—enabling comparisons between participants' views of evolutionary theory and the other four. Participants were provided with a brief description

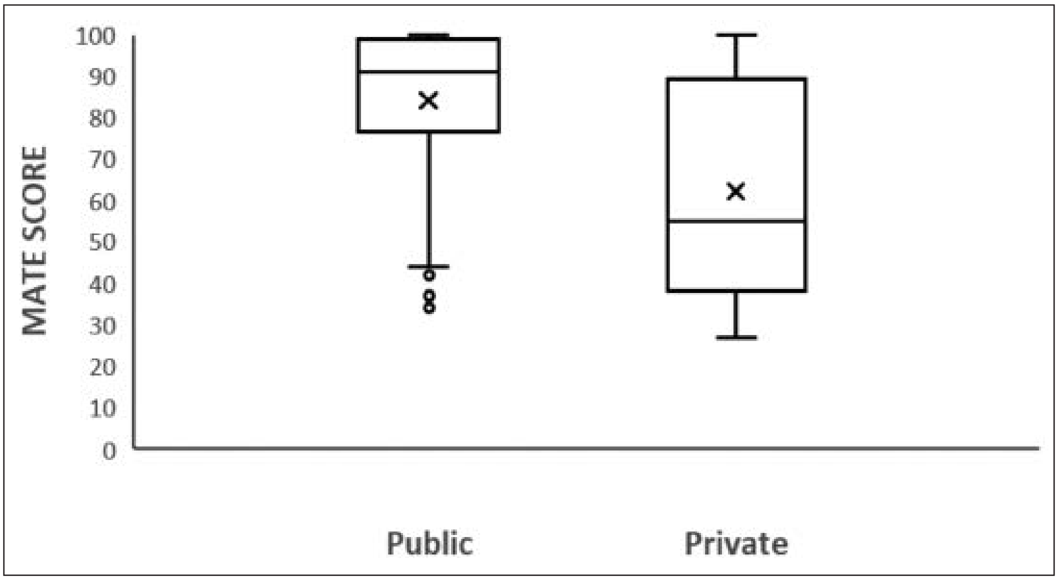

Figure 1. Box-and-whisker plots depicting scores on the MATE for public and private school teachers.

of each theory and asked to report their degree of agreement on whether each theory is scientifically valid.

Finally, Likert-scale items asking participants about their treatment of topics within evolution were included: speciation, diversity, descent with modification, evidence, natural selection, pace and rate, and human evolution.

Recruitment of Participants

High school biology teachers employed in public and private high schools in Arkansas were invited to complete our survey via the online service, SurveyMonkey, during the Spring 2021, Fall 2021, and Spring 2022 semesters. Reminder emails were sent two weeks after the initial invitation. Due to low response rates from private schools, paper surveys with a stamped return envelope were mailed during the early fall semester, 2021. Ninety-eight public and 16 private high school teachers returned completed surveys. Incomplete surveys were omitted from our analysis. To ensure that only data from biology teachers was used, surveys were screened with the first question of the survey: "I teach one or more biology classes (Yes/No)."

Statistical Analysis

Average MATE scores were calculated for both public and private teacher responses and were compared using Mann-Whitney U. We used Mann-Whitney U and Wilcoxon signed-rank tests to investigate differences in teachers' acceptance of evolutionary theory compared with the other theories. Mann-Whitney U and Wilcoxon signed-rank tests were also used to assess differences in public and private teachers' emphasis of evolutionary concepts.

○ Results

Research Question 1: Acceptance Among Public and Private School Teachers

We hypothesized that public school teachers (n = 98) would have higher acceptance of evolutionary theory than private school teachers (n = 16). Box-and-whiskers plots showing distributions of MATE scores are provided in Figure 1. There were obvious departures from normality, with strong negative skew for scores from public school teachers and slight positive skew for private school teachers, who also showed greater variability in scores. Because these issues could be problematic for typical parametric significance tests (e.g., ANOVA, Student's t) all analyses on these and other measures reported below were conducted with nonparametric methods (Mann-Whitney U, Wilcoxon signed-rank) and a stringent criterion for statistical significance ($p < .001$). The difference between the two groups on the MATE (medians of 91 and 55) did reach this level of significance for the Mann-Whitney U, indicating that public school teachers were more accepting of evolution than were private school teachers.

Research Question 2: Acceptance Across Five Scientific Theories

We hypothesized that private school teachers' acceptance of evolution's validity would be significantly lower than their acceptance of four other scientific theories, but that this trend would not be observed in public school teachers' responses. Teachers were asked to identify their level of agreement with statements describing five scientific theories on a five-point scale ("Strong Disagreement" to "Strong Agreement"). Relative frequency distributions showing percentages of public and private school teachers selecting each level are provided in Table 1. Teachers from public and private schools were in agreement with statements related to gene, germ, cell, and atomic theories. However, the percentage of teachers showing agreement with evolutionary theory was lower, especially for those teaching in private schools. We used separate Mann-Whitney U tests to compare the two groups' responses toward each theory. They differed significantly only on evolutionary theory and, consistent with MATE scores, public school teachers showed greater agreement than did private school teachers.

Wilcoxon signed-rank tests were used for pairwise comparisons of responses to theories for all teachers. Comparisons of evolutionary theory with each of the others were significant, indicating less agreement with evolutionary theory. None of the other six possible pairwise comparisons among the four other theories were significant.

Table 1. Frequency distributions for public school ($n = 98$) and private school ($n = 16$) teachers' acceptance of five central scientific theories.

	SD	D	U	A	SA
Evolutionary Theory					
Public	5%	3%	4%	22%	65%
Private	19%	19%	13%	13%	38%
Atomic					
Public	0%	0%	2%	17%	81%
Private	0%	0%	0%	19%	81%
Germ Theory					
Public	1%	1%	1%	13%	84%
Private	0%	0%	6%	13%	81%
Cell Theory					
Public	0%	0%	0%	15%	85%
Private	0%	0%	0%	13%	88%
Gene Theory					
Public	0%	0%	0%	12%	88%
Private	0%	0%	0%	19%	81%

Table 2. Frequency distributions for public school ($n = 98$) and private school ($n = 16$) teachers' treatment of seven topics within evolution.

	None	Little	Some	Moderate	Strong
Human Evolution					
Public	15%	28%	23%	19%	14%
Private	31%	19%	25%	25%	0%
Pace and Rate					
Public	15%	22%	33%	15%	14%
Private	31%	6%	31%	31%	0%
Speciation					
Public	2%	6%	22%	34%	36%
Private	13%	13%	19%	31%	25%
Descent					
Public	1%	6%	15%	32%	46%
Private	6%	25%	13%	25%	31%
Evidence					
Public	2%	4%	15%	26%	53%
Private	6%	19%	13%	31%	31%
Diversity					
Public	1%	2%	9%	36%	52%
Private	6%	6%	13%	31%	44%
Natural Selection					
Public	1%	1%	5%	19%	73%
Private	6%	25%	0%	31%	38%

Research Question 3: Teachers' Treatment of Topics Within Evolutionary Biology

Arkansas' current science standards are a modified version of the NGSS and include significant treatment of evolution (Arkansas Department of Education, 2016). We hypothesized that public school teachers would teach and emphasize themes within evolutionary biology more than private school teachers. Teachers were asked to indicate the degree of emphasis given to seven concepts in evolution (speciation, diversity, descent, evidence, natural selection, pace and rate, and human evolution) on a five-point scale ("No Emphasis" to "Strong Emphasis"). Relative frequency distributions of teachers' responses are provided in Table 2. Mann-Whitney U statistics were calculated to compare public and private school teachers' responses for each of the seven concepts. Public school teachers reported significantly greater coverage for natural selection than private school teachers. No significant differences were observed for the remaining six concepts. Comparisons between coverage of the seven concepts for the entire sample of 98 teachers were conducted using Wilcoxon signed-rank tests. The two concepts receiving significantly more coverage than the others were diversity and natural selection, while the two receiving significantly less coverage than the others were human evolution and pace and rate.

○ Discussion

Limitations

All survey-based studies have limitations, including the potential for self-selection bias. Public school teachers should know that they are mandated to teach evolution based on the state science standards. Our data may not be representative because some teachers may have elected to not complete the survey, or to misrepresent what they are actually teaching in their classrooms, once they learned that questions about how they teach evolution comprised part of the survey. This may have affected response rates from private school teachers also; however, we suggest that because they are not required to adhere to state standards, questions about how they teach evolution should not have served as a deterrent. Additionally, teachers who feel strongly about their rejection of evolutionary theory could be as motivated to complete our survey as those who feel more strongly about the value of teaching evolution in their classrooms.

The small sample size from private schools makes meaningful inferences more difficult. Sixteen private schools (23% of those in Arkansas) submitted completed surveys, prompting our decision to use nonparametric statistical tests. Return rates for public school teachers are difficult to calculate because contact information for science teachers was unavailable in many cases, so email invitations were sent to whole schools. However, personal communication with the Arkansas Department of Education suggests that 98 completed surveys represent 15% of the total number of certified biology teachers in K–12 public schools in Arkansas.

Finally, though the MATE was recently revised because of concerns over conflation of constructs (Barnes et al., 2022) MATE 2.0 was not available until after this study was completed. It is possible that some participants' responses were influenced by constructs not directly related to acceptance. However, because the original MATE was designed to measure high school teachers' levels of acceptance and has been validated and deemed psychometrically sound (Rissler, 2014; Romine et al., 2017; Rutledge & Warden, 1999), we regarded it to be satisfactory for informing us about differences between private and public school biology teachers' views.

○ Conclusions

Teaching evolution in public school classrooms has been contentious in the United States for decades. Though recent data suggests an increase in acceptance of evolution among adults in the United States, a significant portion are still unsure of or reject the theory of evolution outright (Miller, 2021).

Recently introduced legislation aimed at diminishing the status of evolutionary theory in Arkansas public schools was supported by more than three-fourths of voting members of the Arkansas State House of Representatives (HB1701, 2021), suggestive of an ongoing anti-evolution climate in Arkansas. Conversely, and consistent with current national trends reported elsewhere (Plutzer et al., 2020), our results suggest that current public high school biology teachers in Arkansas are generally accepting of evolutionary theory, while private high school teachers have a somewhat lower acceptance.

As independent organizations, private schools are not obligated to adhere to state educational standards, nor are private school teachers required to obtain state-issued licenses. Most of them are religiously affiliated, and correlations between religiosity and acceptance of evolution have been documented (Heddy & Nadelson, 2013; Jensen et al., 2019; Rissler et al., 2014). Religiosity is only one aspect of a group of interrelated factors, however. Researchers also suggest that personal beliefs and convictions often have more influence than church doctrines, both religiosity and religious affiliation can impact how one views evolution (Jensen, 2019). Such factors may influence who chooses to apply for positions at private schools, and private school teachers' religious affiliations and religiosity may influence their acceptance of and teaching of evolution in the science classroom (Schulteis, 2010).

Moreover, whether private school students are taught evolution may have effects moving forward: students who were taught evolution and not creationism have been shown to be significantly more likely to accept the validity of evolutionary theory (Moore & Cotner, 2009; Rissler, 2014), and pre-course knowledge and acceptance has been found to correlate with course achievement in introductory college biology (Carter et al., 2015). Acceptance of evolutionary theory has also been found to correlate with students' abilities to negotiate biology-based socio-scientific issues (Fowler & Zeidler, 2016). Additionally, college-level instructors would benefit by understanding these effects. Disentangling specific motives driving private school teachers to teach or to not teach evolution presents questions for future research.

We were not surprised that acceptance of evolutionary theory was significantly lower than the other four scientific theories (cell, germ, gene, and atomic) among private school respondents. However, we were somewhat surprised that public school teachers also showed this trend. Consistent with Rutledge's (2011) findings, it appears that university students and teachers alike are more likely to accept other scientific theories over evolution. The reluctance of science teachers to fully accept these scientific theories as settled science raises questions about the reasons behind this observation; identifying these also presents questions for future research, and addressing this question across other demographics may provide some interesting insight into the reasons behind these differences.

Finally, our assessment of biology teachers' emphasis on seven concepts specific to evolutionary theory did not yield surprising results, as public and private school biology teachers reported less emphasis on human evolution than natural selection. Human evolution may be considered more controversial than other topics, making private school teachers more reluctant to include it in their instruction. In public schools, the high emphasis on natural selection may be a result of how it is addressed in Arkansas science standards (Arkansas Department of Education, 2016).

An understanding of evolutionary theory is fundamental to scientific literacy, and the high school science classroom is the last opportunity many have to learn about the nature of science and the mechanisms of evolution. Thus, secondary science educators play an important role in developing a scientifically literate populace, including bridging the gap between settled science and resistant sectors of the public (Nehm & Schonfeld, 2007). Long-term progress in closing this gap would be best achieved by recruiting high-caliber high school graduates and early-career college students into quality science education programs. Such programs should provide students with a solid foundation in content, including evolutionary biology and the nature of science, and the instructional tools to teach these topics (see Ziadie & Andrews, 2019, for an excellent resource). Pre-service science teachers are an especially important group for consideration (Glaze, 2018; Glaze et al., 2014; Larkin & Perry-Ryder, 2015; Vaughn & Robbins, 2017): pre-service teachers' understanding and acceptance lead to a higher likelihood that they will give treatment to evolution in their own classrooms. We consider in-service teachers and college professors as having much potential in this endeavor and call on them to continue to serve as positive role models for our future science teachers.

Historically, Arkansas' treatment of evolution in state education standards was found to be unsatisfactory (Lerner, 2000). In 2005, Arkansas revised state science standards with strengthened evolution content goals, and in 2016 implemented a modified version of the NGSS (Arkansas Department of Education, 2016). We view these steps as progress and are hopeful that teachers' efforts in the classroom will continue to close the gap between settled science and public perception.

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INQUIRY & INVESTIGATION

Elephant Tusks and Natural Selection: Leveraging Naïve Student Models to Identify and Address Misconceptions Surrounding Biological Evolution

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Abstract

Evolutionary theory is foundational to the life sciences because it unifies complex ecological principles and explains variation observed between and within species. Students at the secondary level often lack deep conceptual understanding of evolutionary theory, which is crucial to grasp topics related to primary drivers within populations such as inter- and intra-specific competition, predation, and reproductive success. Nonetheless, evolution remains a contentious topic in the United States. The prevalence of pseudoscientific belief among the U.S. populace warrants a calculated approach to deconstructing student misconceptions. This article puts forth an action-research-supported instructional strategy through which educators can identify and address core student misconceptions regarding evolutionary theory and other complex scientific phenomena, utilizing real-world and student-generated models to drive instruction.

Key Words: *Evolution, Misconceptions, Modeling*

○ Introduction

Biological evolution remains an instructional challenge for U.S. science educators. A 2019 Gallup poll, for example, reports that nearly 40% of American adults continue to reject evolutionary theory in favor of purely creation-centered worldviews (Brenan, 2019; Miller et al., 2022). For the science teacher, who must already navigate the philosophical and theological objections of parents and students, a contentious public discourse only generates additional pedagogical challenges. By the time students enter the secondary science classroom, they have often inadvertently adopted common but false beliefs regarding the rate of evolutionary change, the amount of scientific evidence in support of evolution, and the capacity for an individual organism to rapidly adapt to its changing environment. These misconceptions not only hamper students' ability to develop accurate conceptual models but serve to buoy the various arguments that teachers must address if students are to ultimately accept accurate models of evolutionary theory (Rudolph & Stewart, 1998; Rutledge & Warden, 2000).

To counter misconceptions within abstract scientific topics such as evolution, national reform documents, including the Next Generation Science Standards (NGSS), prescribe lesson sequences that simultaneously mimic the work of scientists and act as critical grounding experiences for students (NRC, 2012; NGSS, 2014). The curricular shift has been noteworthy, with many contemporary lesson sequences centering concrete exploratory and explanatory activities (e.g., Janney et al., 2022; Kidd et al., 2023). Evolutionary theory, unsurprisingly, has received significant curricular attention, resulting in numerous concrete instructional interventions that target key conceptual ideas such as natural selection, coevolution, inheritance, and genetic variation (e.g., Reynolds, 2019; Wilcox et al., 2017).

Alone, however, even exceptionally designed instructional sequences are unlikely to fully eliminate deeply held misconceptions surrounding evolution and other complex topics, unless they are explicitly addressed. Longstanding research in student conceptual change suggests that for learners to avoid rejection or false accommodation (accepting a modified version of the accurate model and/or accepting the accurate model only in "school" settings), they must first identify inconsistencies within their own models (Appleton, 1993). To do so, science teachers must not only draw out student thinking through questioning, but simultaneously scaffold students toward the rejection of false ideas. Unfortunately, when it comes to abstract science concepts, students are adept at "classroom camouflage," capable of imitating a deep level of understanding, even if it is only surface level. Thus, it can be difficult for science teachers to assess and address individual misconceptions in large classrooms in real time.

This article puts forth Naïve (pre-instruction) Student Modeling as a simple, yet effective instructional strategy designed to draw out early student sensemaking, and present opportunities through which students begin to question the legitimacy of their explanations surrounding complex scientific phenomena such as evolutionary theory.

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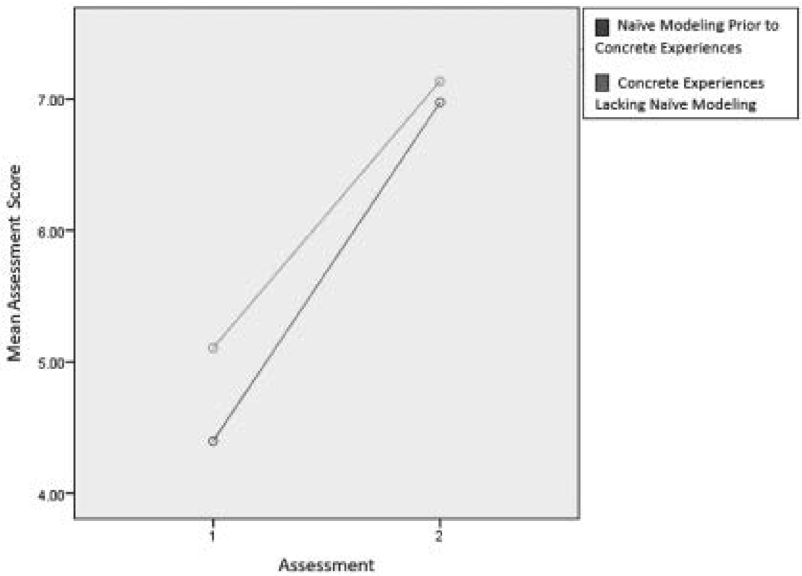

Figure 1. *Student Learning Growth: Naïve Model Development.*

Note: Seventh-grade honors student learning growth between time 1 (pre-assessment) and time 2 (post-assessment). The figure indicates a statistically significant difference in growth between units in which students engaged in naïve model development prior to concrete experiences (bottom) and units that solely utilized concrete experiences (top).

○ Some Evidence for the Positive Impact of Naïve Model Development

Class action research conducted during the 2019 spring semester in seventh-grade honors science classes found some benefit to engaging in naïve model development prior to moving into more concrete experiences. A mixed-design ANOVA indicated that mean conceptual growth between pre- and posttests was statistically significantly greater for lesson sequences that utilized naïve modeling *before* a concrete experience than sequences that *began* with a concrete experience [$F(1,1) = 7.508$, $p = .007$]. (Figure 1).

○ Lesson Overview

In the following example of Naïve Student Modeling, biological data surrounding tusk growth patterns in elephants in response to poaching is used to reveal early student misconceptions surrounding evolutionary theory (Table 1). As students clarify, present, and scrutinize their initial models, they not only begin to identify inconsistencies within their own thinking, but they also provide critical insight to the instructor prior to engaging in more content-specific activities.

○ Background Information for the Instructor: Leveraging Elephant Tusk Reduction to Reveal Student Thinking About Evolution

Genetic bottlenecks arising from anthropogenic environmental disturbances can offer an effective way to explore evolutionary ideas in the science classroom. Perhaps most famously, a dramatic uptick in the phenotypic frequency of industrial melanism in "Peppered Moths," due to heavy environmental damage caused by the newly industrialized United Kingdom of the 19th century, offers an often oversimplified but powerful example of natural selection driving population change (Cook et al. 1999).

A less well-known, yet similar evolutionary phenomenon has occurred in some African elephant populations. Global demand for ivory has long driven catastrophic poaching activity, with some estimates suggesting an annual mortality rate of nearly 30,000 (Bale, 2020). However, intense ivory harvesting, which resurged during the 1970s and 1980s, resulted in even more significant regional African elephant declines, with some populations experiencing losses upward of 50–90% (Chiyo et al., 2015). Though there is significant natural variability in tusk size due to genetic and environmental factors, poachers are thought to more frequently target older adult males. This results in an age-striated downward selection pressure, making smaller tusks dramatically more prevalent in these populations. Specifically, these elephant populations have observed an overall reduction in tusk length and circumference, and an increased expression of the typically rare and potentially X-linked tusklessness gene among females, which causes them to have no tusks at all (Campbell-Station et al., 2021).

An overall reduction in tusk size may have unpredictable ecological implications for African elephants. Large tusks are useful for foraging, clearing access routes to nutrient-rich vegetation, stripping bark from trees, and digging for water. By using their tusks, elephants regularly reshape their environments, producing downstream effects for smaller organisms. It is unclear how significant alterations to elephants' physiology and behavior may impact their broader ecosystem (Maron, 2018). For the biology student, the story of the African elephant is compelling, not only because elephants are well-known charismatic megafauna, but

Table 1. Naïve Student Modeling Overview.

Grade/Content Area	Secondary Life Sciences
Objectives	Students will: • Crystalize pre-instructional thinking surrounding evolutionary theory. • Create pre-instructional explanatory models, leveraging real-world data on African elephants. • Reveal pre-instructional thinking for instructor use. • Test the resilience of pre-instructional models through classmate critique.
Materials	• Trends in African Elephant Physiology in Response to Poaching Pressure – Dataset • Student Model Development Page
Instructional Sequence	
Step 1:	Prepare the Classroom to Explore Naïve Models
Step 2:	Present Poaching as an Ecological Threat
Step 3:	Introduce the Phenomenon of Elephant Tusk Reduction
Step 4:	Elicit Naïve Student Models
Step 5:	Test Model Resilience Through Classmate Scrutiny
Step 6:	Leverage Naïve Models to Tailor Instruction

also because this case-study perfectly illustrates the convergence of anthropogenic evolutionary pressures with currently advantageous, yet almost certainly detrimental, trait expression.

Step 1: Prepare the Classroom to Explore Naïve Models About Evolution

Requiring that students expose their thinking surrounding a new topic is nontrivial. For one, students rarely consider scientific phenomena in their everyday lives. They also place themselves at heightened social risk by expressing potentially incorrect lines of thought. It is crucial that educators seeking to make effective use of naïve student models increase opportunities for students to honestly reveal their thinking while maintaining a classroom environment in which they feel comfortable doing so.

Prepare students to engage in naïve modeling. We have found it useful to spend the preceding class period introducing the structure of the activity, establishing classroom norms, and discussing expectations for participation (Table 2). As students will ultimately evaluate the ideas of their classmates, it is crucial that they are shown how to properly critique ideas without falling into personal attacks. Historical examples from science including the purported rivalry of Sir Issac Newton and Robert Hooke, debates about the structure of the solar system, and the complex politics surrounding Watson and Crick's triple helix model of DNA can be useful stories to draw on when discussing proper and improper strategies for scientific debate.

Step 2: Present Poaching as an Ecological Threat

Introduce *poaching* as a key ecological threat to African elephants and other megafauna. The level at which poaching is explored may vary according to student age and previous exposure to global ecological challenges. Various media sources can function as useful introductory material (articles, videos, etc.). For example, Fobar (2023) offers insight into potential economic drivers specifically related to elephant poaching. The introductory material should meet the following objectives:

1. Identifies poaching as a detrimental human activity driven largely by economic factors.
2. Discusses the role of poaching in funding regional conflicts.
3. Discusses the ecological implications of heavy poaching in terms of population dynamics and trophic-level interactions.

Table 2. Strategies to Foster an Effective Classroom Environment to Explore Naïve Sensemaking.

• Place students into teams of no more than four. If the class makeup is heavily differentiated in terms of ability, stagger groups so that lower-performing students are supported by a more knowledgeable team member. Similar pairings can be used for particularly shy students by pairing them with more outspoken individuals.
• Establish reasonable classroom norms surrounding student participation, questioning, and respect for classmates including raising hands when asking questions, not interrupting other classmates, and listening quietly while others are speaking.
• Spend time before the activity discussing key differences between criticism of an idea and criticism aimed at an individual.

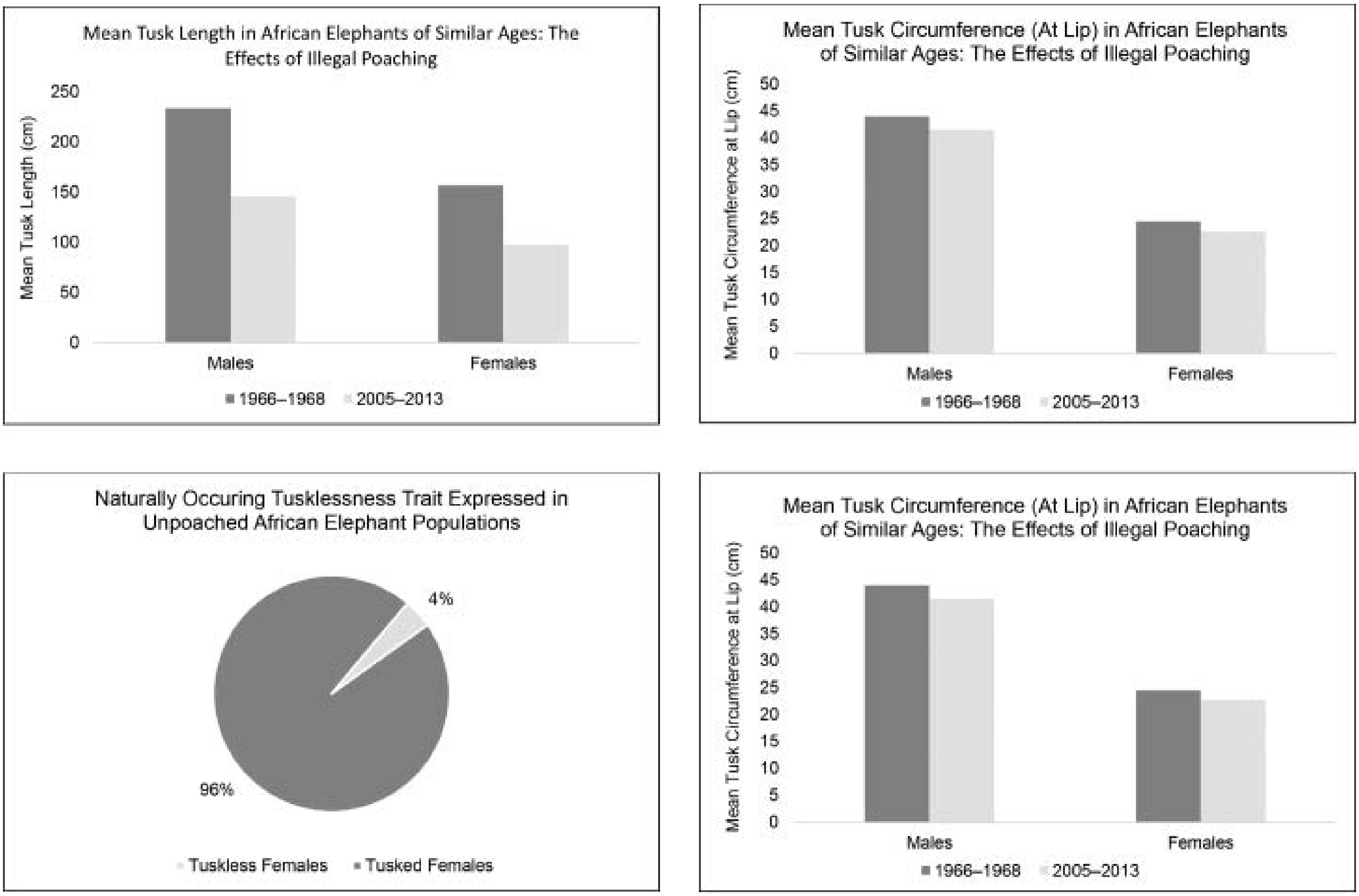

Figure 2. Trends in African Elephant Physiology: The Result of Poaching Pressure (adapted from Chiyo et al., 2015; Maron, 2018).

Note: At this stage, students should not be presented with any introductory material that describes behavioral or physiological changes that elephants have experienced because of poaching pressures. It is crucial to leave this unaddressed for students to first attempt to unpack on their own.

Step 3: Introduce the Phenomenon of Elephant Tusk Reduction

Pose two overarching questions to students: (a) What have been the historical impacts of poaching on the African elephant? and (b) How is poaching causing these changes? Use a projector or whiteboard. Provide each student group with the set of data presented in Figure 2 (adapted from Chiyo et al., 2015; Maron, 2018). Additionally, provide each student an "Initial Model Development" page (Figure 3) and ask students to record the overarching questions. The figures highlighted in this lesson are only examples. At the instructor's discretion, alternative data sets may also be useful in displaying long-term trends in African elephant physiology.

Present the following set of instructions:

1. Examine the data carefully. Interpret each piece of data individually, then as a group.
2. Discuss any conclusions that can be reached from each figure.
3. Work with your group to respond to the following prompts: (a) What have been the historical impacts of poaching on the African elephant? and (b) Explain how poaching is causing these changes.
4. Using the "Initial Model Development" page, sketch or describe your group's responses. Include as much detail as possible.

Step 4: Elicit Naïve Student Models

In our experience, students typically require 30–60 minutes to (a) make sense of the data, (b) discuss its implications, and (c) put forth a detailed explanatory model. Monitor student groups carefully by listening to discussions, interpretations, and conclusions to ensure that groups remain focused. Ask clarifying questions (Table 3) to each group, allowing students to speak freely without correction or input, utilizing neutral verbal and physical responses to increase student response rate. Make mental note of their explanations. It is not uncommon for students who are accustomed to a Teach-Practice-Apply classroom model to look to the instructor for approval, and some students may become visibly uncomfortable with the lack of a conclusive response. However, the objective at this stage is not to ensure total comprehension, but to provide students the opportunity to explore their own sensemaking. That students experience some discomfort with their own explanations will be useful in this activity.

With guidance, most groups will correctly ascertain that African elephant populations under heavy poaching pressures tend to experience an overall reduction in tusk size and circumference, and

Initial Model Development Page

Question:

Brainstorming: Fill this section with ideas and explanations. Use sketches, bulleted points, etc. to develop your model.

Explanation: Write a brief description of your model.

Supporting Evidence:

1.
2.
3.
4.

Figure 3. Student Model Development Page.

an increased expression of the tusklessness phenotype (no tusks at all). However, when prompted to explain this connection, student answers often fail to show the evolutionary connection between natural selection and gene expression. Some common student explanations include:

1. As poaching increases, African elephants grow smaller tusks to reduce their chances of being targeted by poachers.
2. As poaching increases, African elephants physically reduce or remove their tusks (typically through some form of "brushing" with rocks or trees) to avoid being targeted by poachers (Figure 4).
3. As poaching increases, African elephants choose to have offspring with smaller tusks to protect them from poachers.

Note how each explanation suggests an intentional avoidance strategy on the part of the elephants rather than the natural outcome of selective pressures. Students often draw on their own experiences when problem-solving and will similarly anthropomorphize animal responses. Even explanation 3, which appears to suggest an at least rudimentary understanding of natural selection, is not drawing a succinct connection between selective culling and reproductive success. Instead, like explanations 1 and 2, explanation 3 inaccurately implies an active form of decision-making on behalf of the animal. Students frequently reference the data or the elephants' desire to survive as supporting evidence despite there being no clear connection between this "evidence" and the explanation put forth in their model. They must be made skeptics of these more obvious logical flaws before proceeding into deeper conceptual ideas of

Table 3. Neutral Instructor Questioning to Elicit and Clarify Student Thinking.

• What patterns did your group discern from the data? • How might poaching have impacted elephant populations? • How could we explain these patterns scientifically? • What evidence do you have for your claims? • What might be some long-term impacts of poaching on elephant populations? • Why would elephant characteristics change in response to poaching?

evolutionary theory. Otherwise, they are at increased risk of false accommodation in which they attribute adaptation to anthropomorphized decision-making.

Step 5: Testing Model Resilience Through Classmate Scrutiny

Ask each student group to present their models to their classmates. As groups present, encourage other students to contribute questions or comments that either support or challenge their classmates' ideas. Carefully monitor participation for respectful and meaningful conversation. Student interactions should foster dialogue and elicit useful and insightful criticism so that individuals may begin to notice potential weaknesses in their proposals—planting seeds of doubt. For students to abandon their prior inaccurate notions surrounding evolutionary theory, they must, of their own accord, begin to doubt their legitimacy. To generate effective student dialogue, prior to beginning the activity, scaffold students toward appropriate interactions by asking questions such as

- How useful would it be for students who share ideas if I just responded with, "Your idea is bad"?
- What if I just said, "I like your idea"?
- What types of questions or comments might be more useful? Why?
- Why might we want to focus our comments on people's ideas and not on their character?

Continue to maintain impartiality during presentations, even if students note key flaws in their own models or those of their classmates. For example, it is not uncommon for students to question the cognitive capability of elephants to connect their tusk length to their risk of being poached when such an idea is presented. However, it is still critical at this stage to withhold approval of specific student ideas to continually foster a classroom environment where learners do not solely rely on the teacher as the sole source of information, but instead become comfortable with intellectual uncertainty. Record student observations and arguments on the whiteboard for later use. Ask students to meet with their groups again to make immediate adjustments to their explanatory models following classmate critique, advising them to be prepared for further reflection and revision as they proceed throughout the unit. With naïve sensemaking regarding evolutionary theory clarified for the student and made visible to the instructor, it is now appropriate to move students into more typical concrete experiences.

○ Leveraging Naïve Models in the Instructional Sequence

When generating naïve explanatory models for a complex phenomenon such as elephant tusk reduction, students will often inadvertently use what are vaguely defined and poorly constructed notions of evolutionary theory. When tested, even by their equally misinformed classmates, these models generally crumble due to inherent flaws in logic. Their weakness is an important tool for the classroom science teacher who, with careful mediation, can leverage the students' desire for understanding as a powerful weapon in combating misconceptions. With seeds of doubt priming students to abandon previously held notions of evolution, subsequent concrete experiences will be significantly more impactful as students have already begun the crucial process of integrating scientifically accurate models. The strategy of using naïve explanatory models preceding a concrete experience is not solely limited to evolutionary theory and can be applied to a variety of complex scientific topics such as the phases of the Moon, Earth's seasons, and states of matter. For these and other topics, the process should be as follows:

1. Introduce a scientific phenomenon with data.
2. Provide students the opportunity to generate naïve explanatory models.
3. Encourage classmate critique, and discussion.
4. Allow students to reconsider their initial models.
5. Begin concrete learning experiences.

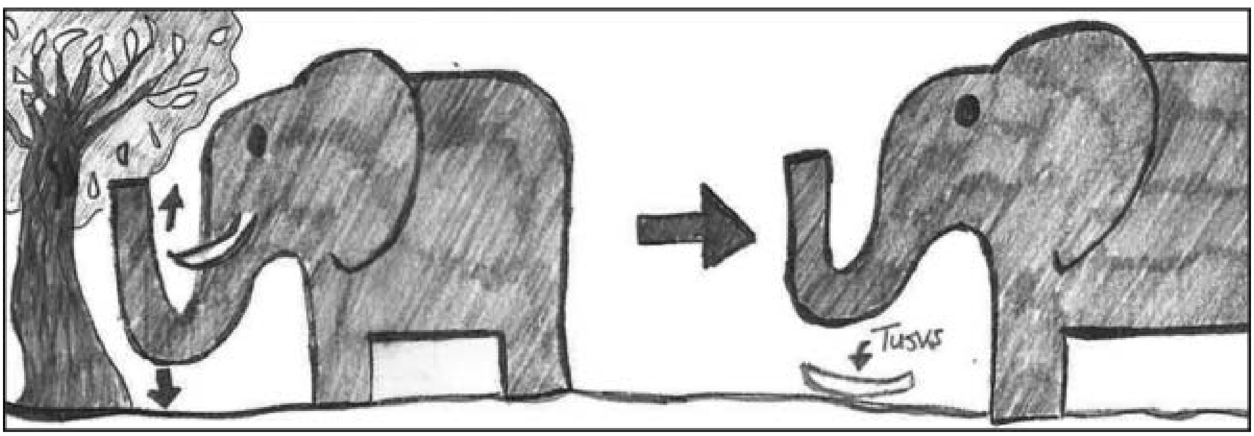

Figure 4. Sample Student Model Depicting Adult Elephants Removing Their Tusks Through "Brushing".

○ Conclusion

Revealing naïve student sensemaking surrounding evolutionary theory and other abstract science topics without significant educator interference requires attentive mediation, a well-established level of trust between the instructor and their students, and careful instructional planning. The strategy, although effective, is accompanied by some risk. If proper scaffolding and support are lacking, students may (a) adopt inaccurate models presented by their classmates, (b) solidify incorrect ideas, or (c) opt out of participation due to frustration, confusion, or fear of judgment. The teacher must be acutely aware of their students' capabilities, provide carefully tailored scaffolding, and monitor their classroom to avoid pitfalls (e.g., students attacking individuals rather than ideas). However, if done properly, students and teachers will find the strategy to be challenging, engaging, and useful in the elimination and replacement of misconceptions with scientifically accepted models.

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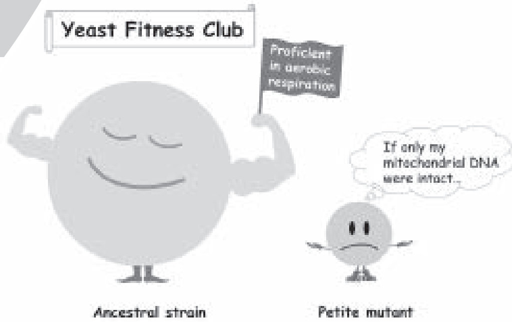
INQUIRY & INVESTIGATION

An Integrated Undergraduate Laboratory Exercise to Demonstrate Microbial Evolution: Petite Mutants in *Saccharomyces Cerevisiae*



RECOMMENDED FOR AP Biology

QIN QI, JEREMY A. C. STACEY, NUREENI WRIGHT, SASHA G. TETU, MICHAEL R. GILLINGS

Abstract

Understanding that evolution progresses through generation of DNA variants followed by selection is a key learning outcome for biology students. We designed an integrated and innovative undergraduate laboratory exercise using Saccharomyces cerevisiae to demonstrate these principles. Students perform in vitro experimental evolution by repeatedly propagating large or small yeast colonies on a weekly basis. Small-colony variants known as petites arise by mutations that disrupt aerobic respiration. To demonstrate the effects of increased mutation rates, half of the selection lines are exposed to ultraviolet irradiation. To understand how the petite phenotype arises, polymerase chain reaction (PCR) is performed to examine mitochondrial DNA, while biochemical assays are used to assess the ability of petites to undergo aerobic respiration. This exercise demonstrates evolution by artificial selection over a suitably short timeframe and links the results to a critical biochemical process: the role of mitochondria in aerobic respiration and ATP production. By implementing these experiments, we successfully demonstrated that the frequencies of petite mutants in evolved populations varied according to the selection pressure we applied, and that petite mutants carried deletions in mitochondrial DNA as anticipated. Through an integrated learning context, this practical exercise promotes fundamental understanding of evolutionary processes and fosters critical thinking skills.

Key Words: *Experimental evolution, Saccharomyces cerevisiae, Aerobic respiration, Mitochondrial DNA, Molecular microbiology*

○ Introduction

Saccharomyces cerevisiae is a useful eukaryotic model organism for demonstrating fundamental principles of various branches of biology (Botstein et al., 1997; Rinaldi, et al., 2010). In recent years, *S. cerevisiae* has been used as a versatile pedagogical resource for designing undergraduate laboratory courses that focus on experimental skills in molecular and evolutionary biology (Chan et al., 2021; Hageman & Krikken, 2018; Marshall, 2019; McDonnell et al., 2022; Ågren et al., 2017). We developed an integrated and highly customizable six- to seven-week practical exercise for undergraduates that capitalizes on the phenotypic plasticity and evolvability of *S. cerevisiae*.

Through this integrated learning context, we hope to promote critical thinking skills and encourage students to reflect on the ways in which various assays complement each other...

In both naturally evolving and mutagen-treated populations of *S. cerevisiae*, small-colony variants (SCVs) of *S. cerevisiae* frequently arise. They have slower growth rates and significantly smaller colony sizes compared with those of their ancestral strains (Garcia et al., 2019; Hess et al., 2009; Osman, et al., 2015). Petite mutants are SCVs that cannot undergo aerobic respiration and must rely on anaerobic respiration as their only source of ATP (Lipinski et al., 2010; Ogur et al., 1957; Powell et al., 2000). Cytoplasmic petites can be broadly classified as *rho*$^-$ mutants that carry deleterious mutations in their mitochondrial DNA (mtDNA) and *rho*^{0} mutants that have completely lost their mtDNA. Nuclear petites (*rho*$^+$) arise due to deleterious mutations in chromosomal genes that cause defects in oxidative phosphorylation. (Ferguson & von Borstel, 1992; Rinaldi et al., 2010) Due to the relevance of petites to fundamental questions in cell biology, research on *S. cerevisiae* petites has been carried out since the 1950s (Gibson et al., 2008; Ogur et al., 1957).

Petites also have practical implications on the biomanufacturing industry. In the brewing industry for example, the accumulation of petite mutants in recycled yeast populations has negative impacts on fermentation, flocculation, and flavor components (Gibson et al., 2008; Lodolo et al., 2008).

Our experimental design was inspired by an integrated pedagogical approach that advocates providing learners an environment through which they can make connections between different concepts across disciplines (Basu et al., 2017; D'Souza et al., 2016). Using this laboratory exercise as a platform, we hope to provide undergraduates a more holistic learning experience by bridging the gap between key concepts in molecular, evolutionary, and cell biology, which are typically taught as independent academic modules at the undergraduate level.

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The core component of this laboratory exercise involves students performing in vitro experimental evolution with the aim of isolating petite mutants. An ancestral population of *S. cerevisiae* BY4741 is repeatedly bottlenecked and passaged on yeast-peptone-dextrose (YPD) nutrient agar on a weekly basis (Brachmann et al., 1998). Variation in colony sizes allows experimental selection based on the two distinct colony sizes (small or large) to be applied. In half of the independently evolving lineages, only the smallest colonies are repeatedly passaged, which allowed the SCV phenotype to be selected for. In the other half of the lineages, only the largest colonies are repeatedly propagated to select against the SCV phenotype.

Exposing yeast to a range of mutagens favors the evolution of petite mutants (Ferguson & von Borstel, 1992; Goldring et al., 1971). To explore the effects of ultraviolet irradiation (UV) on the evolution of petites and the experimental outcomes, half of the experimental lineages in this exercise are irradiated with UV to increase mutation rate. Students receive training in PCR to detect cytoplasmic petites that have lost all or part of their mtDNA. Various phenotypic assays are performed to assess whether the evolved lineages are bona fide petites that are deficient in aerobic respiration. The exercise presented in this work adds to the growing literature of modern undergraduate-level experimental courses that focus on the roles of mutations in yeast (Marshall, 2019; McDonnell et al., 2022; *Ågren et al.,* 2017). Through this integrated learning context, we hope to promote critical thinking skills and encourage students to reflect on the ways in which various assays complement each other in terms of linking phenotypic traits of petite mutants to their genotypes.

○ Learning Objectives

The learning objectives can be customized according to the curriculum requirements of the teaching institution. In the core component on experimental evolution, it is recommended that all students acquire technical competence in the following skill areas:

- Effectively use aseptic techniques that are applicable across general microbiology to minimize microbial contamination.
- Perform serial dilution on microbial cell suspensions in liquid nutrient medium.
- Perform spread-plating on nutrient growth agar for cultivation of microbes.
- Perform streak dilution of microbes on designated nutrient growth agars.
- Collect and plot quantitative data on yeast colony numbers.
- Perform PCR using standard molecular biology reagents and oligonucleotide primers.
- Perform gel electrophoresis of PCR products on an agarose gel and interpret experimental results.
- Understand the importance of including appropriate positive and negative control reactions when designing PCR experiments.

Upon successful completion of the exercise, students are expected to demonstrate conceptual understanding of the following topics at the interface between evolutionary, molecular, and basic cell biology:

- Explain the concept of a population bottleneck and its roles in experimental evolution and natural selection.
- Outline the main processes of UV-induced DNA damage and its effects on mutation rate.
- Explain the concepts of deleterious mutations and compensatory adaptation.
- Describe the role of glycolysis in producing pyruvate that is required for both aerobic and anaerobic respiration.
- Understand the roles of mitochondria in generating energy via aerobic respiration.
- Outline the role of oxidative phosphorylation in ATP production.
- Explain why anaerobic respiration is less efficient than aerobic respiration in terms of energy production.

○ Methods

Experimental Evolution Assays

Students work in pairs throughout the experiments. Figure 1 provides an overview of the main procedures for the experimental evolution core component. Comprehensive protocols that are suitable for student use and an accompanying "Notes for Demonstrators" can be found in the Supplementary Materials provided with the online version of this article. During the Week 1 session, each pair of students pick a very small-colony and a very large-colony of *S. cerevisiae* BY4741 from a YPD nutrient agar plate for resuspension in YPD liquid medium and serial dilution according to the schematics in Figure 2 (Brachmann et al., 1998). Diluted cell suspensions of each colony size are spread plated onto YPD nutrient agar plates. Plates are incubated at room temperature for one week. In the Week 2 session, each colony size is passaged in duplicate sets on fresh YPD plates. One of the two sets for each colony size is irradiated with ultraviolet (UV) light of 254 nm wavelength for 25 seconds to increase mutation rate in these lineages.

In Weeks 3 and 4, the passaging procedure for the four independently evolving lineages corresponding to four different selection pressures is repeated. Each week, the number of SCVs are counted and calculated as a percentage of all the colonies for each of the four lineages. The frequencies of SCVs in the small-colony lineages are expected to increase over time. In contrast, the frequencies of SCVs are expected to decrease in the large-colony lineages, as SCVs in these lineages are repeatedly selected against. UV-irradiated lineages are expected to show higher rates of phenotypic evolution due to the activation of low-fidelity translesion synthesis (TLS) DNA polymerases (Guo et al., 2001; Lawrence & Christensen, 1976). In Week 5, quantitative data on the frequencies of SCVs across all available time points are pooled for a class-based data analysis exercise.

Phenotypic Characterization of Petite Mutants

To test whether the evolved SCVs are bona fide petites in terms of their deficiencies in aerobic respiration, resuspended endpoint colonies from each evolved lineage are streaked on yeast-peptone-glycerol (YPG) and yeast-peptone-ethanol (YPE) plates during the Week 5 teaching session. Petites cannot grow on media that only contain nonfermentable carbon sources such as glycerol and ethanol (Lipinski et al., 2010). During these procedures, students are shown how to perform streak dilution using inoculation loops.

A complementary phenotypic assay involves staining colonies using the 2,3,5-triphenyl tetrazolium chloride redox indicator. Yeast colonies that can perform aerobic respiration are stained dark

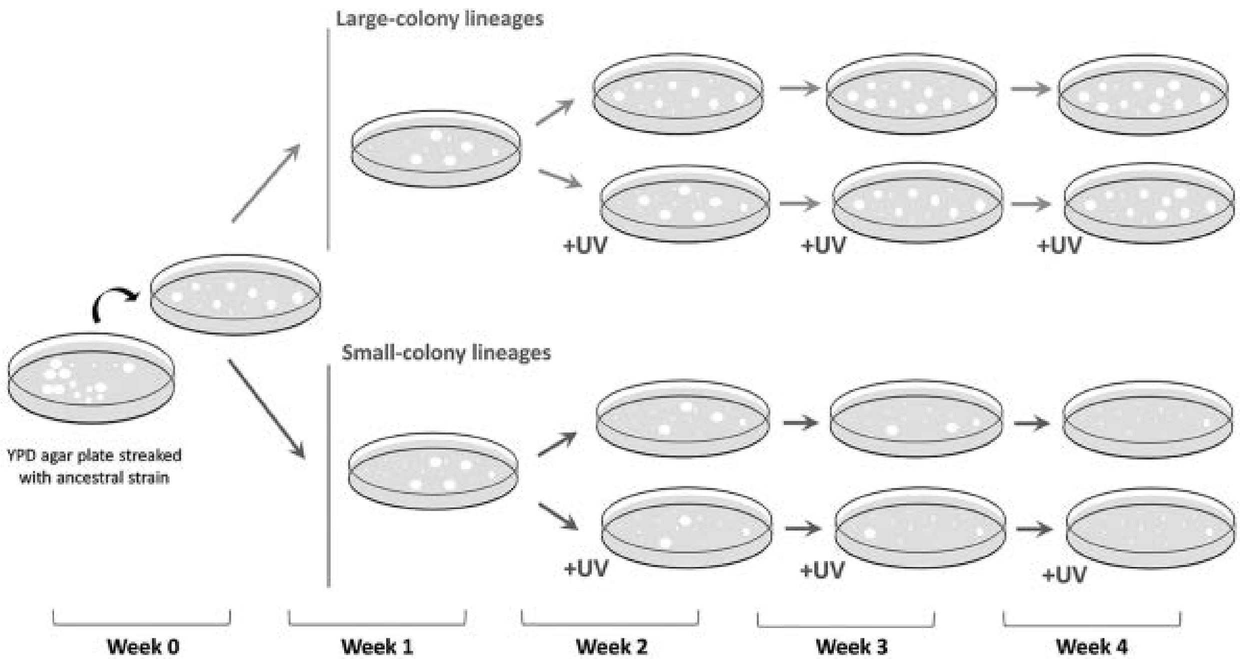

Figure 1. Overview of the procedures for experimental evolution of petite mutants of *S. cerevisiae* during the weekly teaching sessions in Weeks 1–4.

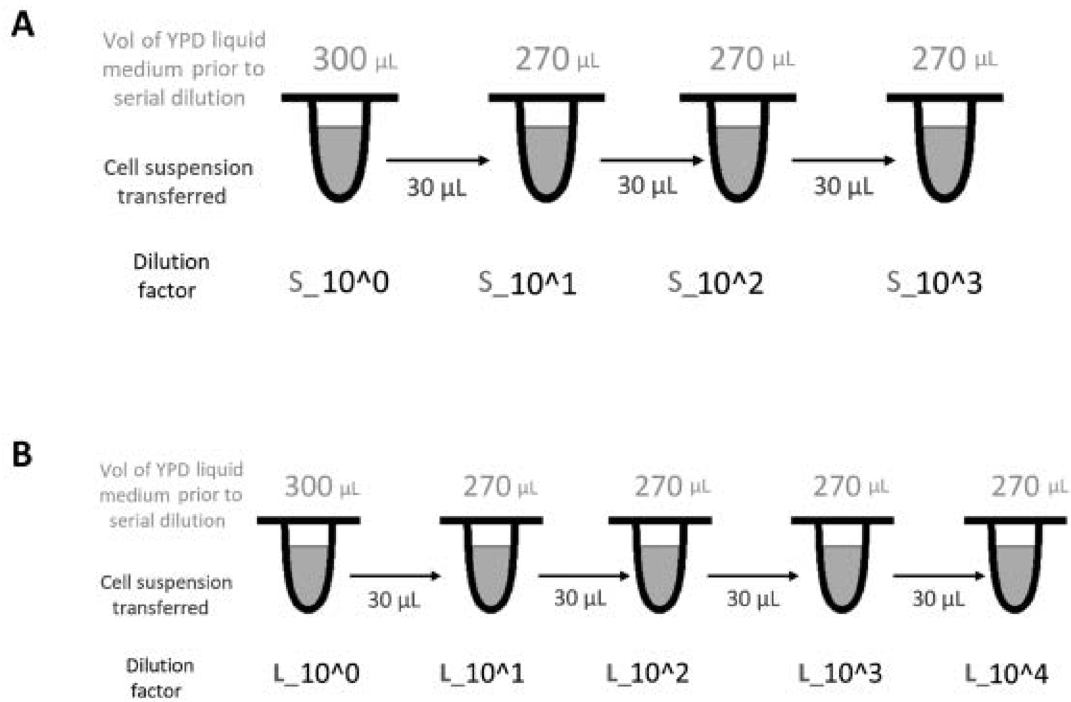

Figure 2. Recommended procedures for the serial dilution of resuspended large or small colonies in YPD liquid medium prior to spread-plating on YPD agar plates. Abbreviations: S = small-colony lineages; L = large-colony lineages.

red when a soft agar containing tetrazolium chloride is poured over the colonies. The color change is based on reduction of the tetrazolium salt by active mitochondrial dehydrogenases into formazan. Colonies that are deficient in aerobic respiration due to nuclear or mitochondrial mutations are unstained (Hess et al., 2009; Ogur et al., 1957). Using this technique, evolved endpoint colonies on YPD plates are screened for their ability to perform aerobic respiration during the teaching session in Week 5.

Most endpoint colonies from evolved small-colony lineages are expected to be bona fide petites that are unstained by tetrazolium chloride. The tetrazolium chloride overlay technique also distinguishes petite mutants from other non-petite SCVs, thus allowing petites to be more accurately identified. Detailed experimental protocols for all three phenotypic assays can be found in the "Notes for Demonstrators" in the Supplementary Materials provided with the online version of this article. The ancestral BY4741 strain should be included as a control strain in all the phenotypic assays.

Polymerase Chain Reaction

Students perform PCR to identify petites that have most likely lost their mtDNA. During the teaching session in Week 6, boiled lysates of glycerol stocks from yeast colonies on Weeks 2 and 4 YPD plates are used as templates for PCR-based detection of two mitochondrial genes (*ATP9* and *COX3*) and a chromosomal gene *ACT1* as positive control (Dirick et al., 2014; Osman et al., 2015).

The loss of *ATP9* greatly increases mtDNA instability and the likelihood of mtDNA loss to form *rho*^{0} petite mutants (Bietenhader et al., 2012). Due to the possibility that some cytoplasmic petites could be *rho*$^-$ mutants that have fixed large deletions in their mtDNA, it is essential to target at least two mitochondrial genes that are spaced far apart in mtDNA, such as *ATP9* and *COX3* (see Figure S4 in the Supplementary Materials provided with the online version of this article) (Osman et al., 2015). The oligonucleotide sequences of the three primers are summarized in Table S2 (see Supplementary Materials provided with the online version of this article). Boiled lysate of the ancestral BY4741 strain should be added to the positive control reactions as template, while negative control reactions without template should also be included.

Subsequently, all the PCR products are analyzed by gel electrophoresis. In all the sample groups, 100 bp bands corresponding to the chromosomal *ACT1* gene fragment should be detected if alkaline lysis of yeast glycerol stocks was successfully carried out. For *rho*^{0} cytoplasmic petites without mtDNA, neither of the two mitochondrial genes will be amplified. Detection of only one out of the two tested mitochondrial genes suggests that the undetected gene was affected by deletion(s) in mtDNA. Demonstrators should emphasize that this PCR test has an inherent limitation in that it can identify neither *rho*$^+$ nuclear petites nor *rho*$^-$ cytoplasmic petites with mutations such as single nucleotide polymorphisms (SNPs) and small insertions and deletions (INDELs) that affect only a few nucleotides in mtDNA.

As a supplementary experiment to confirm that evolved Δ*ATP9* Δ*COX3* mutants are *rho*^{0} petites rather than *rho*$^-$ petites with large deletions in their mtDNA, Δ*ATP9* Δ*COX3* petites can be treated with 4',6-diamidino-2-phenylindole (DAPI) nuclear stain and imaged on a fluorescent microscope according to previously described experimental procedures (Amberg, et al., 2006; Dirick et al., 2014; Williamson & Fennell, 1979). Given the technical complexity of fluorescence microscopy procedures, we strongly recommend that this experiment should be performed by the demonstrators. Students can observe the processes of sample preparation, image requisition, and image analysis under guidance. As possible further extensions to this laboratory exercise, optional higher-level activities, such as Nanopore long-read sequencing of genomic DNA and mtDNA extracted from selected petite mutants, can be performed by technical staff.

○ Implementation and Results

This laboratory exercise was trialed and implemented by undergraduates in Biological Sciences under the laboratory guidance of a postdoctoral researcher at Macquarie University in 2022. The experimental evolution assay was performed in three independent lineages (n = 3) for each of the four selection pressures (large or small-colony lineages with or without UV-treatment), resulting in 12 evolved lineages in total. SCVs with distinctively small-colony sizes were observed since Week 1 of the experiment (Figure 3A), which allowed small-colony variant lineages to be established at the start of the experiment as planned.

None of the endpoint populations from the small-colony lineages were able to grow on YPG agar and YPE agar plates, which suggests that yeast cells in those lineages had lost their ability to undergo aerobic respiration. We then performed tetrazolium chloride staining of the endpoint populations. Almost all the colonies of the *S. cerevisiae* BY4741 founding strain and the endpoint colonies of a representative large-colony lineage (L2) stained dark red (Figures 3B and 3C). In contrast, the endpoint colonies in a representative small-colony lineage (S2) were SCVs that were mostly unstained by tetrazolium chloride (Figure 3D).

In Weeks 3, 4, and 5, the frequencies of SCVs on YPD plates from the previous week were quantified and calculated as percentages of all the counted colonies on the same plates for each of the four selection pressures. The average frequency of SCVs in the small-colony lineages (n = 3) increased over time, which is consistent with the principle that sustained artificial selection leads to fixation of the selected trait (Figure 4A). The frequencies of SCVs decreased in the large-colony lineages (n = 3) over time because the SCV phenotype was repeatedly selected against (Figure 4B). By the end of the experiment, the average frequency of SCVs in the UV-irradiated small-colony lineages (n = 3) were significantly higher than that in the non-irradiated small-colony lineages (unpaired two-sample t-test: t = 3.31, d.f. = 4, p = .0297). This is consistent with our expectation that the rate of phenotypic evolution increases with higher mutation rate in the UV-irradiated lineages. The continued presence of non-SCV colonies in the small-colony lineages that are deficient in aerobic respiration could potentially be due to compensatory adaptation that ameliorates the fitness defects of petites (Garcia et al., 2019).

Our PCR results showed that loss of mtDNA had most probably occurred in four out of six small-colony lineages (Table 1) because neither *COX3* nor *ATP9* can be detected in these lineages. Interestingly, only *COX3*, but not *ATP9*, was detected in the remaining two UV-irradiated small-colony lineages. This was most likely due to deletion mutations that resulted in the loss of *ATP9*, but not *COX3*, thus resulting in *rho*$^-$ cytoplasmic petites. In contrast, all the large-colony lineages retained their mtDNA as expected. In terms of the general trend for all the three pairs of primers we tested, the PCR results for Week 4 colonies were identical to those from Week 2 (Table 1). In the positive control reactions, the chromosomal *ACT1* gene was detected in all the samples with the exception of the no-template negative controls. This suggests that alkaline lysis of yeast cells and PCR were both correctly performed. Taken together, these results corroborate our phenotypic results for the evolved lineages and showed that parallel evolution can be observed in the three independent lineages for the four selection pressures.

To help demonstrators assess whether students have acquired the theoretical concepts that underpin this laboratory exercise, we compiled two sets of assignment questions that focus on laboratory general knowledge—molecular and evolutionary biology—basic aspects of cellular microbiology (see Supplementary Materials provided with the online version of this article). A standardized set of grading criteria and sample answers are available upon request.

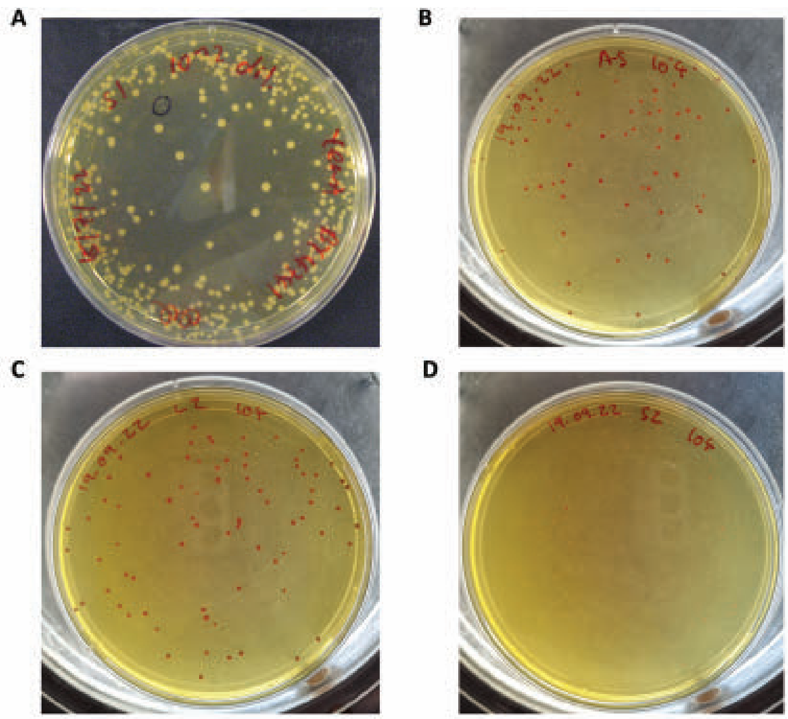

Figure 3. Phenotypes of *S. cerevisiae* BY4741 colonies on YPD agar plates. **(A)** SCVs with distinctively small-colony sizes were observed from Week 1 of the experiment, an example of which is circled in black. **(B)** Almost all the colonies of the *S. cerevisiae* BY4741 ancestral strain were stained dark red by the tetrazolium chloride overlay method, which demonstrates their ability to undergo aerobic respiration. **(C)** Similarly, most endpoint colonies of a representative large-colony lineage (L2) were stained dark red by tetrazolium chloride. **(D)** The endpoint colonies of a representative small-colony lineage (S2) showed the characteristic SCV phenotype and were mostly unstained by tetrazolium chloride.

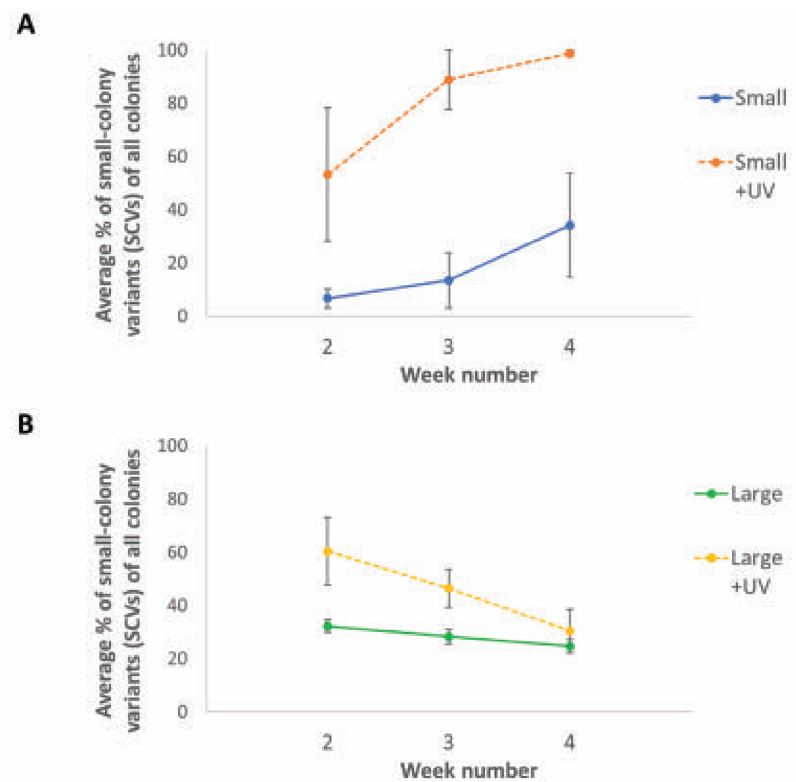

Figure 4. Average frequencies ($n = 3$) of SCVs expressed as percentages of all counted colonies in **(A)** small-colony lineages with and without UV-treatment and in **(B)** large-colony lineages with and without UV-treatment. The lines representing UV-irradiated lineages are shown as dotted lines. The average frequency of SCVs in the UV-irradiated small-colony lineages were significantly higher than that in the non-irradiated small-colony lineages (unpaired two-sample *t*-test: $t = 3.31$, d.f. $= 4$, $p = .0297$).

Table 1. PCR results for small- and large-colony evolved lineages. "+" in the table indicates detection of the respective PCR products of the correct band sizes. The "-" sign indicates their absence.

Week	mtDNA or chromosomal DNA target	Gene	Small-colony lineages						Large-colony lineages					
			No UV			With UV			No UV			With UV		
			S1	S2	S3	S1_UV	S2_UV	S3_UV	L1	L2	L3	L1_UV	L2_UV	L3_UV
2	mtDNA	*COX3*	-	-	-	-	+	+	+	+	+	+	+	+
		ATP9	-	-	-	-	-	-	+	+	+	+	+	+
	Chromosomal	*ACT1*	+	+	+	+	+	+	+	+	+	+	+	+
4	mtDNA	*COX3*	-	-	-	-	+	+	+	+	+	+	+	+
		ATP9	-	-	-	-	-	-	+	+	+	+	+	+
	Chromosomal	*ACT1*	+	+	+	+	+	+	+	+	+	+	+	+

○ Precautions and Safety

Detailed safety assessments must be performed prior to the start of the practical class in accordance with the health and safety regulations of the teaching institutions. All students require hands-on training from demonstrators on the safe use of Bunsen burners to create an aseptic working environment while completing microbiological procedures. Tetrazolium chloride is a chemical irritant that can cause skin and eye damage or corrosion. It may also cause respiratory irritation if accidentally inhaled. Therefore, it is recommended that demonstrators should prepare tetrazolium chloride stock solutions from powder in designated chemical hoods in advance. During the gel electrophoresis experiment, students need to wear disposable safety gloves when working with pre-stained agarose gels that contain any DNA-binding dye.

○ Acknowledgments

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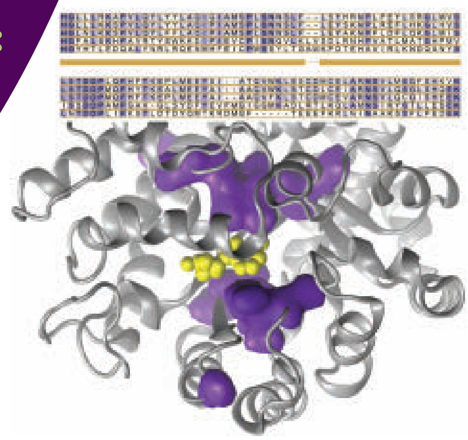
TIPS, TRICKS & TECHNIQUES

A Tool to Teach Evolution of Protein Sequences and Structures: Prediction of Protein Structure by Building Homology Models



AGNIESZKA SZARECKA,
CHRISTOPHER DOBSON

Abstract

Computer modeling and protein structure visualization tools are effective and engaging ways of presenting various molecular biology concepts to high school and college students. Here, we describe a series of activities and exercises that use online bioinformatics databases and programs to search for and obtain protein sequence and structure data and use it to build homology models of proteins. Exercises in homology modeling can serve the pedagogical purpose of introducing and illustrating the concept of homology within gene and protein families, which results in conservation of the 3D structures of proteins and allows us to predict structures when experimental data are not available.

Key Words: *Bioinformatic databases, Protein structure prediction, Homology modeling*

○ Teaching Evolutionary Relationships Among Proteins

Introduction of the concept of homology is critical to teaching evolution. On a molecular level, homology (i.e. the evolutionary relationship between two or more gene/protein sequences) is reflected by measurable similarities in their sequences and structures. Homologous proteins form families and have similar sequences. Namely, many of the amino acids in their sequences are identical or similar (in chemical character), and conserved motifs (segments of amino acids that are present in all the related proteins) can be revealed by sequence alignments. Protein structures, within families, tend to be even better conserved than the corresponding sequences. This conservation of 3D structure among homologs (proteins that share a common ancestor) constitutes the basis for homology (or comparative) modeling, one of the most commonly used and most successful methods of predicting protein structure. Homology modeling predicts a structure for a protein with known sequence but unknown structure (target) if the sequence and structure for a close homolog (template) are known. The main steps of homology modeling are finding template structure(s), alignment of target and template sequences, building the 3D structure for the target, loop and side chain modeling, optimization, and evaluation. Most of the current bioinformatic resources for homology modeling integrate and streamline these steps (Hameduh et al., 2020; Hati & Bhattacharyya, 2016). Homology modeling has helped bridge the gap between the large amount of sequence data and limited structure data currently available to us. It facilitated studies of protein dynamics or mutations (e.g., Ikemura et al., 2019). It has allowed for structure-based drug design for proteins that were/are challenging to structural biology methods such as X-ray crystallography or Nuclear Magnetic Resonance (NMR) (Leelananda & Lindert, 2016). In the classroom, homology modeling is a relatively easy, inexpensive, and engaging experience with protein modeling tools that illustrates vital concepts in molecular biology and genetics. Herein we describe a lesson plan, in which students (1) learn about a selected protein family (their cellular functions and implications to human health), (2) identify one member of this family for which structure is not available, (3) use bioinformatic databases to obtain the sequence of this protein, (4) use a homology modeling program online to predict the structure of this protein, and (5) use a visualization software to analyze the predicted model.

○ Choosing the Protein Family

The first step to implement this activity in the classroom setting (or as a project) is to decide which protein or domain family to use, taking into account the protein domain's function, size, availability of structural data, and availability of both ortholog and paralog sequences. Orthologs and paralogs are two types of homologs; the former are present in different species, while the latter arise within a single species through gene duplication (Koonin, 2005). Sequence or structure data can be obtained from public databases, such as UniProt (https://www.uniprot.org/) and Protein Data Bank (PDB; https://www.rcsb.org/). We have described using PDB in lesson plans in our previous paper (Szarecka & Dobson, 2019). Homology modeling programs are available online and free of charge as well, but many servers impose limitations on the size of the proteins to model. We would recommend choosing sequences of up to 1000 amino acids for this activity. Depending on the protein

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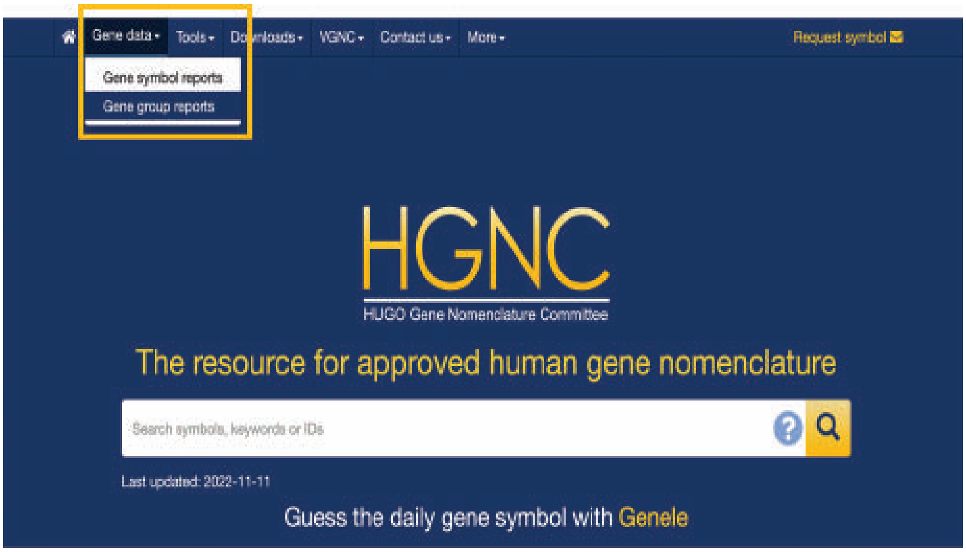


Figure 1. HGNC database homepage. Search can be conducted directly or more easily, using the *Gene group reports* tab.

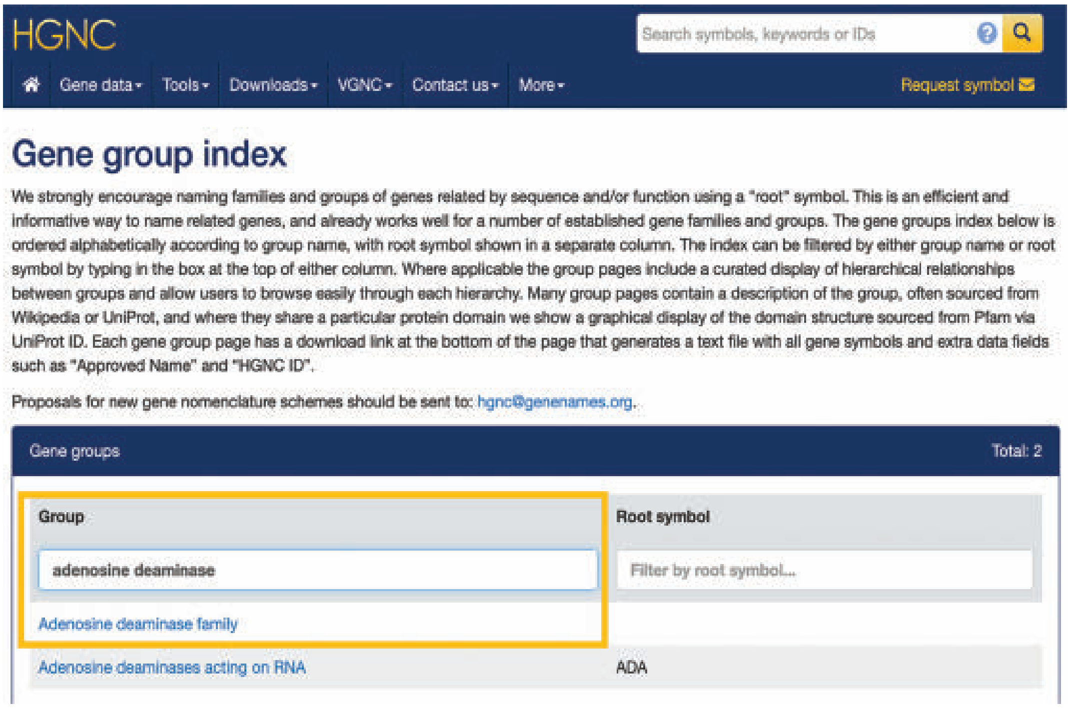


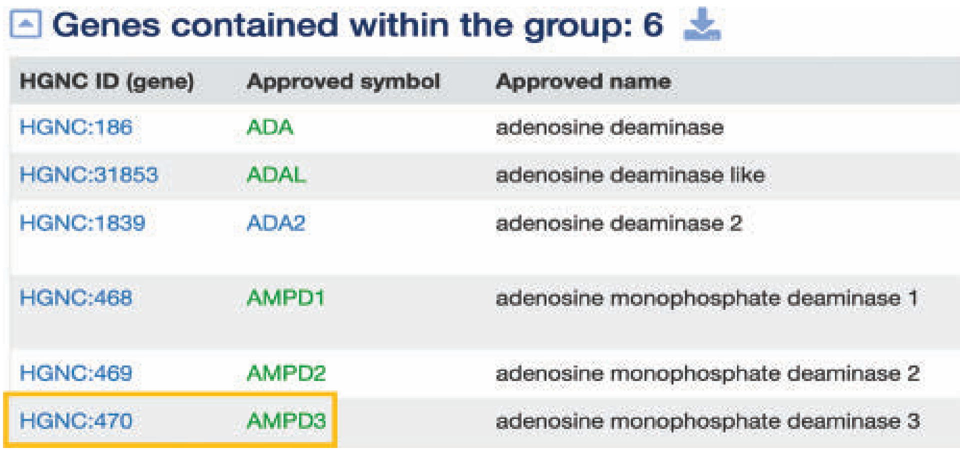
Figure 2. HGNC database, searching the *Gene group reports.*

sequence selected and the computational capacity of the server, the results will be typically returned within a few days, thus the activity could be implemented as two class periods or one class period and homework.

Here we suggest using the adenosine deaminase family. Interesting connections to other information presented in class could include enzymes, metabolism, purine nucleotide cycle, expression of different paralogs in different tissues, and a variety of mutations causing a broad range of disorders. For example, mutations in ADA genes are involved in immunodeficiency disorders, while those in AMPD genes affect muscle function and neurodevelopment. More information on ADA/AMPD mutations and related disorders can be found in UniProt records P00813 and P23109 (links and screenshots are provided in the Supplemental Material provided with the online version of this article), and also in (Whitmore & Gaspar, 2016) and (Hayes et al., 2013). The UniProt records provide a list of sequence variants that will be very interesting and convenient to discuss with students.

The family members can be found in the HGNC database (Figures 1 & 2; https://www.genenames.org/).

Of the six family members, AMPD1, AMPD3, and ADAL are good candidates for homology modeling as the experimental structures are not available (Figure 3). Selecting an HGNC ID link for a gene provides valuable information and links to other databases, for example access to sequences, links to PDB (to find experimental structures if available), and to OMIM (to find information on genetic disorders and mutations linked to a particular gene).



Genes contained within the group: 6

HGNC ID (gene)	Approved symbol	Approved name
HGNC:186	ADA	adenosine deaminase
HGNC:31853	ADAL	adenosine deaminase like
HGNC:1839	ADA2	adenosine deaminase 2
HGNC:468	AMPD1	adenosine monophosphate deaminase 1
HGNC:469	AMPD2	adenosine monophosphate deaminase 2
HGNC:470	AMPD3	adenosine monophosphate deaminase 3

Figure 3. HGNC database. Adenosine deaminase gene family.

Teacher or students can also choose other protein families depending on their interests and connections to other parts of the class material. Good candidates may, for example, include serpins, histone deacetylases, and hydroxysteroid 17-beta dehydrogenases. For ideas and inspiration, we recommend PDB Molecule of the Month collection, browsing through the *Gene group reports* or through OMIM database (https://www.ncbi.nlm.nih.gov/omim) to look for proteins involved in genetic disorders. Specific protein sequences can be found through a search of the UniProt database by the gene or protein name. UniProt also provides information on whether a given sequence is represented by a structure in the PDB.

○ How to Obtain Protein Sequences for Modeling

The easiest way to find and download a protein sequence is through a search of the UniProt database. In this lesson plan, students can find the sequences either by a direct search of UniProt (Figure 4) or through a link provided in HGNC (by clicking on AMPD3 link shown in Figure 3 and scrolling down for a link to UniProt). The UniProt accession number for AMPD3 is Q01432 (Figure 4, box 5). The sequence is obtained by scrolling down the UniProt entry to the protein sequence segment and downloading the sequence in the FASTA format (Figure 5).

An interesting addition to this part of the lesson would be for students to select the family members' sequences (by checking the boxes on the left, by each entry, Figure 4, box 4) and perform a sequence alignment (Figure 4, box 1). Sequence alignment will reveal the similarities among the sequences within the family, conserved amino acids, and family motifs, as well as differences that occurred during evolution of various paralogs (an example of an alignment is shown in the Supplemental Material included with the online version of this article).

○ Building Homology Models

There are several servers that can be used to build homology models online free of charge. The Supplemental Material provided with the online version of this article includes a list of resources for homology model building and assessment. Here we suggest using Swiss-Model (Waterhouse et al., 2018) and/or I-TASSER (Yang & Zhang, 2015). Swiss-Model requires three steps: input of a protein sequence to model, search for templates, and building models (Figure 6). It is advisable that students enter their email addresses to receive notifications from the server as well as bookmark the runs if they need to step away from the project.

This step is critical in the modeling process, and here also—pedagogically. Prediction of a protein structure through homology modeling hinges on availability of a homolog protein whose structure is available in the PDB (template). Successful (i.e., with reasonable accuracy) prediction of protein structure depends on this structural homolog to have as high sequence identity to the target as possible and as high quality structure as possible. Sequence identity is a measure of similarity between two sequences; it is defined as percent of identical amino acids when two sequences are aligned. Sequence similarity is a related term, defined as the combined percent of identical and similar (in physico-chemical properties, such as polarity or size) amino acids. The Supplemental Material provided with the online version of this article shows an example of sequence alignment with identical/similar amino acids marked.

The threshold for sequence identity between two sequences (of lengths >100 aa) to consider the two proteins to be homologs is typically 30%. The accuracy of structure modeling increases as the sequence identity goes up (Hati & Bhattacharyya, 2016). For a scientist modeling a protein structure, examining the available templates, target-template sequence alignments, and "coverage" (does the template sequence and structure cover all or most of the target?) is vital. Swiss-Model searches for templates and provides the user with a summary of available templates and their suitability for modeling (Figure 7). Students can scroll through the results and note what sequences and structures have been identified and note the optimal template(s). They can also create various models and compare their structures and quality assessments. Toward the overarching goal of this activity, we would recommend discussing the following aspects: (a) evolution of protein sequences produces multiple lineages; homology is recognizable by a sufficient sequence identity among them and the presence of conserved residues and motifs even if other segments of the sequences diverged; and (b) while homologous proteins will have the same fold type and show similar secondary and tertiary structures, the accuracy of homology modeling depends critically on the level of sequence similarity (in our case ~50%).

Predicted models can be viewed and downloaded from the server as shown in Figure 8.

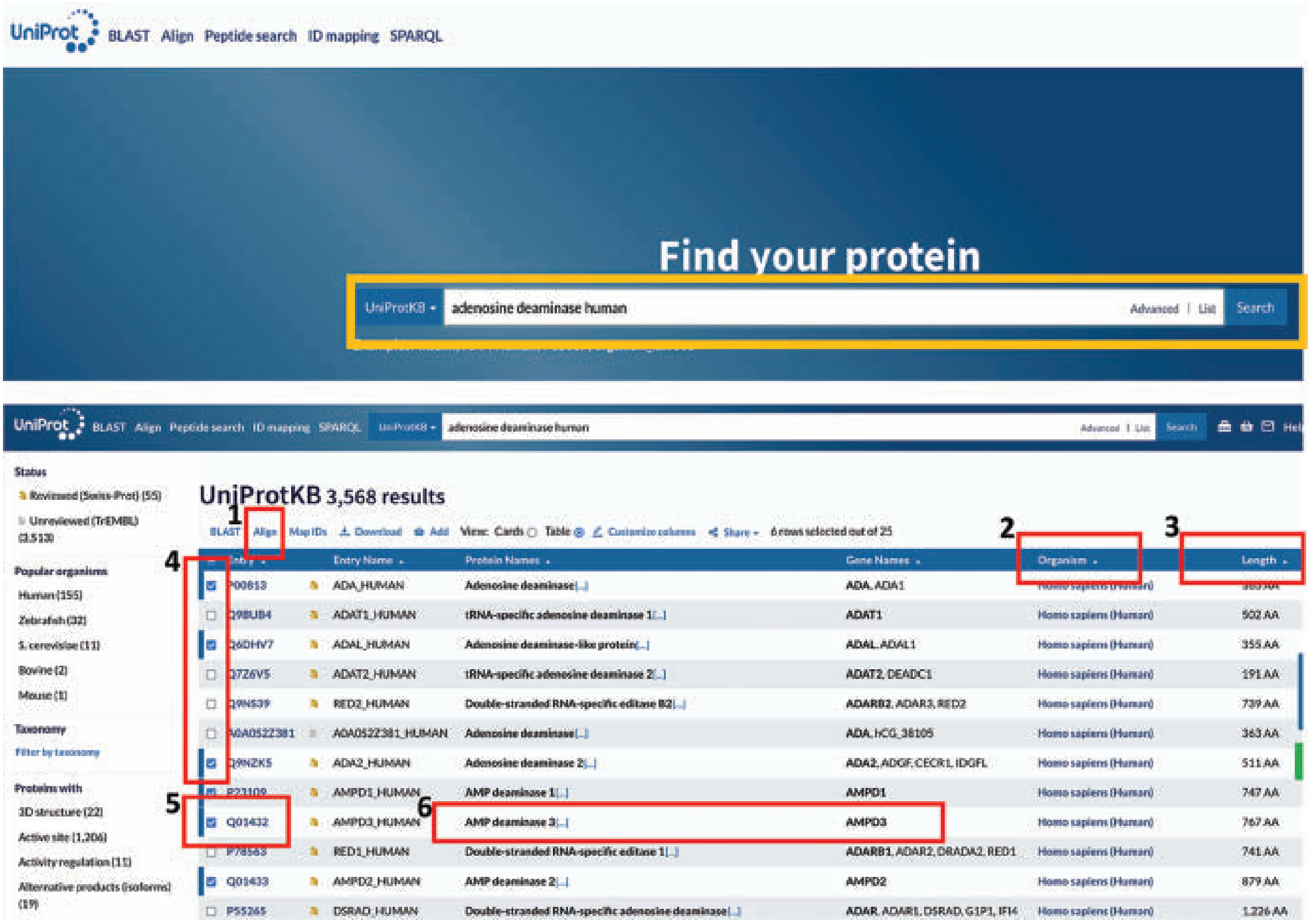


Figure 4. Searching UniProt Database. Results table shows the sequence accession number, e.g., Q01432 (box 5), protein name (box 6), organism and sequence length (boxes 2–3). Sequences can be selected (box 4) and aligned (box 1). An example of alignment is shown in the Supplemental Material included with the online version of this article.

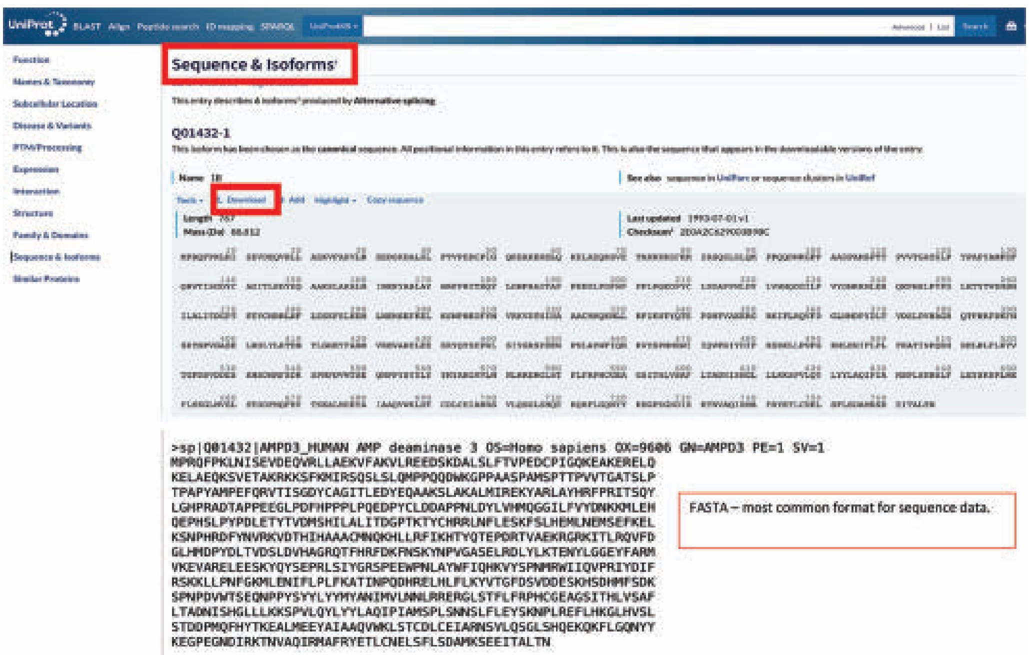


Figure 5. Obtaining protein sequence from a UniProt database entry file Q01432. "Sequence & Isoforms" segment of the file allows the user to download the sequence in the FASTA format (bottom panel) that can be copied and pasted in as input into bioinformatics programs.

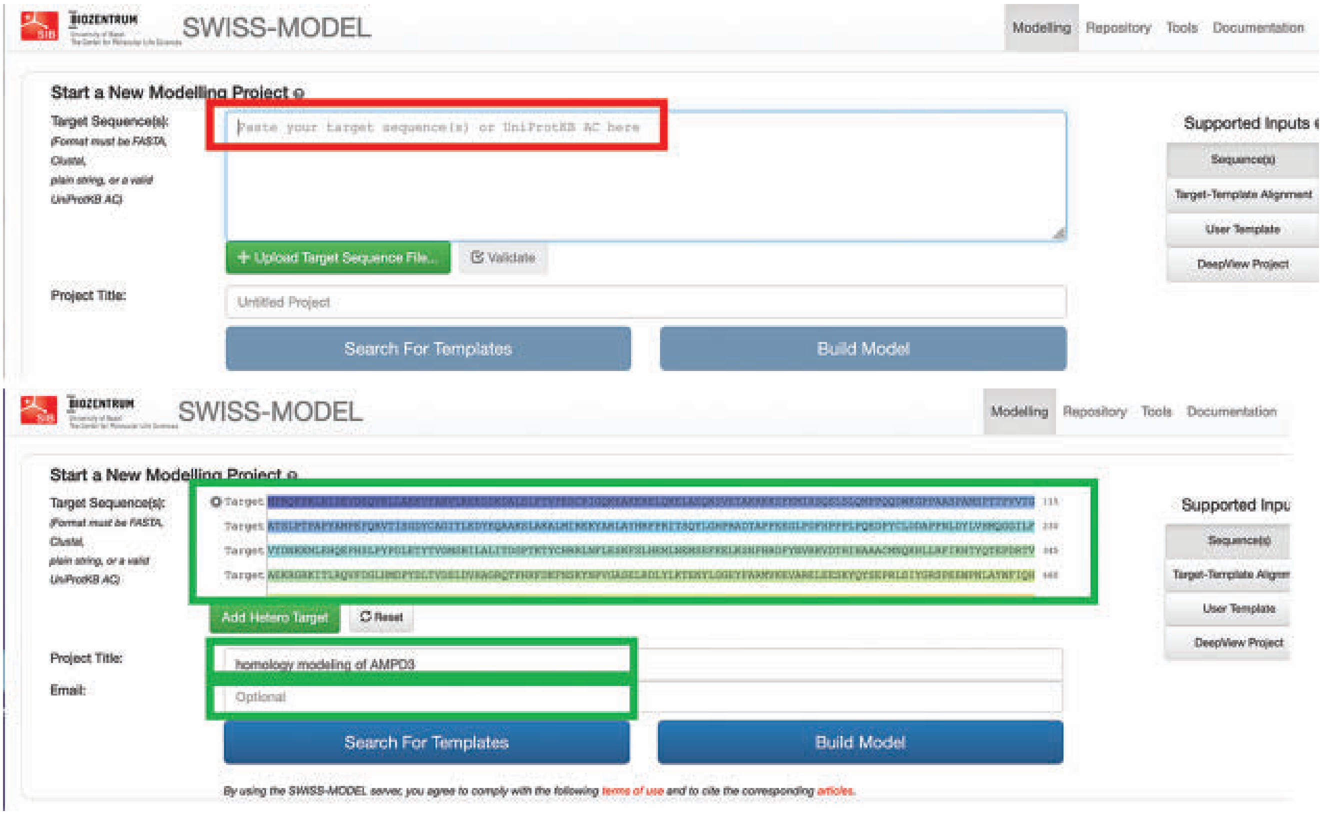


Figure 6. Setting up a homology modeling run for human AMPD3. After pasting in the input sequence, select project title, enter email address, and select "Search For Templates."

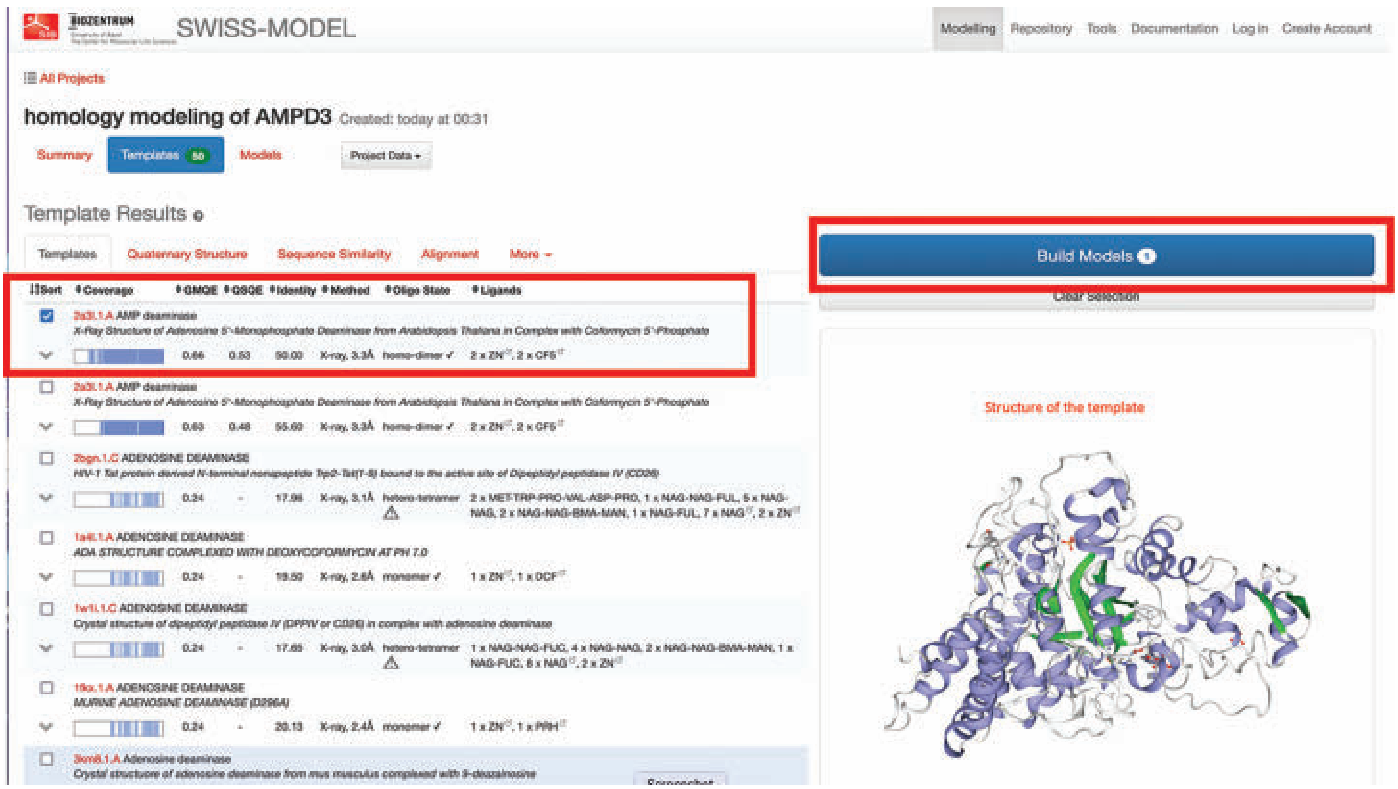


Figure 7. Templates identified for AMPD3. The best template is PDB structure 2a3l with sequence identity of 50% and coverage 79%. Click "Build Models" after selecting a template.

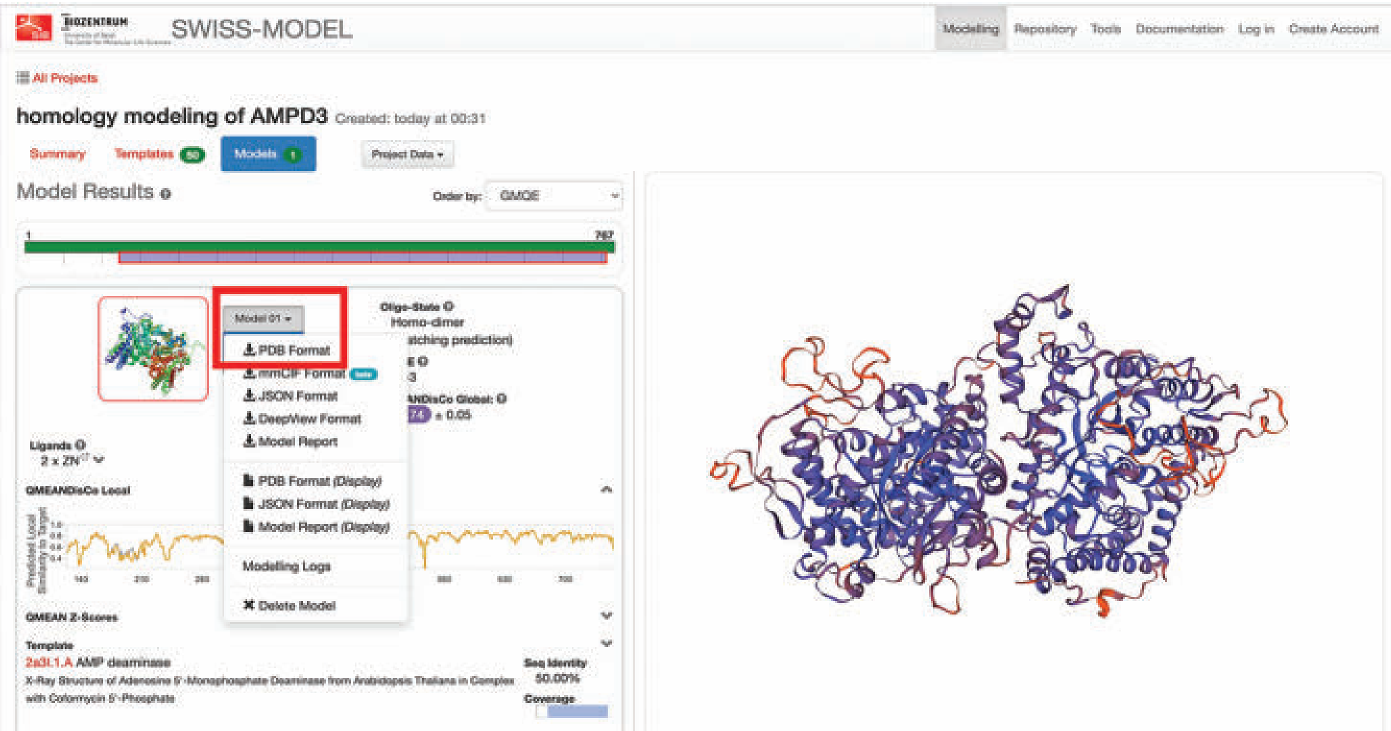


Figure 8. Homology model of AMPD3. Note that multiple models are typically created and compared. Teacher can choose whether to work with a single model or extend the project. Structure of the model should be downloaded as PDB file as highlighted.

○ Obtaining Homology Models from the AlphaFold Database or UniProt Database

Recently, great progress was made in the field of homology modeling by an AI-based method called AlphaFold (Jumper et al., 2021). Many (but not yet all) sequences in UniProt have been modeled using this algorithm, and the predicted structures are available for download from the respective sequence entry files (e.g., here Q01432). We recommend that students search for protein structures predicted by AlphaFold through UniProt (Figure 9). It could be an interesting part of the modeling exercise in the classroom to compare the models they built with those predicted by AlphaFold. Note that homology models may have errors due to many factors, but accuracy assessment is provided for every model, which is an interesting aspect to discuss with students. For example, if the model has a structural segment of low confidence, this segment would not be a reliable structure to use in protein-ligand or protein-protein docking.

○ Analysis of Evolution of Protein Structures

Similarity between two homologous protein structures is best assessed by structural superposition that can be calculated by free programs, such VMD (Humphrey et al., 1996) or USCF Chimera (Pettersen et al., 2004), the latter being easier to use. More information on protein visualization software can be obtained from (Barber, 2021) and from the Supplemental Material included with the online version of this article.

Students can import any pdb files (for example the AlphaFold model and the Swiss-Model structure pdb files) into Chimera using File→Open and then Tools→Structure Comparison→MatchMaker. One of the structures can be highlighted as a reference leaving all other settings at default. Students can observe that Model 1 from Swiss-Model is a dimer (complex of two proteins); one of the protein chains can be visualized by selecting and hiding the other. The superposition of the template and structure predicted by Swiss-Model is presented in Figure 10A. Figure 10B shows a superposition of the template structure and the one predicted by AlphaFold. As the students will observe, there are differences between the predicted models, and areas of low confidence should be considered carefully—for example, whether they would like to use any of these models for their drug design projects. From the evolutionary perspective, a comparison of AMPD3 and ADA1 (Figure 10C) is valuable in showing that, while the two protein structures have diverged, the central core is similar between the two. Students can observe that the beta-sheets of the two proteins superimpose very well with the Zn ion and Zn-binding residues occupying the same positions (Figure 10D), although alpha-helices are not so well conserved and there is a number of structural segments unique to AMPD. This is consistent with their shared catalytic mechanism, but distant evolutionary relationship (Maier et al., 2005). Further analysis of the superposition (Figures 10C and 10D) can be found in the Supplemental Material provided with the online version of this article.

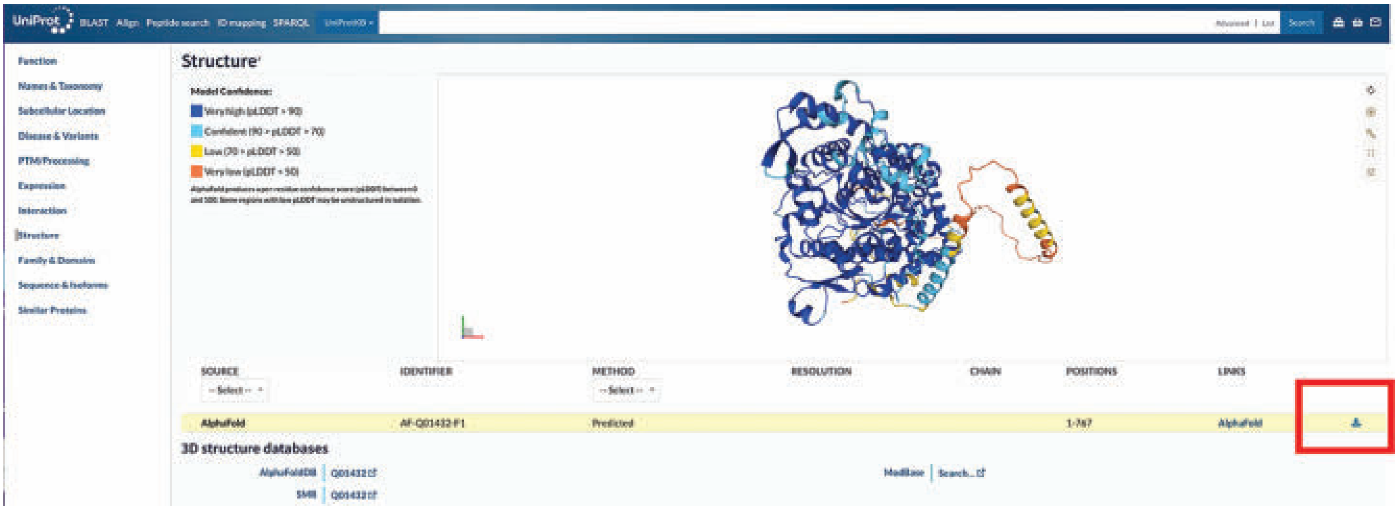


Figure 9. Homology model of AMPD3 created using AlphaFold. The structure of the model can be downloaded using the icon as highlighted.

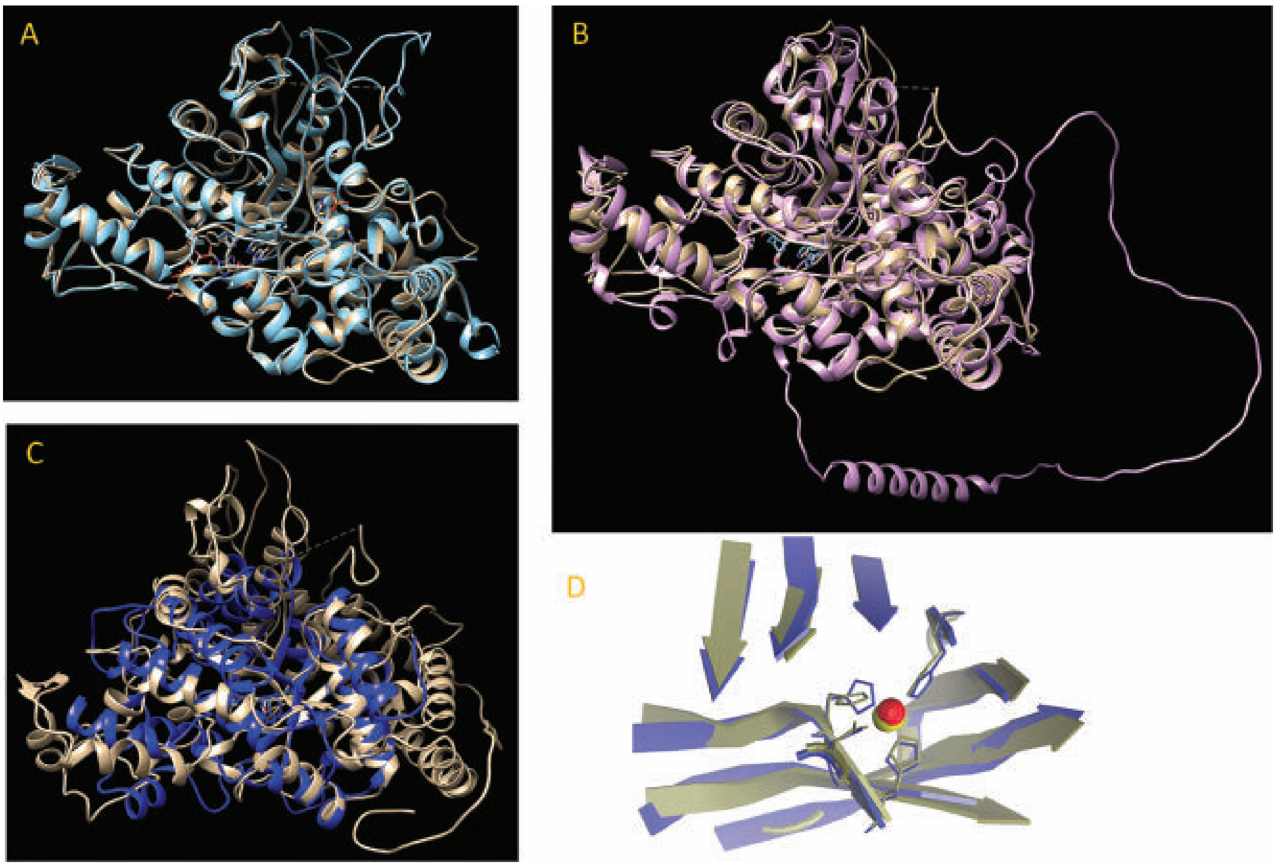


Figure 10. (A) Structure of AMPD3 predicted by Swiss-Model overlayed with the homologous template 2A3L (AMPD2 from *A. thaliana*). (B) Structure of AMPD3 predicted by AlphaFold overlayed with 2A3L. (C) Superposition of 2A3L with human ADA1 (7RTG). (D) Comparison of the core beta-sheets in 2A3L (tan) and 7TRG (dark blue) with Zn ion (shown as yellow/red balls) and Zn binding residues (shown as sticks). Images A–C were created with USCF Chimera, D - with VMD.

○ Conclusions

In our experience, computer modeling techniques are inexpensive and highly effective methods of engaging students and presenting a variety of topics in biology. Here, we emphasize use of bioinformatic databases, familiarizing students with biomolecular sequence and structure data, discussing homology and evolutionary relationships among proteins (for example, difference between ortho- and paralogs), and discussing gene families in the context of gene expression and disease-causing mutations, particularly using an enzyme family involved in diverse disorders from muscle fatigue to immunodeficiencies. 3D protein structure conservation among homologs is a powerful illustration of evolutionary relationships and the foundation of homology modeling as a method of structure prediction. Identical protocol can be applied to other protein families and sequences, allowing for diverse projects. The additional benefit is for students to become familiar with protein structure visualization software that can also be used for analysis of binding pockets, protein-ligand interactions, and mapping sequence alignments to structural data (examples are presented in the Supplemental Material provided with the online version of this article). This exercise can also be used to expand the lesson plan for computational drug design and protein-ligand docking that we described previously (Szarecka & Dobson, 2019). The activity proposed in our previous paper relies on availability of an experimental protein structure that serves as a target for drug design; homology modeling (as described here) can be done when such structure is not available.

Assessment of student learning can be focused on students' ability to retrieve correct sequence data, create a high-confidence homology model, and discuss the structural features and similarities among predicted and experimental structures.

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NABT BioClub

CHAPTERS

Abington Heights High School, Clarks Summit, PA
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Bloomington High School South, Bloomington, IN
Brentwood Academy, Brentwood, TN
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Colonia High School, Colonia, NJ
Coronado High School, Colorado Springs, CO
Cuyohoga Community College, Macedonia, OH
Darnell-Cookman School of the Medical Arts, Jacksonville, FL
DeVry Advantage Academy, Chicago, IL
Divine Savior Holy Angels High School, Milwaukee, WI
Dora R-III School, Dora, MO
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Frontier Regional School, S Deerfield, MA
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Grandville High School, Grandville, MI
Greater Lowell Technical High School, Tyngsborough, MA
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Snow College, Ephraim, UT
Southeast Community College, Lincoln, NE
South Central Jr Sr High School, Elizabeth, IN
South Garner High School, Garner, NC
Southern Wells High School, Poneto, IN
St. Andrew's Episcopal School, Potomac, MD
St. Clair High School, St. Clair, MI
State Library of PA, Lykens, PA
Stillwater High School, Stillwater, OK
Stouffville District Secondary School, Whitchurch-Stouffville, ON, Canada
The Summit County Day School, Cincinnati, OH
Sunlake High School, Land O'Lakes, FL
Taylor University, Upland, IN
Tiffin Columbian High School, Tiffin, OH
Troy High School, Troy, MI
Unionville High School, Kennett Square, PA
University Christian High School, Hickory, NC
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Vincennes University, Vincennes, IN
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Westdale Secondary School, Hamilton, Ontario
Western Piedmont Community College, Morganton, NC
West Mifflin Area High School, West Mifflin, PA
Wheeling Park High School, Wheeling, WV
Worthington Christian High School, Worthington, OH

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.

CLASSROOM MATERIALS & MEDIA REVIEW

JEFFREY D. SACK, DEPARTMENT EDITOR

Reconciling Evolution
Brigham Young University
www.recoevo.byu.edu

With much of the United States population being religious, how we as educators approach teaching evolution can be beneficial to bringing more knowledge and understanding to the students in our classrooms. For educators teaching science and evolution to populations with high student religiosity, the Reconciling Evolution website at Brigham Young University (recoevo.byu.edu) has many resources that can help alleviate the perceived conflict students may feel learning about evolution. Although the website has a strong emphasis on a single Christian belief (Church of Jesus Christ of Latter-Day Saints), the authors of the website have done a great job including resources (lesson guides, curricular material, and videos) from other religious sects and denominations such as Judaism, Baptists, Non-denominational, and Non-sectarian, just to name a few. These resources broadly talk about religion and evolution for all denominations. This website has teaching material, resources, and tools that will benefit educators, students, and curious individuals from any age group and worldview.

The website is formatted with drop-down tabs on the top of the webpage, which help navigate through the website. By clicking on the "About Us" tab, the visitor can learn more about the authors and contributors of the website, along with research papers, research presentations, YouTube videos, and podcasts explicitly talking about the intersection between religion and evolution. They are not all necessarily from the authors, but come from different sources and people. For example, the RecoEvo website has an embedded YouTube video, *Evolution and God – Can you believe in both?* In this video, two scientists discuss how science and religion differ from one another and claim that you can be both religious and a scientist at the same time. The content in this section would be helpful for anyone who is generally interested and may want to understand evolution and religion better.

The most helpful section of the website is the "Resources" tab. Here, educators can view curricular materials (e.g., PowerPoint slides, Instructor Guides) developed by biologists, religious educators, and community religious leaders. The instructor guides are meant for teaching evolution to religious students in a biology or theology class from different religious perspectives. For example, if an educator teaches biology in a strong Pentecostal population, the Pentecostal instructor guide, "A Pentecostal Perspective," gives a list of activities, outcomes, and reading that could help teachers and students from this specific background learn about the science of evolution. The instructor guide also gives a brief background of the potential cultural barriers to consider when teaching evolution to students of different populations. These instructor guides may also improve teacher/parent interactions when parents might be concerned that their children are learning about evolution. By using the resources on the website, the teacher will have additional background knowledge on specific religious beliefs to better inform their discussions about evolution with parents.

Other resources in this section include a video that follows a made-up student, Carlton Smith, as he goes throughout his day. This video, although hypothetical, is based on actual experiences shared by students interacting with and learning about evolution. This video can be used as an in-class resource that teachers could share to help ease students' minds in learning about evolution. There are also videos where scientists and religious individuals share their beliefs and thoughts about connecting the hard science of evolution and the beliefs of religion. These reconciliation videos could be a helpful resource for high school and college students, teachers, and parents.

The tools section has tools for educators in junior high and high school wondering about the perceived conflict religious students may feel when learning about evolution. "RecoEvo for Secondary Educators" is a free course that walks educators through religious history and scientific thought. This free course aims to help educators learn how to reduce the perceived conflict students feel learning about evolution. There is also a survey instrument, "pFEAR survey," that educators can give to their students to help educators better know what influences their students' worldviews and whether they perceive a conflict between science and religion. The survey may influence whether resources from the RecoEvo website are needed.

A significant gap between students and educators can be seen when approaching human evolution, as many students seem to struggle with this topic the most. The RecoEvo website has a whole section dedicated to human evolution, including videos describing evolution, the scientific method, and the basics of human evolution. Not only does it have basic information, but it also has more in-depth videos about how scientists obtained evidence and why that evidence is essential to understanding human evolution. These videos include famous paleoanthropologists (i.e., Dr. Lee Berger, Dr. John Hawkes, and Dr. Richard Potts) who describe their research in different localities, such as in the museum or the field. The videos do an excellent job of tying in religion, along with the words and evidence from the scientists describing their research. These videos would benefit anyone looking for information about human evolution and wondering how to reconcile it with evolution.

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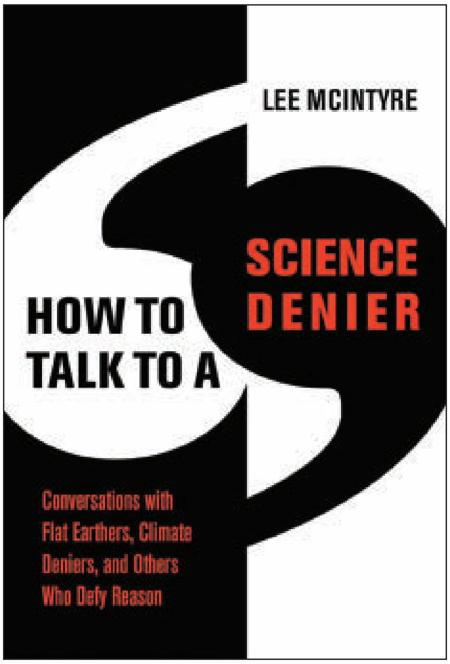

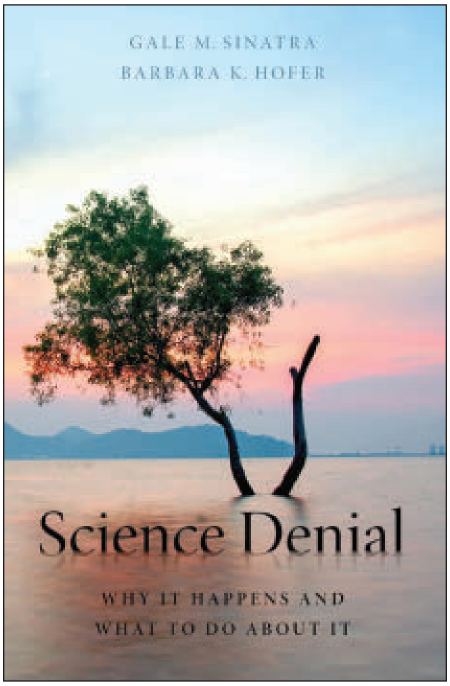

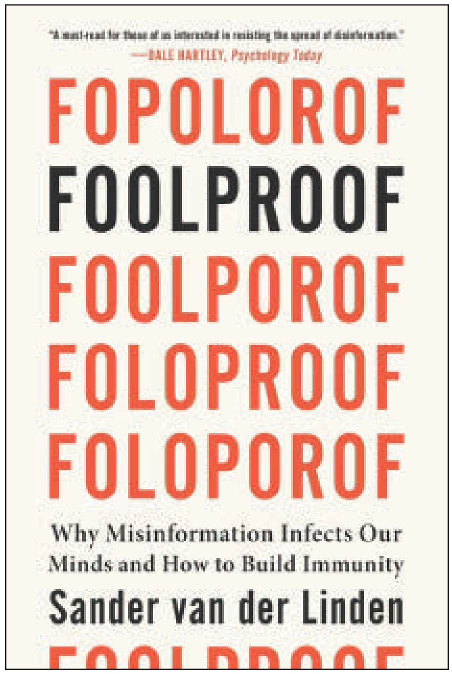

How to Talk to a Science Denier: Conversations with Flat Earthers, Climate Deniers, and Others Who Defy Reason. By Lee McIntyre. 2021. MIT Press. (ISBN 978-0-262-04610-7). 264 pp. Hardcover, $24.95. Paperback and ebook also available.

Science Denial: Why It Happens and What to Do About It. By Gale M. Sinatra & Barbara K. Hofer. 2021. Oxford University Press. (ISBN 978-0-19-094468-1). 193 pp. Hardcover, $37.99. Paperback and ebook also available.

Foolproof: Why Misinformation Infects Our Minds and How to Build Immunity. By Sander van der Linden. 2023. W.W. Norton. (ISBN 978-0-393-88144-8). Hardcover. 358 pp. $30.00. Ebook, paperback and audio also available.

Verified: How to Think Straight, Get Duped Less, and Make Better Decisions about What to Believe Online. By Mike Caulfield and Sam Wineburg. 2023. University of Chicago Press. (ISBN: 978-0-226-82984-5). 280 pp. Ebook (PDF), $13.99. Hardcover and paperback also available.

Four recent books address the current misinformation crisis, science "denial," and trust in the scientific consensus. They raise important questions for biology teachers: Are some approaches to science "denial" guilty of blaming the victim? Can we provision students to make judgments about scientific evidence on their own, or must they ultimately rely on experts? How are consumers of science beguiled by persuasive techniques? Should media literacy now be an integral part of science teaching?

In *How to Talk to a Science Denier*, philosopher Lee McIntyre seems quite proud that he could walk into a Flat Earthers' convention and not say a word for 24 hours. He has written previously on "the skeptical attitude" and the problem of "post-truth," so his effort to "reach out" to those he once disparaged seems to have been a personal challenge. McIntyre seeks out conversations with climate-change naysayers, coal miners, anti-vaxxers, and GMO-skeptics. His primary "revelation"? After meandering through the philosophical literature and psychological research, he concludes that to achieve conceptual change, you should engage in respectful dialogue. "Empathy, warmth, and human understanding" (p. 160), he concludes, are tools that open trust. Of course, this is well known by experienced constructivist teachers! Once there is a personal relationship, one can entertain more rational discussion: graphs, appeals to consensus, and rebuttals based on describing logical fallacies or persuasive techniques (pp. 171–174).

The lessons from the second volume, *Science Denial*, may be familiar to *ABT* readers, from a December 2022 editorial by its two authors, educational psychologists Barbara Hofer and Gail Sinatra. That short essay is an abridged section from their book's final chapter and summarizes their message well. Like McIntyre, they focus on "denial, doubt, and resistance," construed as deviations from normal (proper) thinking. They provide five explanations: cognitive biases, flawed basic epistemological beliefs, motivation, emotions, and social identity.

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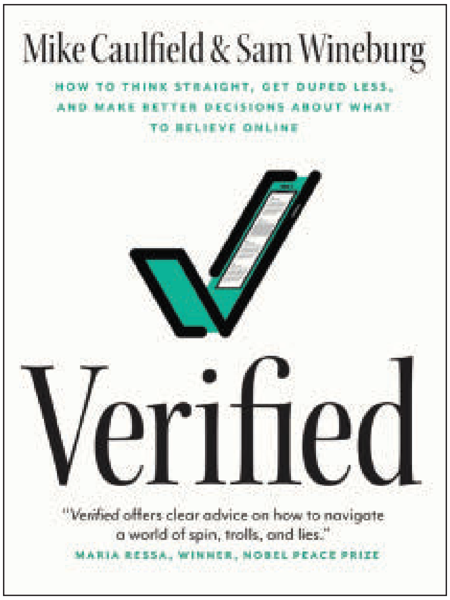

Hofer and Sinatra's discussion is richly informed by their classroom research and illustrated with numerous personal anecdotes. Each chapter ends with explicit advice on "What You Can Do," sorted by different roles in the educational ecosystem. The book is well structured and easy to read episodically. However, I found their approach often reflecting a deficit model of science communication and education. There seems little benefit in confronting naïve students as misguided or "wrong," or maligning some imagined "lazy" thinking. Instead, we might engage, inform, and reshape existing motivations and emotions. The authors do not delve deeply here into *why* we should trust science—or why anyone should *want* to trust science, even while they acknowledge that it is important. What might it mean to focus on how to make science inviting and show how it earns trust, rather than bemoan those who reject it or merely expect trust as a default?

In *Foolproof*, social psychologist Sander van der Linden offers an analogy for the spread of misinformation that biology teachers can readily appreciate: he likens it to a virus of the mind, explaining that we can be infected with beguiling falsehoods that are transmitted across the media landscape and through the population. Van der Linden's proposed solution, therefore, is to imagine psychological vaccinations, or inoculation against being victimized—also known as "prebunking." This is achieved by helping consumers become familiar with the various stratagems used as "weapons of mass persuasion": how others try to hijack our intuitive cognition into believing, and then sharing, information that may not be true. This prophylactic tactic has been tested and seems effective. The manipulative techniques described in *Foolproof* are nicely packaged in the mnemonic acronym DEPICT: Discrediting, Emotion, Polarization, Impersonation, Conspiracy, and Trolling. Van der Linden also distinguishes misinformation—typically a result of relying on inaccurate information (by living in a filter bubble or echo chamber)—from conspiratorial thinking; different strategies are needed to address each. I found the writing crisp and well organized, easy to read and digest in fragments with each chapter ending with a bullet-point list of simple take-home messages. Forewarned is forearmed, and this book is a good "how-to" primer on defending against manipulative persuasion.

In *Verified*, Caulfield and Wineburg, a historian/educator and an information literacy specialist, focus more narrowly, perhaps: just on online reasoning. Their acronym is SIFT: Stop, Investigate the source, Find better coverage, and Trace the claim (or quote or video) to the original context. "Stop" is an antidote to our tendency to race ahead, whether you call it jumping to a conclusion, hasty generalization, relying on first impressions, confirmation bias, or predictive neural programming. "Investigate the source" reflects the importance of credibility, not just the erroneous evidence or selective argument that a purveyor of misinformation wants you to hear. "Find other coverage" because the internet is awash with information and you can easily check questionable claims or consult better sources. And, finally, "trace the original" to establish context and meaning, and to expose misrepresentation. Namely, separate authentic science from bogus *reports of* science. A key overall theme is "do what fact-checkers do": leverage the vast information on the web to disarm the *mis*information also on the web and social media. The authors demonstrate these skills and their variants through numerous authentic examples, both humorous and profound. You learn the cautionary practices that Sinatra and Hofer merely allude to. The language is lively and pithy, suitable for students.

Verified indirectly raises a provocative question for science teachers: what is the relationship between scientific research in a lab or field site and media literacy? Both strive to ascertain the facts—the "real" facts, not illusions or mere plausibilities. Both involve evidence, although of different kinds: science focuses on observations and empirical results, while an online search focuses instead on the evidence for the credibility and expertise of the source. Examining the credentials and track record is parallel to checking the reliability of an instrument or lab procedure. Consulting a second source is like calibrating an instrument or running a controlled experiment. Finally, tracing originals is akin to checking experimental assumptions or the legitimacy of proxy variables. Both science and media literacy have methods to root out possible errors and ensure reliability. And for most of our students—those who will not pursue professional careers in science—who seek scientific information, which seems most important? Namely, what role does learning skills in science media literacy have in a science classroom if the aim is to develop competent citizens and consumers of science? Caulfield and Wineburg also delve into the foundational issues of what makes a claim, scientific or otherwise, reliable in the public sphere: expertise and consensus (Chapter 5) and peer review (Chapter 6), critical topics often missing in the science classroom. My verdict on *Verified*? Engaging, insightful, and useful.

How to Talk to a
Science Denier:
Science Denial:
Foolproof:
Verified:

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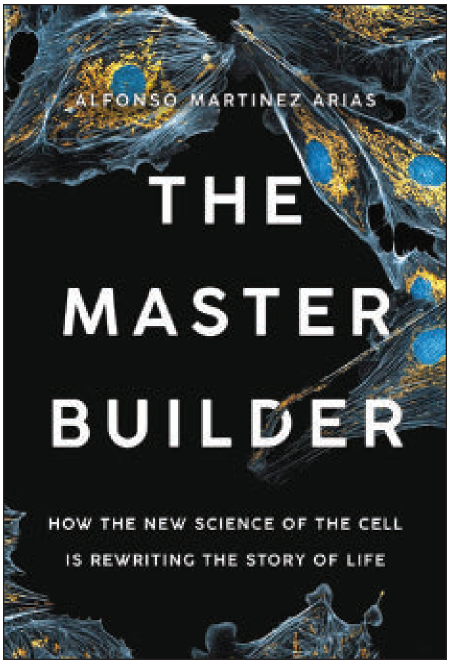

The Master Builder: How the New Science of the Cell is Rewriting the Story of Life. By Alfredo Martinez Aria. 2023. Basic Books. (ISBN 978-1541603271). 352 pp. Hardcover, $22.05. Paperback and ebook also available.

If you haven't yet met a *gastruloid*, it's probably time you did, especially if you cover aspects of embryology, genetics, or evolution in your classes. Gastruloids are small (<1mm) groups of cells that go through significant changes that result in their having a body axis, a more-or-less head end and a more-or-less tail end, and even some apparent tissue concentrations that look like early heart cells—all despite the fact that they're not embryos and did not result from a fertilization. Over the last decade or so, they have been produced from embryonic stem cells (tissue cells that have been transformed into pluripotent embryonic stem cells by exposure to a small cocktail of transcription factors) derived from tissues of humans, mice, frogs, and zebrafish—and they all look more or less the same.

With that, I've just given away the climactic conclusion of *The Master Builder: How the New Science of the Cell is Rewriting the Story of Life*, by Alfonso Martinez Arias. On the way to introducing gastruloids, Martinez Arias wants to convince us that we're on the verge of much deeper understanding of how complex multicellular animals develop, and how they evolved, but to accomplish this he has to move us beyond our gene-centered view of life to learn more about the behavior of cells. Early in the book he claims that "[g]eneticists have been so successful at finding changes to genes associated with dysfunction that we've fallen into the trap of equating correlation with causation" (p. 6). This is not the assertion of an armchair biology enthusiast. Martinez Arias is the ICREA Research Professor in the department of Medicine and Life Sciences of the Universitat Pompeu Fabra in Barcelona, Spain. From 2003 to 2021, he was Professor of Developmental Mechanics in the University of Cambridge Department of Genetics.

The Master Builder is a three-part report from a frontier of knowledge, one in the midst of which many researchers know something of huge significance is going on, but for which existing language is not yet adequate to explain precisely what. Part One cites evidence that cells actively employ genes to send messages among themselves and to direct activities that result in building important structures. Part Two narrows the focus to the spectacular cell behaviors that occur during gastrulation, especially the "choreography" by which vertebrate embryos set up their body plans and axes. Part Three introduces the discovery of a new sort of "creature," the gastruloids, that hold out hope for new research pathways to understand just how organisms develop and how they evolved.

In Part One, Martinez Arias replaces the "blueprint" analogy commonly applied to genes and DNA with a "hardware store catalog" image: the genome is better viewed as the catalog from which the cell orders up the appropriate plans for the protein tools and materials it needs. He repeatedly asks us to consider that genes are used by cells to generate three dimensional, sometimes symmetrical, complex structures, emphasizing always that genes themselves have no concept or perception of space or time. Knowing that the movements of cells during gastrulation are spectacular and not yet well explained, Martinez Arias spends Part Two digging in to evidence that cells respond to all sorts of things—their physical substrate, its hardness, softness, geometry, the number and density of adjacent cells, and even the order of cellular events—as they reach into their catalog of available genes to accomplish complex multicellular constructions. The astonishing activity of the neural crest cells provides his strongest example:

> Positioning relative to other cells turns out to be one of cells' most valuable assets and is very much in evidence in the actions of a wondrous group that arises shortly after gastrulation: the neural crest. … The migration of these cells is as precise as it is mysterious, following cues that must be hidden in the cellular territories they traverse. Recent studies suggest that these cues are not simply chemical; instead, inputs include the hardness or softness of the territories and the density or looseness of the cell populations they invade. (163–164)

Martinez Arias doesn't see evidence that the neural crest cells are simply gene-driven. Rather, they snap into streaming migratory behavior just after vertebrate gastrulation, establish resident populations at particular locations, and set about building vital, complex structures (e.g., the complex, integrated anatomy of our head and face). Traditionally, the language used to describe this involves movements of neural crest cells to locations adjacent to epithelial cells where the migrants then receive signals that "induce" them to transform their activities and create a specific organ or shape. Martinez Arias sees these neural crest cell movements as far more dependent on the "choices" of the cells. He asks why, and how, the neural crest cells "choose" to migrate in the first place.

In Part Three, Martinez Arias engagingly tells the story of how a number of different researchers stumbled on the conditions that stimulate formation of gastruloids, little "creatures" with a predictable shape and a predictable gene expression geography. They're not embryos, they don't represent a known stage of embryonic development, and they won't proceed through full development. Instead, they seem to present a bare "outline" of a bilaterian vertebrate body plan. Manipulating them in the lab allows the probing of questions about just what sort of conversations are going on among the cells to get them to this point, and what conversations are *not* going on, thwarting their further development. They present a gold mine of possible laboratory investigations (and, for now at least, avoid the political/legal complications of studying human embryos).

Throughout the book, Martinez Arias hints strongly at how his view of cellular activity fits with evolution. He sees the advent of multicellularity, at least among animals, as the watershed moment at which genes—which had been functioning as "simple" tools of single-celled organisms, providing appropriate responses to certain environmental limitations and opportunities—began to be used in cell-to-cell conversations using new gene products as signals, creating unlimited levels of complexity. He believes those conversations led to the movements of gastrulation and the resultant great diversity of animal body plans, likely happening during the Cambrian period.

A major problem for *The Master Builder* is that we don't yet have adequate language to describe what's happening in and among cells as they go through complex embryonic development. Martinez Arias regularly employs active verbs that imply intelligence and will, using "choose" and "decide" frequently as cells order up the appropriate tool or material from the genomic catalog, then selectively employ the molecular product to accomplish an essential end. Unless we think cells are conscious, there must be some mechanistic link that looks like the "choosing" that's going on. Martinez Arias has observed that many features of a cell's immediate environment (the number, type, density, or texture of the neighboring cells, and physico-chemical properties of any substrate along which or in which the cells are acting) seem to trigger specific cell responses. But is that an example of the cell choosing or simply reacting? The mechanisms for these interactions are not fully known, as he freely admits. Despite the difficulty of talking of cells' agency in developmental processes, Martinez Arias never suggests that there is some vital force behind development, and he is not sympathetic with any sort of intelligent design argument (p. 177). Martinez Arias simply suggests that we are only beginning to figure out how cells work together in the processes of building multicellular organisms. (For a quick information-packed introduction to this line of research, see Gorfinkiel and Martinez Arias, 2021.[1] But reading *The Master Builder* is a lot more fun.)

Gastruloids open a window into asking a series of new questions that couldn't be asked before. *The Master Builder* is a great read and an exciting introduction to this field of study.

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[1] N. Gorfinkiel and A. Martinez Arias. 2021. The cell in the age of the genomic revolution: Cell regulatory networks. *Cells and Development*. 168 (2021): 203720.

NABT

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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, 2024.**

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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 2024. 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, 2024.**

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GENETICS EDUCATION AWARD ... Sponsored by 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 $500 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, 2024.**

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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. Supported by Bedford, Freeman, and Worth as well as private contributions, 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, 2024.**

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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 **March 1, 2024.**

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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, 2024.**

TWO-YEAR COLLEGE BIOLOGY TEACHING AWARD ... Sponsored by NABT's Two-Year College Section, 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, 2024.**

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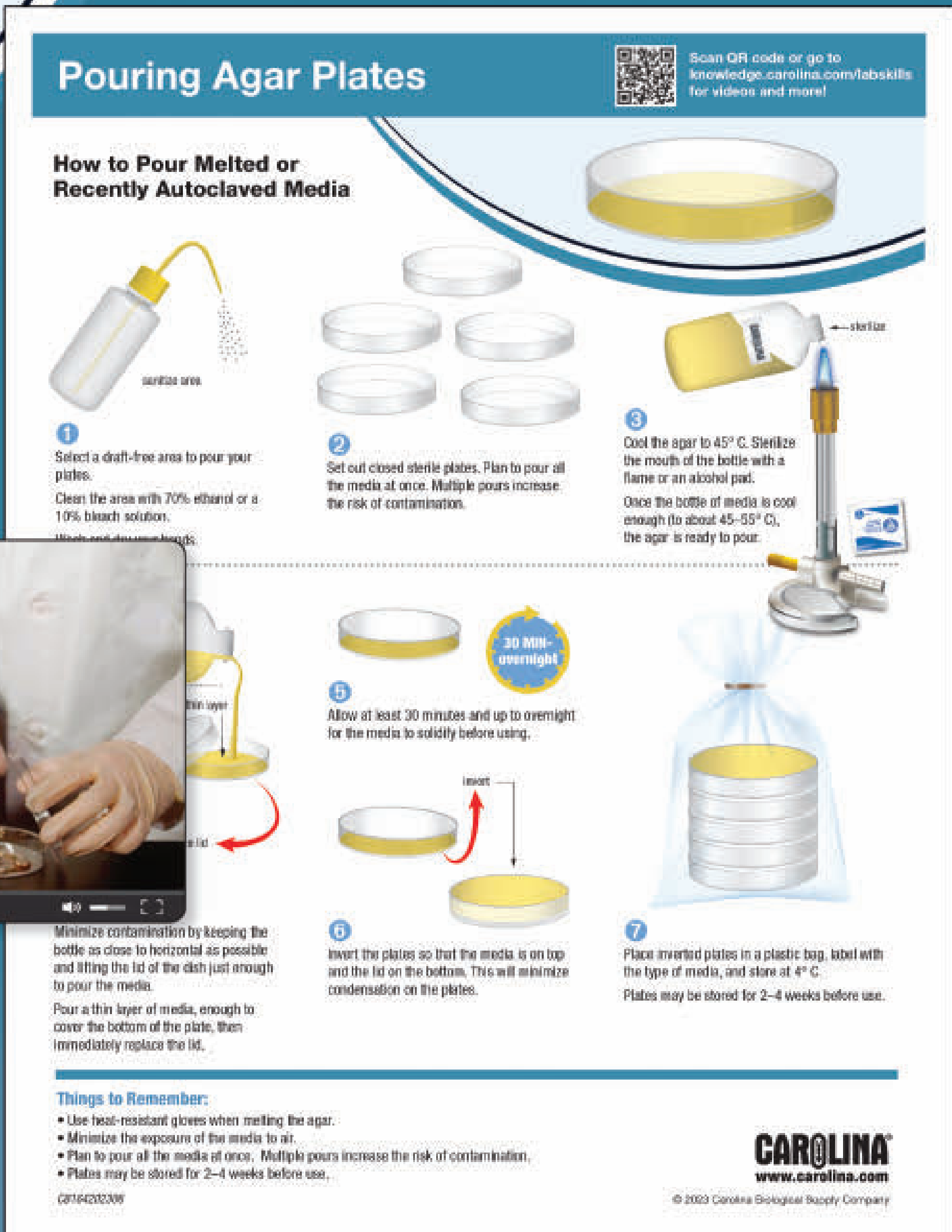

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sanitize area

1 Select a draft-free area to pour your plates.

Clean the area with 70% ethanol or a 10% bleach solution.

2 Set out closed sterile plates. Plan to pour all the media at once. Multiple pours increase the risk of contamination.

sterilize

3 Cool the agar to 45° C. Sterilize the mouth of the bottle with a flame or an alcohol pad.

Once the bottle of media is cool enough (to about 45–55° C), the agar is ready to pour.

thin layer

lid

Minimize contamination by keeping the bottle as close to horizontal as possible and lifting the lid of the dish just enough to pour the media.

Pour a thin layer of media, enough to cover the bottom of the plate, then immediately replace the lid.

30 MIN–overnight

5 Allow at least 30 minutes and up to overnight for the media to solidify before using.

invert

6 Invert the plates so that the media is on top and the lid on the bottom. This will minimize condensation on the plates.

7 Place inverted plates in a plastic bag, label with the type of media, and store at 4° C.

Plates may be stored for 2–4 weeks before use.

Things to Remember:

- Use heat-resistant gloves when melting the agar.
- Minimize the exposure of the media to air.
- Plan to pour all the media at once. Multiple pours increase the risk of contamination.
- Plates may be stored for 2–4 weeks before use.

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