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Theme Issue: Evolution Education

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National Association of Biology Teachers

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About Our Cover

The Defense Team and John T. Scope in 1925 "Monkey" Trial
This one-hundred-year-old photograph taken by an unknown newspaper photographer in the summer of 1925 features many of John Scopes's defenders in the most famous court case in science education, The Scopes "Monkey" trial.

Here we see the white-shirted defense lawyer Clarence Darrow leaning forward with his hands on the table, with co-counsel Dudley Malone standing behind with arms crossed. Almost lost in the middle of the image is John T. Scopes, the young coach, science and math teacher gazing expectantly forward, who volunteered to be charged with breaking Tennessee's Butler Act. This law made it illegal for teachers to teach any theory that denied the Bible's account of the divine creation of man.

The trial and its aftermath are benchmarks in the history of evolution education, thus making this image a perfect accompaniment to ABT's annual issue on Evolution Education as it commemorates 100 years since the "trial of the century." As we will see in the commentary accompanying this issue, in one way the trial changed nothing, but in others the world was a different place upon its conclusion. Nonetheless, we should remember what is written on Scopes's gravestone, that he was "a man of courage," as are all those who seek and teach the truth.

This photograph is in the collection of the Library of Congress filed as *Clarence Darrow at the Scopes evolution trial, Dayton, Tennessee, July*. Tennessee Dayton, 1925. July. Retrieved from the Library of Congress Prints and Photographs Division Washington, D.C. 20540, https://www.loc.gov/item/97512300/.

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Editorial Staff

Editor: William F. McComas
Parks Family Professor of Science Education
University of Arkansas
ABTEditor@nabt.org

Cover Photo Editor: Julie Minbiole
jminbiole@colum.edu

Reviewing Editors: Julie Minbiole
Columbia College Chicago
jminbiole@colum.edu
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University of Arkansas
knwalker@uark.edu
Emily Weigel
Georgia Institute of Technology
emilygweigel@gmail.com
Suann Yang
SUNY Geneseo
yang@geneseo.edu

Managing Ediror: Valerie Haff
managingeditor@nabt.org

ABT Production Office: P.O. Box 3363
Warrenton, VA 20188
phone: (888) 501-NABT
Web site: www.nabt.org

Contributing Columnist: James Morris, Brandeis University
(jmorris@brandeis.edu)

Department Editors

Book Reviews: Kirstin Milks & Frank Brown Cloud, Kirstin.milks@gmail.com
Classroom Materials & Media Reviews: Jeffrey D. Sack, sack.jeffrey@comcast.net

Advertising Sales

Charlton Calhoun, NABT Account Executive,
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EDITOR'S COMMENTARY

William F. McComas
Editor, *The American Biology Teacher*

Teaching Biology and the Long Shadow of Scopes

For years, the February issue of *The American Biology Teacher* has focused on Evolution Education in honor of Charles Darwin in his birth month. This year we also reflect on a century-old trial, the consequences of which still resonate in classrooms. Of course, I am talking about the Scopes "Monkey" Trial that took place in Dayton, Tennessee, between July 10 and July 21 in 1925.

Tennessee and two other southern states—Arkansas and Mississippi—passed laws that prohibited the teaching of human evolution. This was the case in Tennessee with the Butler Act named after the legislator who proposed it. The law was not particularly detailed and passed without much fanfare but made it illegal for teachers in public schools to "teach any theory that denies the story of the Divine Creation of man as taught in the Bible," but many interpreted this to extend to evolution generally.

The American Civil Liberties Union (ACLU) was concerned and searched for a defendant to challenge the law. Several leading citizens in the small town of Dayton, which had fallen on economic hard times, believed that having a trial there might bring attention to their fair city and boost the local economy. They responded to a newspaper ad from the ACLU and found a willing party who agreed to "break" the law and stand trial. That man was local coach and science and math teacher, John T. Scopes, and the rest, as they say, is history.

Sadly, much of that fascinating history has become muddy with time, but here are the main points. Scopes did use a state-approved biology book that contained a few pages about evolution mostly featuring what is called the "great chain of being," but probably taught nothing contrary to the "divine creation of man." Scopes was never really arrested, did not testify at the trial, never spent a night in jail, and most certainly never dated the daughter of a local fire and brimstone minister, as depicted in the stage play and movie, *Inherit the Wind*.

Quickly, Scopes was tried with defense support from the ACLU, but not before former Secretary of State, three-time presidential candidate and fundamentalist William Jennings Bryant volunteered to assist the prosecution. Clarance Darrow, arguably one of the most famous lawyers in the country, soon signed on to aid the local defense team. To make things even more interesting and contentious, Darrow and Bryant knew each other and traded barbs throughout the trial effectively putting them centerstage. Scopes, the jury, and the main arguments in the case were often forgotten.

The judge quickly ruled that the law was not on trial, and all the assembled scientific experts were excused from testifying. This brought the ironic comment from John Butler, author of the anti-evolution law, that perhaps some discussion of evolution would be useful because he didn't know anything about it, a view that is likely true among many who oppose evolution today.

The eight-day trial was shared with the world through the relatively new medium of radio and the breathless newspaper reports including those from the sardonic H. L. Mencken. All this focused attention on the tiny town and biology education to such a degree that neither has ever been the same since. Newspaper reports and live radio streamed over new telephone and telegraph lines while scores of individuals flooded into the local station on special excursion trains to witness history in the making. The crowd grew so huge that the trial moved outside the courthouse with fear that the floor might collapse and, to the relief of those in attendance, because it was stifling hot.

Much has been written about the trial, including in the Pulitzer Prize–winning book *Summer for the Gods* by Edward Larson and Brenda Wineapple's new account *Keeping the Faith*. In due course Scopes was found guilty, and the judge levied a $100 fine that was later deemed inappropriate because the jury was not consulted. Ultimately the Tennessee Supreme Court, on appeal, dismissed the case saying "nothing is to be gained by prolonging the life of this bizarre case." So, little changed from a legal perspective. The law remained on the books in Tennessee and other states for more than four decades. However, many issues from the trial still remain.

The case brought up the important issue of majority rule when local control of education contrasted with the minority rights of those who wanted their children to learn about topics such as evolution. The bombastic writing of Mencken, who left before the conclusion of the trial, painted an inaccurate view of southerners calling them "yokels," "hillbillies," and "ignorant," words that still sting. Moreover, the fact that a scientific idea was on trial suggested that there must be something wrong or evil about evolution, and publishers quickly removed all such content from their biology textbooks. Even today we find that some biology teachers fail to emphasize this key dimension of the life sciences for fear of backlash, or worse because they personally do not accept the validity of evolution. Sadly, countless laws continue to be proposed in one state after another attacking teaching of the science of evolution with a variety of schemes that curiously often mirror one another.

It is time to lift the shadow of the Scopes trial and rededicate ourselves to teaching evolution and honor Scopes who allowed himself to be a vital test in a trial of ignorance vs. reason during a sultry summer in 1925. We hope that this theme issue of *The American Biology Teacher* contributes to both goals.

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Recognize Excellence in Teaching

NABT is accepting nominations for the 2025 awards program.

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

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

PROF. CHAN TWO YEAR COLLEGE AWARD FOR ENGAGED TEACHING OF BIOLOGY ... Sponsored by Sarah McBride and John Melville, the Prof. Chan Two-Year College Award for the Engaged Teaching of Biology is given to a two-year college faculty member who has successfully developed and demonstrated an innovative, hands-on approach in the teaching of biology and has carried their commitment to the community. This award includes $500 toward travel to the NABT Professional Development Conference, a plaque to be presented at the NABT Professional Development Conference, and one-year complimentary membership to NABT. The nomination deadline is **May 1, 2025.**

ECOLOGY/ENVIRONMENTAL SCIENCE TEACHING AWARD ... Sponsored by Vernier Software & Technology, the Ecology/Environmental Teaching Award is given to a secondary school teacher who has successfully demonstrated an innovative approach in the teaching of ecology/environmental science and has carried that commitment to the environment into the broader community. Vernier's sponsorship of this award includes up to $500 toward travel to the NABT Professional Development Conference, and Vernier equipment. The recipient also receives a plaque to be presented at the NABT Professional Development Conference and a one-year complimentary membership to NABT. The nomination deadline is **March 15, 2025.**

EXCELLENCE IN ENCOURAGING JEDI AWARD ... The NABT Excellence in Encouraging Justice, Equity, Diversity, and Inclusion (JEDI) Award recognizes efforts to promote equity in life science education. The recipient/recipients demonstrate a passion and commitment for JEDI through their teaching and outreach while also identifying successful strategies that increase enthusiasm for biology. Sponsored by NABT, this award includes a $500 honorarium, a recognition plaque to be presented at the NABT Professional Development Conference, and a one-year complimentary NABT membership. The nomination deadline is **March 15, 2025.**

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, 2025.**

FOUR-YEAR COLLEGE & UNIVERSITY SECTION BIOLOGY TEACHING AWARD ... This award, sponsored by NABT's Four-Year College & University Section, recognizes creativity and innovation in undergraduate biology teaching. These innovations may include curriculum design, teaching strategies, and laboratory utilization. Additionally, award winners will agree to present their work during the NABT Conference. The award is open to NABT members and includes $500, a recognition plaque to be presented at the NABT Professional Development Conference, and a one-year complimentary membership to NABT. The nomination deadline is **May 1, 2025.**

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HONORARY MEMBERSHIP ... The highest honor bestowed by NABT, this award recognizes individuals who have "achieved distinction in teaching, research, or service in the biological sciences" as Honorary Members. Those selected become lifetime members of the Association and receive recognition in NABT publications and at the NABT Professional Development Conference. Nominations may be made by any NABT member and must include (1) a description of the candidate's qualifications, (2) a detailed biographical summary, and (3) supporting letters from at least nine NABT members. The nomination deadline is **March 15, 2025.**

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, 2025.**

KIM FOGLIA AP BIOLOGY SERVICE AWARD ... The Kim Foglia AP Biology Service Award recognizes an AP Biology teacher who displays a willingness to share materials, serves as a mentor to both students and professional colleagues, creates an innovative and student centered classroom environment, and exemplifies a personal philosophy that encourages professional growth as an AP biology teacher. Sponsored by NABT, the Kim Foglia AP Biology Service Award includes a $500 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, 2025.**

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, 2025.**

OUTSTANDING NEW BIOLOGY TEACHER ACHIEVEMENT AWARD ... This award, sponsored by NABT, recognizes outstanding teaching (grades 7-12) by a "new" biology/life science instructor within their first three years of teaching (when nominated) who has developed an original and outstanding program or technique and made a contribution to the profession at the start of their career. The award includes a $500 honorarium, recognition plaque to be presented at the NABT Professional Development Conference, and a one-year complimentary membership to NABT. The nomination deadline is **March 15, 2025.**

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, 2025.**

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, 2025.**

Nominate a teacher today!

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FEATURE ARTICLE

How Do Species Change Over Time? Designing a Hybrid Teaching Unit on Five Factors of Evolution

BERRIT K. CZINCZEL, DANIELA FIEDLER,
UTE HARMS

Abstract

Evolution is the central concept of biology and key to a comprehensive understanding of any complex biological interaction. It has proven to be a particularly difficult subject for both teachers and students. Hybrid teaching environments have the potential to support students in learning about complex topics and simultaneously enable researchers to collect data on students' learning, giving them the opportunity to diagnose concrete obstacles for learning. Thus, we developed a teaching unit on five factors of evolution that utilizes digital tools in a traditional biology classroom for a hybrid teaching environment. The teaching unit is theoretically founded on biology education research (e.g., about students' misconceptions) and utilizes validated teaching materials. Using various kinds of data from the unit, we expect to be able to gain a deeper understanding of students' learning by analyzing students' individual learning trajectories. This will lay the foundation for enhanced individualized feedback in the future.

Key Words: *evolution education; digital teaching and learning; secondary school; factors of evolution; genetics.*

○ Introduction

Evolution as the overarching explanatory principle of biology provides the conceptual foundation for all biological processes (Dobzhansky, 1973). These processes are crucial for biology education as they are at the core of prevalent challenges threatening humanity's survival, such as multi-resistant pathogens or biodiversity loss. This makes evolution not only the central topic of biology education but the connective thread that should run through the entire biology curriculum to link the various subdisciplines (Brewer & Smith, 2011; German National Academy of Sciences Leopoldina, 2017).

> *Using methods of learning analytics, we aim to trace students' individual trajectories taking into consideration various data generated through the hybrid teaching unit with a particular focus on concept mapping tasks.*

In order to understand evolutionary change, students must understand the processes that cause it. Processes that can change the frequency of alleles in a gene pool over time are known as *factors of evolution*. They encompass mutation, selection (both natural and sexual), genetic drift, and gene flow (Sadava et al., 2019). Mutations in the germ lines, for instance, cause intraspecific variation, which lays the foundation for selection processes. Selection processes cause directed change in populations that can lead to greater fitness in specific habitats or enhance traits desired in mating partners (Storch et al., 2013). Genetic drift triggered by randomly occurring natural events can also cause changes in allele frequencies independent of individuals' fitness. This process is often offset to an extent by the genetic exchange caused by migration of individuals between populations (Storch et al., 2013).

Despite the importance of the topic, evolution has proven to be difficult for students to learn and educators to teach (Nehm, 2019). A full understanding of evolution necessitates the understanding of several key and threshold concepts, which are in themselves challenging (Tibell & Harms, 2017). Students may also hold a variety of misconceptions about evolution and biological phenomena that can hinder learning (Coley & Tanner, 2012). Moreover, they often struggle with the timeframe in which evolution happens as well as the idea that there is no underlying design (see Ayala, 2013). Additionally, the interactions between processes on the cellular, individual, and population levels can complicate matters for students (Nehm, 2019), as does transferring concepts between different biological kingdoms, especially to the plant kingdom (Nehm, 2018). All of these difficulties have to be carefully considered by researchers and educators aiming to successfully develop teaching materials covering evolution.

There are several established theoretical learning progressions on the topic of evolution that were taken into consideration for the

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development of the unit. For instance, Furtak (2012) developed a learning progression on natural selection, while Zabel and Gropengießer (2011) described different conceptions students might hold while striving toward a scientifically correct one. Moreover, Catley and colleagues (2005) traced evolution learning over the entire K–12 range while Wyner and Doherty (2017) focused on macroevolution. Students' individual paths along these theoretical learning progressions are called learning trajectories. They describe the order in which concepts within a topic are ideally learned. Using methods of learning analytics, we aim to trace students' individual trajectories (see Kubsch et al., 2022) taking into consideration various data generated through the hybrid teaching unit with a particular focus on concept mapping tasks.

Concept mapping is a method that can be used to assess students learning about a topic and reflect conceptual change if recursively applied over time (Kern & Crippen, 2008). Over the course of the unit, we incorporated recursive concept mapping tasks preceded by a method training into the unit to (1) support students' conceptual learning about evolution and (2) trace their individual learning trajectories throughout the unit and analyze them using learning progression analytics (LPA; Kubsch et al., 2022) approaches.

In this article, we describe a hybrid teaching unit for upper-secondary level that aims at supporting students in learning about the factors of evolution as well as collecting data on students' learning in order to provide better formative feedback in the future. The teaching unit is implemented in the digital learning platform Moodle, which is used in a traditional classroom setting. The digital teaching unit (1) contains digital learning tools that help support students in their learning, (2) allows teachers to see students' answers in real time on their own screen, and (3) collects data that can be analyzed by researchers to improve knowledge about students' learning. The data will be analyzed as a part of an interdisciplinary project called ALICE (Analyzing Learning for Individualized Competence development in mathematics and science Education) that aims at examining students' learning trajectories for one central topic in each of the natural sciences and mathematics.

○ The Teaching Unit

Learning Objectives

After completing the unit, the students will be able to explain the processes causing intraspecific variation. They can name intraspecific variation as a prerequisite for evolutionary change and explain differential reproductive success as a cause for species' adaptation to their respective environment. They can also explain adaptations that seem maladapted to the environment as a result of assortative mating. Students will be able to explain genetic drift as a random process that causes undirected evolutionary change and gene flow as the process that can counteract genetic drift. Moreover, they can critically evaluate the consequences of human actions on evolutionary processes.

Conceptual Prerequisites

In order to successfully engage with the teaching unit, students need to be familiar with the following concepts from their lower secondary biology classes: (1) the central dogma of molecular biology, (2) sexual reproduction and inheritance, (3) meiosis, and (4) Darwin's theory of evolution. They may also already have a basic understanding of the following processes: (1) mutation and (2) natural selection.

Digital Learning Environment

In this hybrid teaching unit, students work in a traditional classroom setting while using the digital learning platform Moodle as an online workbook. The unit combines published materials for teaching evolution with digital media (i.e., videos, images, and digital worksheets) and digital tools (i.e., concept mapping tool or a collaborative whiteboard tool; see following chapters).

For the teachers, the digital workbook provides a better overview of students' progress and enables them to catch misconceptions or a lack of understanding early, especially in students who might otherwise not volunteer in group discussions. It also simplifies assessments, as multiple-choice and short-answer quizzes can be evaluated quickly.

Alignment with NGSS

Within the unit, instruction is structured into lesson sets that follow cycles of activities that emulate the process of knowledge generation in science (Figure 1). These inquiry cycles (Kubsch et al., 2022) consist of the activities (1) asking questions, (2) planning and conducting investigations, (3) analyzing and interpreting data, (4) developing and using models, and (5) developing and arguing about explanations. The activities are adapted from the NGSS Science and Engineering Practices (Next Generation Science Standards; NGSS Lead States, 2013) for the use in the digital learning environment and our teaching unit (Kubsch et al., 2022).

Each inquiry cycle is structured around a driving question in a project-based learning approach (Krajcik & Shin, 2014; Kubsch et al., 2022). Project-based learning is designed to help students construct integrated knowledge and develop a deeper understanding of scientific concepts (Krajcik & Shin, 2014), making it a promising method for teaching evolution. In the unit, students work on predefined driving questions while engaging with real-life examples and problems such as habitat fragmentation in forest trees and the emergence of antibiotic-resistant bacteria strains (Krajcik & Shin, 2014). They work collaboratively on the tasks, supported by digital learning technologies embedded in the Moodle platform (Krajcik & Shin, 2014).

Figure 1. Inquiry cycle (Kubsch et al., 2022).

Outline of the Unit

The unit is structured into four lesson sets consisting of nine 90-minute periods in total (Figure 2) and preceded by a 90-minute concept mapping crash course (see next section for more details). The unit includes a variety of tasks designed for individual, partner, and group work using different kinds of materials, all of which are described in detail in the Supplemental Material provided with the online version of this article.

The first lesson set covers recombination and crossing-over contextualized through intraspecific variation in doves (*Columba livia*). Students are confronted with the phenomenon by watching a video of doves in a courtyard and prompted to form hypotheses on the origin of their varying colors and patterns. This is followed by a modeling activity in which students recreate recombination and crossing-over using paper templates (Homburger et al., 2019). At the end of the lesson set, students are prompted to explain the origin of intraspecific variation using the concepts recombination and crossing-over. This lesson is designed to activate prior knowledge on genetics and challenge the misconceptions that traits do not change when they are inherited (Gregory, 2009).

In the second lesson set, students start with the first three factors of evolution: mutation, natural selection, and sexual selection. Students formulate questions about the origin of intraspecific variation in guppies (*Poecilia reticulata*). To answer their questions, they work in groups on the topics of gene, chromosome, and genome mutations (Markl, 2010) and collect results on an interactive whiteboard (Menzel et al., 2022). Afterward, students recreate a simplified version of John Endler's guppy experiment in different environmental settings and interpret the outcome (Staatsinstitut für Schulqualität und Bildungsforschung München, 2016). After this, the initial questions are revisited and students formulate their answers using the concepts of mutation and selection. This lesson set challenges the misconceptions that mutation and selection are not necessarily directional processes and that selection processes lead to the prevalence of just one type of individual (Gregory, 2009). The use of an example in which natural and sexual selection favor different traits supports students in focusing on differential reproductive success rather than just survival (Spier & Dauer, 2023). In addition, this lesson set also emphasizes the random character of evolutionary processes such as mutations (see Tibell & Harms, 2017).

The third lesson set covers the remaining two factors of evolution, genetic drift and gene flow as a counteracting factor. Students work on the topic of habitat fragmentation in forest trees, its origins, and its consequences (Hansen, 2001). Through a simulation with a population of colored sticky notes, students encounter three processes that cause genetic drift (i.e., the founder effect, random deaths, and the bottleneck effect; Lee et al., 2017), and explore the underlying issues and consequences of habitat fragmentation by referring to these processes. In addition, they encounter gene flow as a process counteracting genetic drift by posing the question why genetic drift is not causing an infinite number of individual tree species. Improving on the simplified island model of gene flow, they are led through the process of model improvement toward the stepping stone model of gene flow (Seitz, 1998). This lesson set tackles the preconceived notion that evolutionary change is directional and progressive (Gregory, 2009) and introduces another instance of randomness in evolution (i.e., genetic drift; Tibell & Harms, 2017).

The fourth and last lesson set recontextualizes the factor's mutation and selection in the socioscientific issue which is the emergence of antibiotic-resistant bacteria strains. Students explain mutation and selection as the underlying factors leading to the development of antibiotic-resistant bacteria strains and explore the consequences of human influence on evolutionary processes. They prepare a classroom discussion in a talk show format, highlighting the societal implications from the viewpoint of three groups (i.e., pharmacists, medical experts, and farmers) that are affected by this issue (Sagmeister et al., 2021). This lesson set aims to help students to realize the relevance of evolutionary processes for their

		Lesson set 1 *(2 periods)*		**Lesson set 2** *(3 periods)*		**Lesson set 3** *(2 periods)*		**Lesson set 4** *(2 periods)*	
Concepts	*Concept Map 1 (unguided)*	Variation Recombination Crossing-over	*Concept Map 2 (guided)*	Variation Mutation Natural Selection Sexual Selection	*Concept Map 3 (guided, reworked from CM 2)*	Genetic drift Gene flow Gene pool Population	*Concept Map 4 (guided, reworked from CM 3)*	Variation Mutation Selection	*Concept Map 5 (guided, reworked from CM 4)*
Example organism *(kingdom)*		*Columba livia* (animal)		*Poecilia reticulata* (animal)		Forest trees (plant)		Bacteria (bacteria)	
Epistemic activities		(1) Asking Questions (2) Developing and using models (3) Developing and arguing about explanations		(1) Asking Questions (2) Planning and conducting investigations (3) Analyzing and interpreting data (4) Developing and arguing about explanations		(1) Asking Questions (2) Developing and using models (3) Planning and conducting investigations (4) Analyzing and interpreting data (5) Developing and arguing about explanations		(1) Developing and arguing about explanations	

Figure 2. Overview of the lesson sets containing relevant concepts, example organisms used, and included activities.

(everyday) lives and enable them to discuss currently relevant issues by introducing the processes that lead to antibiotic resistance (Williams et al., 2018).

Concept Mapping in the Unit

The teaching unit is preceded by a 90-minute concept mapping crash course to prepare the students for the recursive concept mapping tasks in the unit. Concept mapping has been shown to have positive effects on students' conceptual learning in biology (Schmid & Telaro, 1990) and support knowledge integration processes (Schwendimann, 2011). Digital concept mapping is particularly useful as it makes it easier for students to move concepts and connections or erase them and create new ones (Brandstädter et al., 2012).

The concept mapping crash course starts with a theoretical introduction, which aims at explaining the application and benefits of the method (Lenski & Großschedl, 2021). This is followed by an explanatory video in which students learn to use the digital concept mapping tool imbedded in the learning platform (Chiu, 2004). After this, students engage in a first concept mapping task in which they collaboratively create a concept map on a well-known topic (i.e., the school system; Chiu, 2004; Lenski & Großschedl, 2021; Schwendimann & Linn, 2016). This is followed by a consolidation phase, in which students find the mistakes in a faulty concept map and are given the opportunity to ask questions (Lenski & Großschedl, 2021; Schwendimann & Linn, 2016). In the final task, students create their first concept map on evolution based on their prior knowledge (Lenski & Großschedl, 2021).

Throughout the unit, four concept mapping tasks are implemented after each of the lesson sets, meaning students will create five concept maps in total (Figure 2). The first concept mapping task does not provide students with concepts or a detailed task in order to assess students' prior knowledge without interference (see above). The consecutive four tasks follow the same basic structure: They provide (1) a task sheet containing the guiding questions of the respective learning set and metacognitive prompts (Cañas & Novak, 2006; Großschedl & Harms, 2013), (2) a concept mapping cheat sheet recounting the process of creating a concept map (Lenski & Großschedl, 2021), and (3) a list of fourteen central concepts available to the students for use in their maps (Ruiz-Primo & Shavelson, 1996). From the third concept map onward, students are redirected to their previous concept map and instructed to rework it to include newly acquired knowledge and change their maps where necessary (see Kern & Crippen, 2008). This provides them with an opportunity to reflect on their knowledge structure and integrate newly learned concepts (Linn, 2006).

Adaptations

The unit is structured into four lesson sets and the concept mapping crash course with limited interdependence between the parts. The concept mapping crash course and the tasks could be omitted entirely, as could the first lesson set if the students are well acquainted with recombination and crossing-over. The second and third lesson set form the core of the unit and could be taught independently of the rest as a short unit on factors of evolution. The fourth lesson set could be added to any unit on factors of evolution as transfer and consolidation. Alternatively, parts of the lessons that are meant as individual or partner work could be worked out with the entire student group and students could be provided with questions, hypotheses, or partial explanations from the script by the teacher. There are many possibilities to adapt the unit to learners' preexisting knowledge, time constraints, or curricular demands.

○ Enactment

A small pilot study was conducted with upper-secondary-level biology courses (*N* = 76) in the school year 2021/22 in Schleswig-Holstein, Germany. Two of the courses completed either the fourth lesson set or the concept mapping training and one course completed both. The lessons were taught by the first author while the respective biology teachers were present. Afterward, the students were asked to fill out a short questionnaire covering students' perception of the tasks, materials, and lesson structure. The teachers were also asked to provide feedback. The answers were analyzed and used to improve the respective lesson sets.

The main data collection was conducted in the school year 2022/23 with upper-secondary biology courses (*N* = 382) in Schleswig-Holstein, Germany. Interested biology teachers received a one-day professional development workshop from the developers of the unit. In the following weeks, these teachers taught the unit to their upper-secondary-level courses. Each teacher was supported by the developers through visits for pre- and post-test, an online seminar after completing half of the unit, and ongoing support as needed. The teachers also received extensive materials on the unit including lesson plans, sample solutions, and additional information and tips for each lesson phase incorporated into the Moodle platform (see Supplemental Material provided with the online version of this article).

Data collection included data from (1) pre- and post-tests, (2) tasks within the unit, and (3) students' interaction with the unit (process data). In order to assess students' prior knowledge on the topic as well as additional variables such as reading competency or motivation, a pre-test was conducted before the first lesson. Students' knowledge before and after the unit was assessed using the CANS (Conceptual Assessment of Natural Selection; Kalinowski et al., 2016) and two ACORNS items (Assessing Contextual Reasoning about Natural Selection; Nehm et al., 2012). In addition, two ACORNS items were given after each lesson set.

○ Conclusion and Outlook

At present, the teaching unit has received mostly enthusiastic feedback from teachers. Preliminary analysis of pre- and post-test results of the first groups hints at an increase in conceptual knowledge about evolution. The ensuing analysis of the learning trajectories will be based on learning progression analytics approaches (Kubsch et al., 2022). We will analyze students' tests, artifacts from tasks, and, most importantly, their concept maps. Through the use of machine learning methods, we plan to integrate as much of the various data generated within the unit as possible into the analyses.

Upon completion of the enactment, the unit will be revised utilizing feedback received from the participating teachers and students as well as the developers' own experiences during data collection. Depending on the data, focal points for revision may be the structure of the tasks (e.g., some small tasks could be combined for better usability), a wider variety of example species used in the lesson sets, and the overall usability of the unit on the learning platform. By developing this unit and analyzing the generated data using machine learning methods, we will gain deeper insights into students' learning about evolution, which in the future will support students and teachers more individually.

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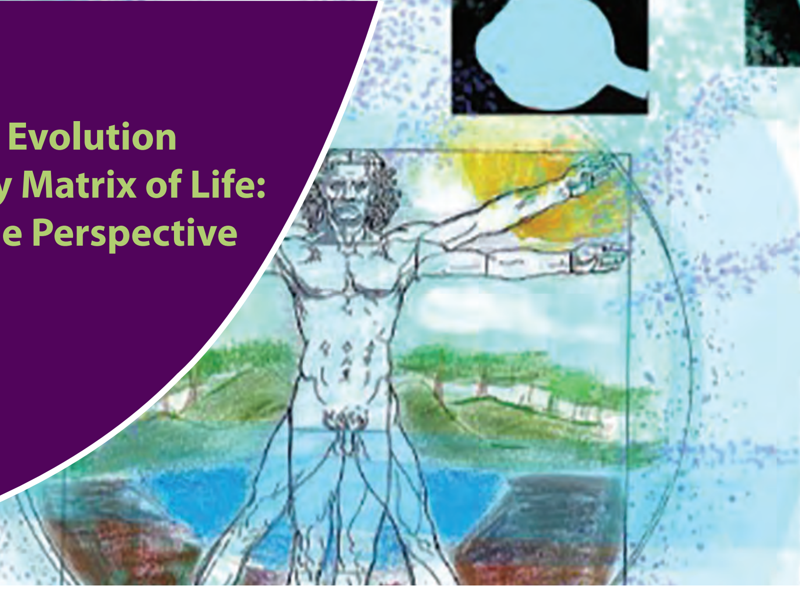
BERRIT K. CZINCZEL (czinczel@leibniz-ipn.de) is a Ph.D. student in Biology Education at the Leibniz Institute for Science and Mathematics Education. DANIELA FIEDLER (dfiedler@ind.ku.dk) is a tenure-track assistant professor in the Science Education department at the University of Copenhagen. UTE HARMS (harms@leibniz-ipn.de) is a professor in the Biology Education department at the Leibniz Institute for Science and Mathematics Education.

FEATURE ARTICLE

Teaching Biology & Evolution Through the Watery Matrix of Life: A Genomic Medicine Perspective



CARYN BABAIAN, SUDHIR KUMAR,
SAYAKA MIURA

Abstract

Water is one of the most common molecules in the universe. Water is polarized, but it has many states besides the normal tetrahedron depicted in standard biology texts. Water is also the most ubiquitous molecule on Earth, the universal solvent. It is the internal and external habitat of cells. Ecologically, water is contiguous with life and the chemistry it nourishes. Water merges with everything from DNA to itself in the vast ocean; it is a constant molecule, and it does not change—or does it? Water, is the planet's unwavering, flowing, but fixed liquid substrate, and it has an elemental and evolutionary story to tell. Water can independently regulate solute transport, entangling with cell proteins to create the aqueous conditions that support life metabolisms and the evolution of other molecules. Water dynamics are rarely mentioned in standard biology discussions, even though biomolecules are strongly influenced by the hydration shells around them. For water to affiliate with all things living requires specialized entry and exiting of water, achievable by a ubiquitous channel protein called an aquaporin. In this article, we will explore water's often neglected complex relationship with all things biological from an aquaporin perspective. The aquaporin family of proteins is ancient and spans the tree of life in archaea, bacteria, protozoa, fungi, plants, animals, and viruses. From DNA to osmoregulation, aquaporins literally channel the water molecule through geological time. We will also explore the bigger picture of the aquaporin as a teaching tool for evolution. Through the genomic medicine paradigm, we examine diseases that manifest from defective aquaporins. From a visual and arts perspective, we reframe biological processes in the light of the most abundant but nominally understood molecule on Earth: water.

○ Introduction

The Aquaporin Teaching Hypothesis

Few concepts unite biological systems like evolution. It is frequently said that "nothing makes sense in biology, except in the light of evolution." This quote from Dobzhansky has become iconic, and while true, biology may make even more of a palpable sense in the light of water. In fact, it's not just biology but evolution itself that could be illuminated through water's diverse conduit, the aquaporin. If we shift the paradigm we can view water's taciturn evolutionary role with life processes through an *Aquaporin teaching hypothesis*. For almost all metabolisms, reactions, divisions, replications, mutations, and conformational changes to occur in a living cell, water is required. Water is biologically ubiquitous, but still underrepresented in conceptualizing the molecular world of biology.

For the earliest metabolisms, the first nucleic acid synthesis needed regulated water concentrations. Once cell membranes are formed, an adjusting conduit from outside to inside becomes a necessity. How and when did this first occur? Can aquaporins help create an evolutionary narrative in biology? Aquaporins were discovered toward the end of the twentieth century. Since then, an increasing number of genome and transcriptome sequencing projects have produced thousands of orthologous channels across the tree of life from archaea through diverse eukaryotic cells, encompassing all the domains of life, including non-cellular viruses (Finn, 2015). From viruses to plants, to fungi, to humans, aquaporins are the interface between watery ecologies inside and outside the cell. From molecular associations to infectious disease transmission, to biomolecule fundamental function, to massive self-associations in aquatic ecosystems, water is the adaptable constant in life-shaping moment-by-moment homeostasis through the longest geological time frame. Most biological processes occur in an aqueous environment. To achieve a cell's aqueous environment, water must be present. The facilitator between water's journeys from outside to inside is the transmembrane amphipathic aquaporin channel protein and its associated membrane, also referred to as a "water channel." This dynamic, interactive interfacing channel is the obligatory accomplice that proceeds everything from larger system metabolisms to DNA stability. Since water is the major prerequisite for life, water is an evolutionary molecule, one that contributes to the evolutionary process, and the aquaporin is the phenotypic byproduct of change reflecting water's sweep into and across all lineages in the tree of Life. Students can learn about water and its cellular connections to life through the art and drawing processes presented in this paper.

> *From viruses to plants, to fungi, to humans, aquaporins are the interface between watery ecologies inside and outside the cell.*

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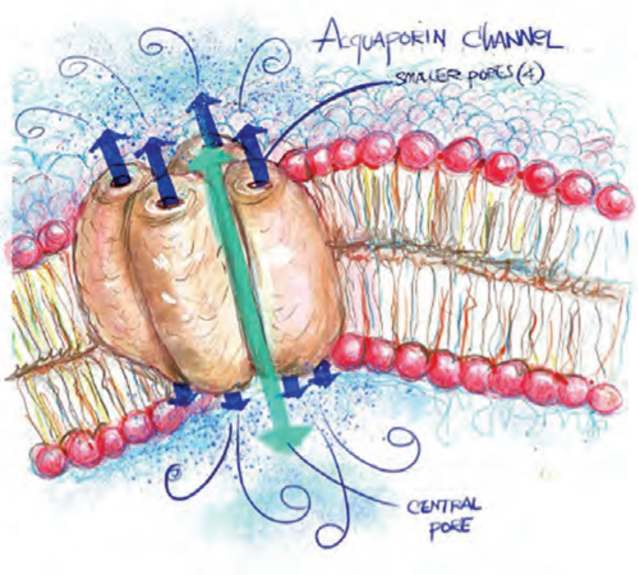

Figure 1. AQP channel with four subunits, arrows indicate movement of water.

The AQP family of proteins passively and selectively shuttle water and other small molecules into and out of cells (Figure 1). They can be divided into two broad categories: the AQP water-only aquaporins and the AQP glycerol aquaporins (King, 2004). Aquaporins are the foundation of homeostasis through osmoregulation and contribute to the overall shape, metabolism, reproduction, biological recognition, and energy states of cells (Fox, 2017). The discovery of aquaporins late in the twentieth century on the surface of red blood cells resulted in a small paradigm shift regarding membrane permeability, one most students never encounter in biology class, imagining that water moves through membranes only via osmosis. The standard discussion on membrane permeability normally does not connect to evolution or the tree of Life, but through aquaporins, a richer, more complex view of evolving cells and the tree of life is possible. Pressured by the necessity of water-biomolecule interfaces leading to increasingly complex molecular relationships, aquaporins have come to occupy niches in physiology, enabling life processes to happen in varying and specific ways as there are many differently sized water channels with a variety of functions. These nuanced requisite reaction interfaces allow organisms to use water and solutes to attune and become one with the place they inhabit, whether terrestrial or aquatic.

Water-specific proteins are typically discussed only in the osmoregulation of the kidneys and related structures, but AQPs are found not only throughout the tree of Life but also throughout the human body (Finn, 2004). From an evolutionary perspective, water is a constant and aquaporins may have been the first communicating conduits of cells such as the hypothetical and elusive LUCA. In an ecological sense, aquaporins through distinct cellular spaces, give organisms physiological plasticity and specific adaptations on an evolving watery planet. Water is involved in DNA-ligand binding, which helps drive evolutionary specializations. In prokaryotes such as lactic acid bacteria, AQPs assist with the fermentation process (Tong, 2019). Water moves through AQPs in protozoans, such as the amoeba and paramecium, and affiliates with specific and specialized vacuoles. In fungi, specific aquaporins are essential for fruiting body emergence and gene expression for spore activation and dispersal (Nehls, 2014). In non-vascular plants such as bryophytes, aquaporins facilitate desiccation intolerance (Oliver, 2008). In ferns, water provides the medium for the movement of sperm and aquaporins mediate ion compositions in varying soil types affecting fern reproduction (Lin, 2021), and in higher plants xylem and phloem are regulated by aquaporins that contribute to the viscosity of dissolved sugars. In our own bodies, aquaporins are found in the membranes of red blood cells, adapting their shape to the viscosity and solute concentration of blood and in the kidneys they help create a salty niche for regulating water. Diseases and mutations in aquaporins can reveal their widespread function throughout the human body providing a more comprehensive view of our intimate relationship with the water molecule. It becomes apparent that across the tree of life, aquaporin's channel the planet's water.

○ Water Across the Tree of Life, from Snowflakes to Raindrops to DNA

Most students are introduced to early life and macroevolution through concepts of a reducing atmosphere on Earth, tumultuous oceans, lightning storms, Miller, and Urey, and ultimately the formation of small biomolecules where somehow electric currents "zapped" life into existence. Electric interactions played a fundamental role for the properties of hydrated bio interfaces where water molecules interact through electric fields. Water is both the source of those charged interfaces and the constituents of them (Shweta, 2018). Bio interfaces through electrostatics and Van der Waal forces are foundational in membrane formation, metabolisms, and DNA binding (Nguyen, 2008). DNA is embedded in a water shell and its shape and function are modeled by the ions around it. "*Water and ions control chemical processes in biological systems thereby directly and indirectly facilitating the functions of biomolecules such as nucleic acids*" (Shweta, 2018; Ball, 2008). Water not only created the conditions for life to emerge and for DNA to replicate, but it could form extensive networks because of its bonding, form crystals to surface tension, and link up with DNA to encourage substrate bonding.

Water is indeed an unusual molecule; it has not changed in billions of years and yet its polarity allows it to dissolve other molecules and dissociate into hydronium and hydroxyl ions generating the pH scale that all students encounter in basic chemistry. Dissolved minerals from rocks created the salty oceans, which flow into the unsalted regions of the hydrological cycle to freshwater basins, through this cycle water becomes part of our physiology and part of our DNA. Genes and chromosomes do not function without water, enzymes don't work without water, blood doesn't exist without water, oxygen isn't generated if water isn't present, and in water's absence, a wide range of desiccation-based adaptive strategies fail to evolve. Is it possible that in teaching evolution we might consider that water co-evolves with proteins, nucleic acids, and cells? Like an enzyme water interfaces temporarily and yet remains unchanged. From an aquaporin perspective water soon becomes the main character in the story of evolution instead of a backdrop and something that can give students a bigger eco-evolutionary picture. *"For long, water was considered as rather a passive background matrix on which only suitable biomolecular structures were thought to permit the mammoth of biological functions"* (Gerstein and Levitt, 1998).

Aquaporin evolution would have to have arisen very early as an absolute necessity of cells to regulate water in specialized spaces to perform its rapid, multitude of interactions. Water contains within its fluid structure a slightly electronegative oxygen and two slightly

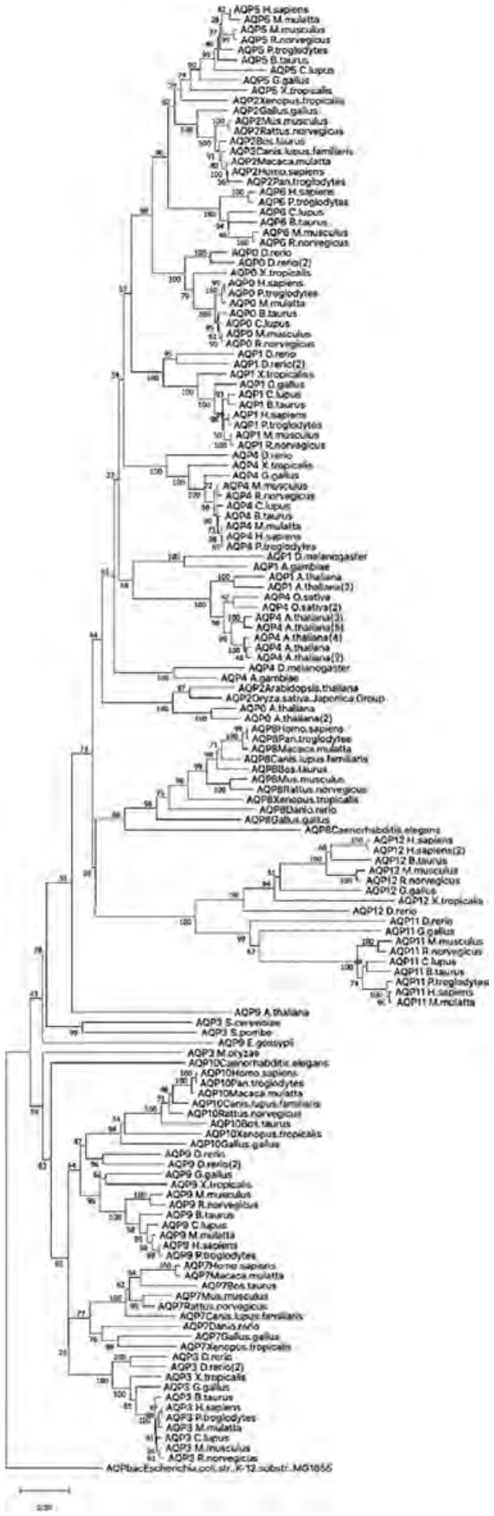

Figure 2. An evolutionary tree of Aquaporin gene family reconstructed using the MEGA software. Aquaporin protein sequences were acquired from the HomoloGene database (https://www.ncbi.nlm.nih.gov/homologene/). They were aligned utilizing CLUSTALW in MEGA, employing the default parameters. The neighbor-joining method was used to build the phylogeny based a Poisson model, with 500 bootstrap replicates used to assess the robustness of the inference. The resulting phylogenetic tree was rooted with a sequence from *Escherichia coli*. Step-by-step instructions are available from igem.temple.edu/genomicmed.

electropositive hydrogens. This is enough to provide a jump start to ion exchange and interfaces the kind that fuel molecular diversity and sophistication. This ubiquitous nature of aquaporins across geological time is visible in the AQP tree (Figure 2). The tree is rooted in a prokaryote (*E.Coli*). Teachers can have students make the tree or they can just examine the various species on the tree and look at relatedness. Phylogenetic trees using such a ubiquitous protein channel give an automatic "big picture" view to evolution and the shared aquaporin protein. A fundamental question to present to students might be "How does the phylogenetic tree make sense of relatedness across the tree of life?" or "Why are aquaporin's found in almost every living thing and in every part of our body?" "Big" questions can reframe the diverse roles of aquaporins and the absolute necessity of water. (Please see our website at the end for activities.)

○ Aquaporins: Current Background

When did AQPs come into the watery scene of Earth? AQP ancestors started in prokaryotes by the duplication of genes that then diversified to eukaryotic cells evolving specific carboxyl terminal functional groups that can be traced to plant and fungal lineages (Ishibashi, 2017). Whole gene duplications and HGT played a role in AQPs spreading through invertebrate and vertebrate linages. Viruses probably acquired the AQP through HGT (Ishibashi, 2020). The necessity of aquaporins throughout the tree of life is clear, living cells and even viruses need to channel water to grow, move, replicate, and evolve. Phylogenetically, aquaporins belong to the major intrinsic protein (MIP) family that is composed of more than 1700 integral membrane proteins (Baiges, 2002). Biochemical analysis of aquaporins indicates a fundamental monomeric structure with a signature amino acid sequence of Asn-Pro-Ala and an amphipathic channel lining. Aquaporins are situated in the membrane as tetramers with each monomer component containing a channel for water, glycerol, small ions, and even simple gases such as carbon dioxide (Kruse, 2006) (see Figure 1). Aquaporins, like all biological molecules, wear many hats and not surprisingly since they are found in so many types of cells and almost all tissues and organ systems of the body. Since sequencing methods have improved radically since the 1990s, a huge diversity of aquaporins have been discovered. Regardless of remarkable structural similarity between all aquaporin channels, it is also not surprising that they contain refined and slight changes to their structures to adapt and adjust water and affiliates to unique cell and tissue requirements as there are 13 known AQP isoforms in humans (Zhu 2016). A gating mechanism strategy employed by aquaporins is a narrowing of their channel, which sits above the bilayer of the plasma membrane, along with a highly conserved arginine residue in a fixed position with a strong negative charge acting as a second gating mechanism (Sachdeva, 2014). Aquaporin permeability can be altered by pH, temperature, concentration, and a host of other variables. The aquaporin family tree roughly correlates with their permeability characteristics indicating that their early transport function was most likely involved in cell evolution and regulation of basic activities (Finn, 2015). Aquaporins can also be very specialized, in some organisms such as mosquitos they can transport sugars such as trehalose, while a mammalian aquaporin AQPb0 ortholog may function as a cell adhesion molecule (Kumari, 2013). Despite being labeled as water channels, aquaporins can accommodate a large diversity of molecules, where and when such diverse lineages and function arose through time is a complex and unresolved subject in aquaporin biology and too tangled for our purposes, but it reveals that there are many unresolved questions about both water and their AQP channels including how water contributed to AQP evolution. We want to explore the potential of aquaporins to inspire critical and divergent thinking in students regarding evolutionary processes and for students to see the connection between the planet's hydrological cycle, evolution, and physiology.

○ Water and AQPs at the Molecular/ Evolutionary Interface

Depictions and descriptions of DNA replication and repair, mutational events and conceptual models and mechanisms of evolutionary and metabolic change almost never include water, and it's easy for students to forget how critical water really is to life because of this absence. For mutations to take place, an aqueous environment is required, the dynamics of such things as DNA repair enzymes require water, but do students actually think about this when studying mutations? In this way aquaporins become the changing, evolving artifact of such dynamics. It might be said that water is essentially the cocreator of the diversity of life on Earth and integral to the molecular evolution hypothesis. Water makes life evolve, it proceeds every reaction and therefore evolution-based processes such as mutations. Water, aside from its osmoregulatory association, participates in decreasing the entropy of a system as it participates in every transitional form through hydration, facilitating conformational change and dynamics such as enzyme and substrate interactions (Davidson, 2013). Students can think about mutational events by hydration shells and by taking a close-up look at how water might regulate such events (see Figure 3). What about the dynamics around the DNA molecule? Can water influence mutations? The rate of evolution? Something students are never taught in units of DNA is that the DNA in their cells exists with vibrational layers of water and that stability of the sugar-phosphate backbone contains what is referred to as "the spine of hydration" (Shweta and Sen, 2018). Many significant facets of DNA's function, that is ligand binding, excision of mismatched bases, and stability, are contingent on water's interface with DNA, which is adjusted and synchronized by aquaporins. Further downstream from DNA's function is the transcriptome and then the proteome, which are further guided by water's availability influencing such critical events as protein folding (Shweta and Sen, 2018).

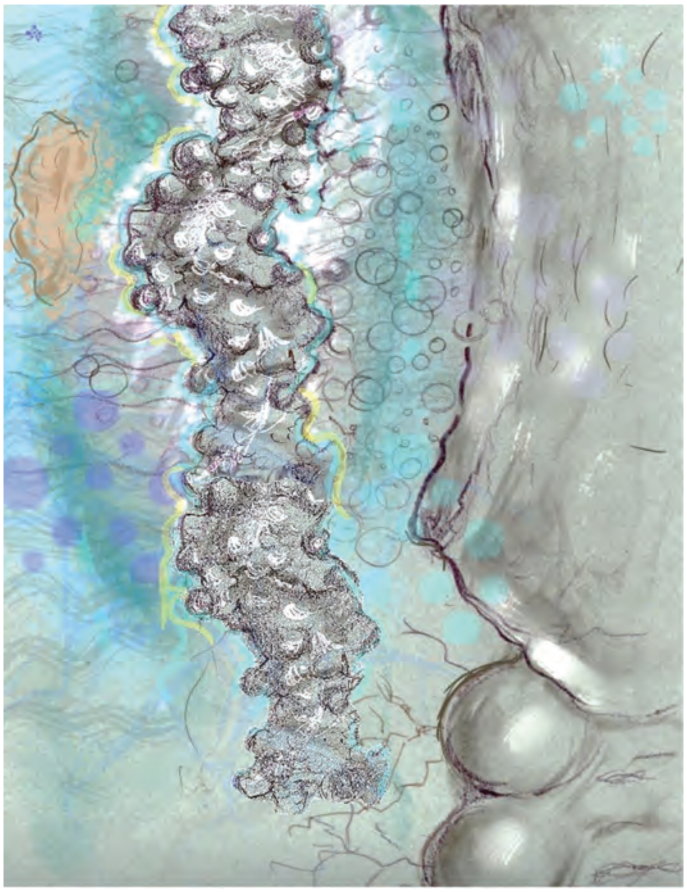

Figure 3. A watery sheath around DNA near an aquaporin.

○ Connecting the Hydrological Cycle to AQPs to the Evolution and Physiology of the Kidney Tubule

Students don't normally connect the hydrological cycle of the planet to their own kidneys and blood, but blood is like a liquid ocean inside our body carrying the ancient solutes in water around to terrestrial environments. Regulating water is often placed under the broad concept of energy gradients with a discussion on osmosis, which does not require energy. Students are given three different environments with three red blood cells. In textbooks the cell's fate in different solutions is visualized as a isotonic, hypertonic, or hypertonic condition. This easy illustration demonstrates solute concentration inside and outside of cells and passive movement of water. Wet labs further enhance the understanding of the effect of water on cells, such as the shrinking or bloating of an elodea leaf. Students are taught with models of salt water, freshwater, and brackish water fish, such as sharks, to illustrate organ systems of vertebrate adaptation to watery and salinity variable environments. This discussion can also move from planarian and flame cells, Malpighian tubules in insects, and ultimately to the mammalian kidney. All these representations of life are linked through the AQP. Like all sequential topics, underlying connectedness, despite being obvious often goes missing. Bridging water and evolution across the tree of life is quite easy if we look at AQPs. Our shifted AQP paradigm focuses on water and aquaporins as the universal drivers of evolution.

○ An Easy AQP Example: The Terrestrial-Aquatic Niche of the Nephron

Some phylogenetic analysis of aquaporins reveals that our mammalian water channels go back to the Devonian period (Finn, 2014). Aquaporins have been used to trace tetrapod evolution through coelacanths, anurans, and Amphibia with mammals retaining the ancestral properties within the aquaporin (Finn, 2014). When students study the comparative anatomy of the osmoregulatory systems they often encounter amphibians who use their skin and kidneys to regulate water. They also encounter a diverse system of waste output from uric acid crystals in reptiles and birds, to urea and ammonia output in fish and mammals. This suggests that aquaporin's evolved gene roles, retaining some functions, losing regions, and localizing expression of specific aquaporins as some survived extinction and were repurposed for various adaptations. As an example, mammals are the AQP6 aquaporin that was retained from reptiles and functions in the intracellular spaces of podocytes in the glomerulus contributing to acid-base balance and glomerular filtration, but have a reduced function compared with other aquaporins (Nishimura, 2013). For terrestrial animals, retaining water is essential. The evolutionary significance of water conservation is visible in the inherited disease diabetes insipidus, which is a nephrogenic disease caused by mutations in AQP2 or the AVPR2 receptor. Large amounts of urine result, causing rapid dehydration (Grunert, 2020). This unresolved evolution to tetrapod evolution segues into a discussion on the amazing configurations in our own kidneys. The nephron of the kidney is perhaps one of the most relatable models for illustrating the specialized niche of water in cells, the evolution of an entire system around the water molecule and the aquaporin proteins, which facilitate and expand the repertoire of water in its diverse capacity for the orchestration of a broad variety of specific molecules and ions in a specific salinity and niche within an organ. The basic functioning unit of the kidney, the nephron is a fascinating brackish vicinity where a clear divergence of habitats has evolved in one place, similar to a biome that contains both a deciduous forest and a wetland. The kidney highlights a complementary evolution of aquaporins in lieu of water. When the circulation of blood enters the afferent arteriole from the renal artery into the glomerulus inside Bowman's capsule, we get an immediate constriction of the renal arteriole into a bunched-up tuft of capillaries, in that constriction, the plasma of blood becomes what we call filtrate and this is further facilitated by fenestrations and podocytes lining Bowman's capsule. Water thus changes its solute-carrying potential and makes its way into the proximal convoluted tubule. The nephron projects into the medulla of the kidney, an environment where concentrations of salt are maintained to facilitate further regulation of the water content of the body. In the nephron we have two opposing tubules, the proximal descending loop and then the distal ascending loop, in the middle connecting them both, the loop of Henle. The proximal side is rich in aquaporins, the distal side is completely devoid of aquaporins and instead contains transmembrane protein channels, which are possibly the evolutionary great grandchildren of the aquaporin. The proximal tubule carefully adjusts water molecules until the loop of Henle is reached. The hairpin turn of the loop of Henle is ensconced in the salty sands of the medulla, which are maintained by its multiple channel proteins, there are a few aquaporins and a few ion channels, once the loop turns upward toward the distal tubule, and then literally no aquaporins regulating various charged particles such as calcium chloride, sodium, and potassium appear to exist. Water is still flowing through this system, until it makes its way to the collecting duct, all the while the saltiness of the sandy medulla is exerting nuanced controls on water via AQPs. In the collecting duct, if there is too much water, ADH exerts its influence on the kidney by genes turning it up a little or down a little, as ADH is antidiuretic, and is there to modulate loss or retention of water until it becomes urine. In this way, water, salts, and other ions are under continual homeostatic control, varying output all the time so the blood osmolality is maintained. How did aquaporins come to reside on one side and not the other? What drove this unique juxta positioning of proteins in tubules? What fostered the divergence of the aquaporin protein into the same tubule making them dissimilar and distinct, with antagonistic functions? Students can think about what pressures drove the nephron tubules and the surrounding space in the urinary system to evolve.

○ AQPs in Disease: A Genomic Medicine Perspective

AQPs are found throughout the body, anywhere mucus is produced, gas exchange takes place, and water is tightly regulated. The old term for the posterior chamber of the eye, the "aqueous humor" was more descriptive, as AQPs are found in the eye, in the lungs, in the digestive system, in the blood-brain barrier, on erythrocytes, in the cerebellum, in cartilage, in the urinary system, and the list goes on. To address this universality, we have created a landscape image of AQP locations (see Figure 4). Teachers can use this figure to answer students' questions about anatomy location and physiology and then they can move into a discussion on disease. Not surprisingly, many diseases from complex to infectious, to Mendelian condition implicate AQPs. In skin disease, the AQPs are expressed throughout the major divisions of the cutaneous skin, from hypodermis, to dermis, to epidermis. In diseases such as psoriasis, there is implication that keratinocyte physiology may involve aquaporins through a proposed down-regulation and mislocalization of an AQP (Bollag, 2020). Even in melanoma, aquaporins are implicated. An autosomal dominant cataract disease involves an APQ0 mutation (Verkman, 2008). In the neurological conditions involving cerebral spinal fluid, AQPs play a central role in edema, traumatic brain injury, brain tumors, and stroke as they become severely disrupted in the regulation of water channels through cell membranes and the blood brain barrier (Filippidis, 2016). Functionally diverged human aquaporins are involved in a wide variety of non-infectious diseases including renal dysfunction, neurological disorders, epilepsy, obesity, metabolic syndrome, and heart disease. Even in infectious diseases, pathogenic bacteria, fungi, viruses, and parasites can cause systemic infections and sepsis, facilitated by compromised aquaporins (Azad, 2021). Bacterial endotoxins have an effect and can even regulate aquaporin expression (Azad, 2021). The Plasmodium that causes malaria can influence aquaporins in the liver of the host as a host membrane sheaths the parasite when it merges in the cells of the host, the aquaporin in this membrane provides the AQP3 aquaporin that enables the transfer of nutrients from the host to the parasite (Liu, 2016). This would make the aquaporin a target for malaria treatment. In the human body, students can explore the tissue-specific distribution and physiological relevance of aquaporins by looking at a visual map of them. Aquaporins are in the lungs, immunity, wound healing, cell proliferation, and transport of waste. Their global, universal appearance and the appreciation of aquaporins suddenly make water a remarkable valuable biological topic.

AQPs have important roles in the host-pathogen interaction (Azad, 2020). Those roles roles include the homeostatic control of inflammatory processes (Azad, 2020). To explore the physiological and pathophysiological landscape of AQPs, see Table 1.

○ Water as We Age: An Aquaporin Aging Hypothesis?

The control of water homeostasis in living cells is facilitated by aquaporins, which can be extremely selective, but the water in cells expands its repertoire of interactions to all cellular components and, therefore, aquaporins cannot be viewed simply as homeostatic for osmoregulation. For their complex role, aquaporins must be able to exclude hydrogen peroxide and hydronium ions, while

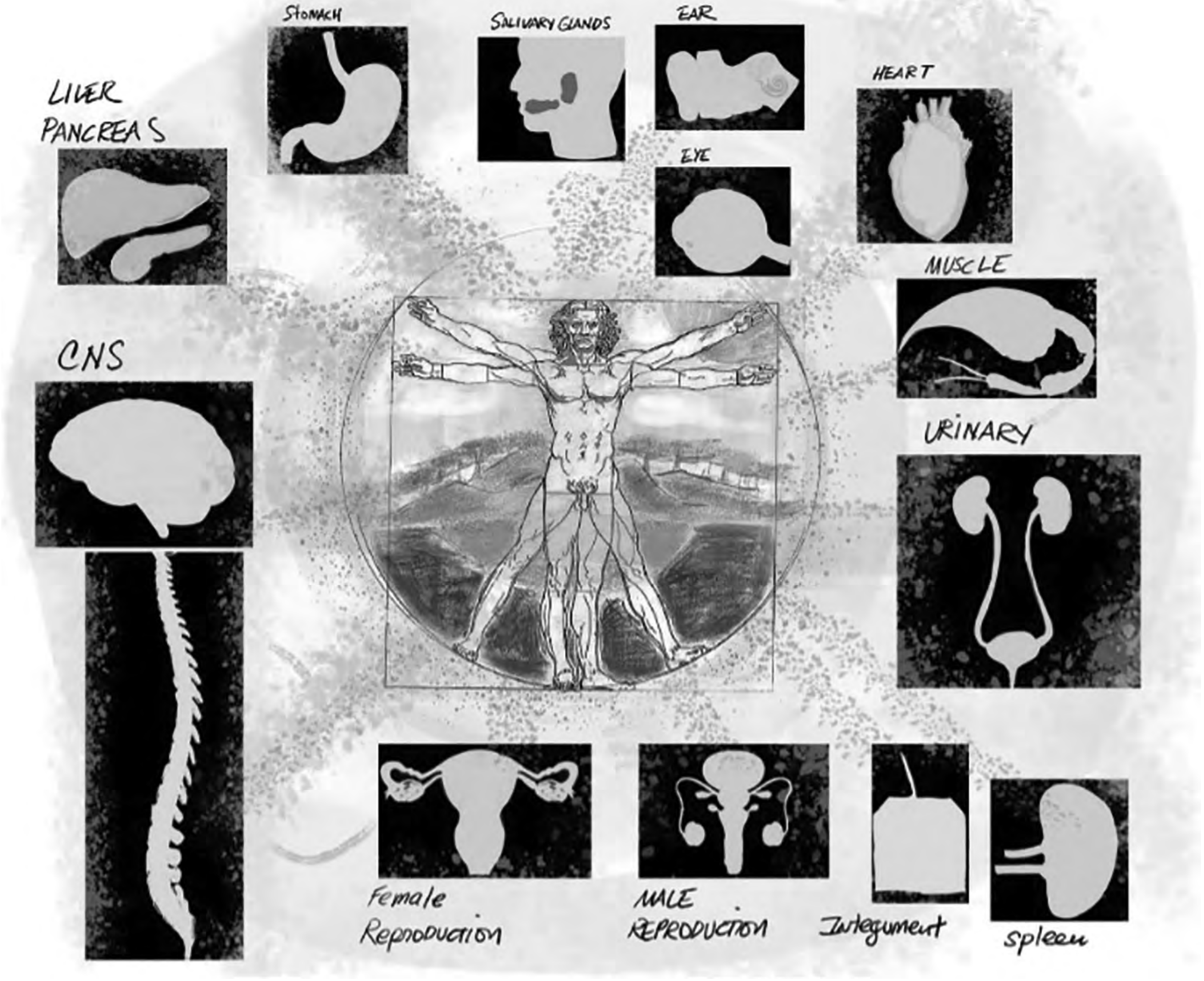

Figure 4. A landscape of the ubiquitous AQP associations with the water cycle depicted behind the Vitruvian man figure. This figure is repeated in black and white in the supplementary section for students to add AQPs to color in, and to label the basic parts of the hydrological cycle.

also preventing the transport of protons. The exclusion of hydrogen peroxide links their functionality to mitochondrial function and to a myriad of other reactions. Proton pumps, chemiosmosis, and energy gradients are, therefore, also dependent on aquaporins (Tesse, 2018). From the GM perspective, mutations in aquaporin genes, as well as the process of aging, reveal the problematic diseases that arise when fundamental channels of homeostasis are disrupted or when senescence of cells occurs. Humans don't desiccate and come to life, but they do dry out as they age. As humans age, aquaporin expression is reduced, fewer and fewer membranes support the functionality of aquaporins. An excellent part of this is in the joints of the body. The plasticity of gene expression and the output of the proteome to make an aquaporin channel, may be timed as well as activated by physical stressors that downturn the expression of the AQP genes through a feedback loop and this might occur throughout the body in eyes, brain, joints, intestines, lungs, and skin (Azad, 2020). Knee cartilage is an aqueous environment filled with synovial fluid. Synovial fluid consists of water, nutrients, and ions that maintain chondrocyte production (Kyung, 2021). Cartilage, a connective tissue, is well hydrated at approximately 75% aqueous water (Kyung, 2021). Aquaporins play a critical role in not only in fibrocartilage functionality but in the inflammatory process. In diseases such as osteo arthritis, gene and protein expression can become profoundly reduced, this prevents chondrocytes from conducting mitosis and differentiating. Responsiveness to reactive oxygen species (ROS) such as hydrogen peroxide can be affected. Expression of aquaporins along with histological images demonstrate that this expression occurs globally, throughout the body cells. Simply put, their surrounding media shrink, and tissues become drier with age (Zhou, 2020). This is another highly visible teaching point that can be immediately be seen in histological images (see Supplementary Material provided with the online

Table 1. Shows a summary of only a few of the known functions of AQP's and related pathophysiology as they occur throughout the body.

	System/Anatomy	AQPs	Function	Associated Disease
Integumentary	Epidermis, dermis, subcutaneous	APQ0, APQ2,3,4,5,6,8,10, & 11	Skin elasticity, cell migration, immune function	Psoriasis, melanoma, wound healing, edema, impaired leukocyte function
Skeletal/Muscle	Striated muscle	APQ 1,3, & 4	Dystrophin associated	
CNS	Brain & spinal cord (blood-brain barrier)	APQ 1,3,4 & 9 (spinal cord) APQ 1 & 4 (brain)	CSF, water homeostasis in the CNS, blood brain barrier metabolism	Edema, hydrocephalus, brain edema, stroke, MD, brain tumors, neurological disorders
Digestive	Mouth, stomach, small intestine, duodenum, jejunum, and ileum, large intestine, liver, pancreas, gallbladder	APQ 1,5, & 8 (salivary glands) APQ 1,3, 5 (oral cavity) APQ1-11 (stomach & small intestine) APQ1,2,3,4,7,8,9,10, 11 (large intestine), APQ1,5,8 (liver & pancreas)	Salivary function, modulation of membrane permeability, mucus production, carbohydrate metabolism, pancreatic exocrine secretions	Sjogren's syndrome, leaky gut, obesity, cholestatic liver disease, liver cancer, IBS
Respiratory	lungs	APQ 3,4,5 (lungs)	AQPs expressed in ciliated columnar cells, support and surround alveoli, pH support, surfactant production, gas diffusion	Lung cancer, asthma, fibrosis, edema, infectious diseases
Circulatory	heart	APQ1,3,4,5,7,9,10, 11 (heart)	Cell migration, pericardial function, inflammatory response, cardiac water homeostasis,	Myocardial edema, arrhythmias, and sudden cardiac death
Urinary	kidney	APQ1,2,3,4,5,6,7,8,11 (kidney)	Glomerular filtration, acid-base metabolism, tubular endocytosis, total water balance	Mutation or functional deficiency of AQP2 leads to severe diabetes insipidus
Reproductive male	Testes, seminal vesicles, vas deferens, epididymis	APQ 0-11	Spermatogenesis, mucus production, seminal fluid	abnormal sperm motility, the abnormal epididymis and infertility seen in cystic fibrosis
Reproductive female	Ovaries, Vagina, uterus, oviduct, placenta, fetal membrane, embryo	APQ 1-12	Mucus production, follicle development, and implantation	Infertility, miscarriage, ovarian cancer
Eye	senses	APQ1,2,3,4,5,7,9,11	AQPs in the eye: water homeostasis, intra ocular pressure. Retina, tear secretion, and lubrication	Autosomal dominant cataracts glaucoma
Ear	Senses	APQ 1,2,3,4,5,6,8,10,11	Fluid balance in semicircular canals, inner ear	Otis media, Meniere's disease, hearing loss
Spleen	Immune	APQ1,3,9	Survival of immune cells, RBC osmolarity	

version of this article). Teachers can also raise questions about water pollutants and recombining chemicals from organophosphates to micro plastics, how do these toxins affect AQP function, what is the implication for the flow of such toxins in the water cycle and for health across the tree of life?

○ Aquaporins, Water Pollution, and Disease

AQPs present another biological relationship between water, the world of modern pollution, and disease. For most people recognizing that water is cyclical is difficult. Water is finite on the planet and most of it is cycled through living things, yet the connection between the modern toxins we produce and disease in our own bodies is a difficult concept to comprehend. Water pollution comes from many sources including air and soil as water cycles through rainfall and ground water. How do conserved, ancient water portals (AQPs) accommodate such a plethora of synthetic toxins? How do these chemicals affiliate with water? How does that affiliation change an aquaporin's response? Since water infiltrates every system of the body, contaminants such as heavy metals can alter AQP function. "AQP4 is a class of aquaporin channels that is mainly expressed in the brain, and its structural changes lead to life-threatening complications such as cardio-respiratory arrest, nephritis, and irreversible brain damage (upon heavy metal exposure) (Maroli, 2019). Nanoparticles used in water treatment have also been shown to have deleterious effects on aquaporins and cause DNA damage (Nallanthighal, 2017). This line of inquiry can lead students into an environmental unit where they can search for studies on the effects of various pollutants in water and their effects on AQPs. Connected to this topic are the yet-undetermined roles of AQPs in cancer, some research suggesting they could be tumor suppressors, other research suggests they might be oncogene triggering.

○ Visualizing and Drawing the Watery Interface

In images of biomolecules and reactions, water is rarely if ever depicted. It is an afterthought once students get passed it in basic chemistry. From excisions of mistaken nucleotides to protein folding, water's associations are not only participatory but pivotal—without them, no such activity would take place (Wspalz, 2009.) Like many oversimplifications in science, water is conveniently omitted from visualizations and discussions on evolving proteins, and rarely factored in as a variable in molecular, genomic, and cellular interactions (Dargaville, 2022). Visualizing water's intimate relationship is a challenge but one some students might find rewarding.

Would students think differently about biology if water were consistently represented in biology? Even dehydration-hydration synthesis reactions are not illustrated with the actual water molecule! Would graduate biology students and medical students have a different view of metabolism, ecology, evolution, and the human body if water were a permanent part of biological subjects, as it is in real life? Larger questions arise regarding conceptualizations and models in biology, which omit water from the equation of life. Visualizing the aquaporin's universality. The panorama of paradigms opposing, facilitating, antagonizing, and promoting metabolic innovations within cells because of water is difficult to conceptualize all at once. Asking students to visualize what DNA would look like and how it would function in a dehydrated state versus an aqueous state raises many questions about replication, binding, DNA's shape, and mutations.

○ Conclusion

Water appears simple to students, and it is mentioned only at the beginning of teaching macroevolutionary concepts, but water has many unique configurations, sheathing molecules, adapting and interfacing with their molecular structure, and providing a liquid system for reactivity making it an essential of life. We live on a watery planet and aquaporins reflect this condition in cells and their adaptations. Aquaporins, not only channel water but many fundamental compounds of life. They are widespread in the human body where their evolution can be studied throughout the tree of life, making them the best candidate to model both micro and macroevolutionary change.

In the geological time scale, through specific cellular adaptations, water and aquaporins can tie together the broad and molecular concepts of biology. Through the aquaporin lens, students can connect the big picture of a water planet over billions of years to the manifestation and an adaptation in a cell membrane to the hydrological cycle. Through a genomic medicine lens students can witness mutations that alter the function of the aquaporin protein and the changes that may produce a disease phenotype, and through phylogenetic trees they can see everything from desiccation strategies to diseases. AQPs reframe our relationship with our planet over its 4.5-billion-year history and the water-based life forms that emerge from it.

○ Supplemental Material

Supplementary teaching material is available on the genomic medicine website at Temple University. Please visit our website www.GenomicMedicine@temple.edu for teaching material and images and activities.

We have 4 activities:

1. Timetree of life: Desert vs non desert animals and aquaporins
2. Color in aquaporins of the human body: distribution and adaptation
3. What's your aquaporin hypothesis?
4. MEGA: Building an aquaporin tree

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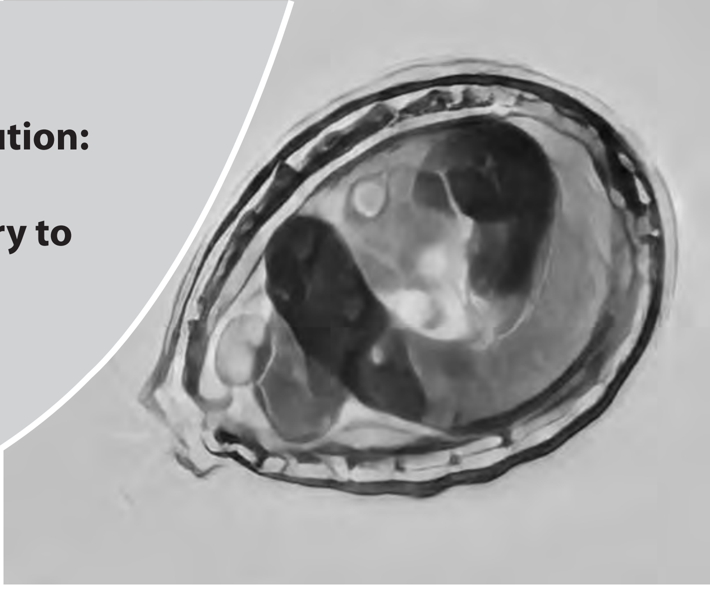
CARYN BABAIAN (caryn.babaian@temple.edu) is an assistant professor of teaching in the Department of Biology at Temple University. SUDHIR KUMAR (s.kumar@temple.edu) is a professor in the Department of Biology and Director of the Institute for Genomics and Evolutionary Medicine, both at Temple University, Philadelphia, PA 19122. SAYAKA MIURA (Sayaka.miura@temple.edu) is an assistant professor in the Department of Biology at Temple University.

INQUIRY & INVESTIGATION

Evidence for Macroevolution: Using a Microbial Phylogenetics Laboratory to Teach Endosymbiosis

JOSHUA S. HOSKINSON, JEREMY G. WIDEMAN



Abstract

The teaching and learning of macroevolutionary processes have received limited attention in the evolution education literature despite their importance in evolution acceptance and evolution understanding. This necessitates the development of pedagogical content knowledge, including best practices in curriculum and instruction, on macroevolutionary processes that support student understanding and acceptance of macroevolution. One promising approach is to consider macroevolution at the microbiological level by teaching endosymbiotic theory while capitalizing on pre-existing pedagogical content knowledge of tree thinking and bioinformatics. Here, we present a computational laboratory activity that guides students through the construction of a phylogeny based on the universal small subunit ribosomal RNA gene. The resulting phylogenetic tree demonstrates that the photosynthetic organelles of the protist Paulinella chromatophora *evolved independently of the chloroplasts of plants and algae. This not only addresses the need for pedagogical content knowledge in macroevolution in an interdisciplinary and integrative fashion, but also serves as a foundation for future research into the teaching of endosymbiosis. This activity is designed for a 15–20 student introductory/intermediate biology laboratory.*

Key Words: *macroevolution; pedagogical content knowledge; endosymbiosis; molecular phylogenetics.*

○ Introduction

Research on the teaching and learning of macroevolutionary processes has received more attention since initial calls for its inclusion (Catley, 2006; Padian, 2010). Educational research has included topics such as fossils and the fossil record (Borgerding & Raven, 2018; Dodick & Orion, 2003), transitional forms (Mead, 2009; Platt, 2009), deep time (Delgado, 2014; Stenlund et al., 2022; Stenlund & Tibell, 2019), speciation (Evans, 2000; Samarapungavan & Wiers, 1997), the evolution of multicellularity (Pentz et al., 2015; Ratcliff et al., 2014), and evolutionary transitions in individuality (Michod et al., 2022). This has facilitated the development of pedagogical content knowledge concerning best practices for teaching macroevolution (Ziadie & Andrews, 2018, 2019). This is especially important since explicit instruction on macroevolution has been shown to increase evolution acceptance (Cotner et al., 2010; Gibson & Hoefnagels, 2015; Nadelson & Southerland, 2010; Walter et al., 2013), which is a major goal of evolution education (Dunk et al., 2019; Smith & Siegel, 2016). Not surprisingly, macroevolution has been identified as a top priority of evolution education research (Harms & Reiss, 2019; Ziadie & Andrews, 2018, 2019). Because students tend to accept microevolution (within-species evolution) but not necessarily macroevolution (evolution of new species or higher-level clades) (Barnes et al., 2021, 2022), it is necessary to further develop macroevolutionary curriculum and instruction strategies.

> *An interdisciplinary approach is necessary to bridge the gaps between the fields of microbiology, cell biology, and evolutionary biology to present macroevolutionary accounts of microbes to undergraduate students.*

Evolution education has largely focused on examples of large multicellular organisms, likely because we are large multicellular organisms and our own species' evolution is important to us. However, given the political and intellectual milieu of our time, human evolution may not be the best nor most convincing starting point (Grunspan et al., 2021; Miller et al., 2006, 2022; Pobiner, 2016). Teaching macroevolutionary processes of microbes may more effectively increase evolution acceptance. Problematically, content experts of both evolutionary biology and microbiology/cell biology are usually not the same person. Evolutionary concepts are not the focus of microbiology or cell biology courses; and similarly, cell biology and microbiology, if even mentioned, are not the focus of evolution courses. An interdisciplinary approach is necessary to bridge the gaps between the fields of microbiology, cell biology, and evolutionary biology to present macroevolutionary accounts

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of microbes to undergraduate students (Burmeister & Smith, 2016; Fraga, 2018). Therefore, any tools created to facilitate this interdisciplinary approach to macroevolution education will likely start at the microbiological level. Here, we outline an introductory-level computational lab to develop a framework for introducing the evidence for endosymbiosis, the macroevolutionary process that led to the evolution of mitochondria and chloroplasts. This activity is grounded in tree thinking, which has a rich history in evolution pedagogy (Gregory, 2008; Meisel, 2010; Novick & Catley, 2016; Phillips et al., 2012), and can be used as an exemplar for teaching macroevolution at the microbiological level. This activity is designed for an introductory biology laboratory with between 15 and 20 students. In our experience, students can follow the instructions and troubleshoot the workflow with little intervention. We highly recommend instructors deliver a short introductory lecture on endosymbiosis and the hypothesis being tested in the activity (slides are provided as Supplemental Material with the online version of this article).

○ Endosymbiosis and Tree Thinking: Overview and Core Concepts

Endosymbiosis as a Microbiological Macroevolutionary Process

What is endosymbiosis? Endosymbiosis is when one cell lives inside another cell. The relationships of endosymbioses can run the gamut of other symbioses; parasites, commensals, or mutualists can live within host cells. In longstanding endosymbioses, the symbiont loses its autonomy and becomes an organelle with only a remnant of its past freedom (McCutcheon, 2021). The two most famous organellar endosymbioses resulted in the mitochondria and the chloroplast. Mitochondria evolved from an alphaproteobacterium over 2 billion years ago (Roger et al., 2017), whereas chloroplasts evolved from a cyanobacterium about 1 billion years ago (Falcón et al., 2010). There are younger endosymbioses that have resulted in relationships that blur the boundaries between organelle and endosymbiont (McCutcheon, 2021), one of which is the focus of the activity presented below (Delaye et al., 2016; Stephens et al., 2021). While this activity focuses on primary endosymbioses of bacteria, more complicated secondary and tertiary endosymbioses have resulted in the many different kinds of unicellular algae spread across the tree of eukaryotes (Burki, 2017; Cavalier-Smith, 1999; Dorrell et al., 2017; Miller & Delwiche, 2015; Petersen et al., 2014; Ševcíková et al., 2015; Stiller et al., 2014). In this activity, we are exclusively looking at a relatively recently discovered young primary endosymbiosis that evolved independent of the plant primary endosymbiosis (Bodył et al., 2007; Kepner, 1905; Macorano & Nowack, 2021; Nowack & Grossman, 2012).

How do longstanding endosymbioses evolve? Any event that ends up with one cell inside of another can result in an endosymbiotic relationship. Cells eat other cells, and sometimes delaying the digestion of photosynthetic food can lead to some extra sugar. Sometimes, digestion can be delayed permanently (McCutcheon, 2021). At some point, the symbiont begins to lose redundant genes and even transfer some to the host nucleus in a process called horizontal gene transfer. Transferred genes are transcribed in the nucleus and translated on cytosolic ribosomes and transported into the symbiont. The process is unidirectional and leads to ever-shrinking gene complement such as those found in the genomes of mitochondria and chloroplasts.

How do we KNOW that mitochondria and chloroplasts are derived from other cells? Endosymbiotic theory is over a century old starting with Merezhkovsky and a few other Russian scientists (Sapp, 1994), but popularized by Lynn Margulis in the latter half of the twentieth century (Sagan, 1967). Mitochondria and chloroplasts were known to contain their own genomes and ribosomes for some time, but the first genetic evidence for the relationship between organelles and specific bacterial groups emerged from Dalhousie University in Canada in the 1970s (Bonen et al., 1977; Bonen & Doolittle, 1975). Researchers were able to show that mitochondrial and chloroplast ribosomes were most similar to particular bacterial groups. Since then, the evidence has only grown and no evidence to the contrary has been presented to our knowledge. Both organelles have two membranes, just like gram-negative bacteria. The inner membranes house electron transport chains, just like the inner membranes of gram-negative bacteria. When phylogenetic trees are constructed using mitochondria and chloroplast genes, the organelles branch near alphaproteobacteria and cyanobacteria, respectively. Since this discovery, several more recent endosymbioses have been identified.

Tree thinking as an approach to study endosymbiosis

One of the most active areas of evolution education research regarding macroevolutionary processes centers around "tree thinking" (Baum et al., 2005; O'Hara, 1988, 1997), or the knowledge and skills necessary to accurately read, interpret, and construct phylogenetic trees (Gibson & Hoefnagels, 2015; Gregory, 2008; Meisel, 2010; Novick & Catley, 2013). Education research thus far has focused on several aspects of tree thinking and student understanding of phylogenetic trees, such as instructional practice (Eddy et al., 2013; Halverson, 2011; Halverson et al., 2011; Marcelos & Nagem, 2012; Novick & Catley, 2016, 2018; Perry et al., 2008; Phillips et al., 2012), assessment (Blacquiere et al., 2020; Blacquiere & Hoese, 2016; Smith et al., 2013), tree style and topology (Catley et al., 2010; Dees et al., 2017, 2018; Podani, 2013), skills for reading trees (Schramm et al., 2019, 2021), common misconceptions (Dees et al., 2014; Gregory, 2008; Kummer et al., 2016; Meir et al., 2007; Meisel, 2010), and teleological issues (Omland et al., 2008; Schramm & Schmiemann, 2019). In addition to studies on various aspects of tree thinking, there has been development of several measures to analyze student understanding of phylogenetic trees, such as the concept inventories (Kummer et al., 2019; Naegle, 2009) and a diagnostic instrument (Jenkins et al., 2022). Therefore, the body of pedagogical content knowledge concerning a tree thinking approach to understanding macroevolutionary patterns has been well-developed.

In the present laboratory activity, we leverage this well-developed body of pedagogical content knowledge on tree thinking, as well as previous activities that use bioinformatic and computational approaches, to construct phylogenetic trees (Campo & Garcia-Vazquez, 2008; Zhang, 2012), to facilitate learning about endosymbiosis as a microbiological macroevolutionary process. This will concern a recent (~ 60 million years ago) endosymbiotic event that resembles the original chloroplast endosymbiosis within the shelled amoeba *Paulinella chromatophora*. Within the *P. chromatophora* cell reside two chromatophores that derive from a symbiosis with cyanobacteria. A simple ribosomal DNA phylogeny can be constructed to demonstrate the close relationship between these chromatophores and cyanobacteria that are distinct from the cyanobacteria that gave rise to primary chloroplasts in other

Protist – a eukaryote that is not a fungus, plant, or animal. Examples include amoebae, ciliates, flagellates, euglenids, algae, and kelp.

Rhizaria – a very large group of eukaryotes that include many heterotrophic flagellated protists as well as shelled amoebae such as *Paulinella chromatophora*. Other examples include forams (Foraminifera) and radiolarians made famous by Haeckel's extravagant drawings.

Paulinella chromatophora – a shelled amoeboid rhizarian protist with photosynthetic organelles called chromatophores.

Microevolution – within-species evolution: changes in gene frequency over generational time.

Macroevolution – evolution beyond the species level. This includes speciation.

Endosymbiosis – a symbiosis in which one organism lives inside the cells of another. Mitochondria and chloroplasts evolved from ancient endosymbioses with alphaproteobacteria and cyanobacteria, respectively.

Primary endosymbiosis – an endosymbiosis in which the endosymbiont is a prokaryote.

Secondary endosymbiosis – an endosymbiosis in which the endosymbiont is a eukaryote that already has a prokaryotic endosymbiont (e.g., green algae living inside an amoeba).

Mitochondria – eukaryotic organelles derived from an ancient endosymbiosis with an alphaproteobacterium, the powerhouse of the eukaryotic cell.

Chloroplasts – photosynthetic eukaryotic organelles. All chloroplasts derive from a single primary endosymbiotic event.

Chromatophores – photosynthetic endosymbionts of *Paulinella chromatophora*.

Primary chloroplasts – chloroplasts in the archaeplastid lineage, which includes red algae, green algae (and all plants), and glaucophyte algae.

Secondary chloroplasts – chloroplasts resulting from a secondary endosymbiosis of red or green algae.

Homology – the case when two traits have a shared ancestry. Bird wings, bat wings, and whale fore-fins have homology to one another as tetrapod fore-limbs.

Homologue – genes that evolved from a common ancestral gene.

Homologous site – nucleotide or amino acid sequences that derive from an ancestral site in an ancestral homologous gene/protein.

16S SSU ribosomal sequence – the small subunit ribosomal gene of a prokaryote.

18S SSU ribosomal sequence – the small subunit ribosomal gene of a eukaryote.

Clade – a monophyletic group.

Monophyletic Group – a group of organisms that include ALL the descendants of a common ancestor.

Paraphyletic Group – a group of organisms that include an ancestor and some of its descendants.

Horizontal gene transfer – in contrast to vertical gene transfer, horizontal gene transfer occurs between lineages rather than within a lineage.

Bootstrapping – in simplified terms, bootstrapping is a kind of replication test that provides confidence estimates of the branches in a phylogenetic tree.

Figure 1. Glossary of important vocabulary.

photosynthetic eukaryotes. The following laboratory activity walks students through the construction of this ribosomal DNA phylogeny to understand the recent endosymbiosis of the chromatophores of *Paulinella chromatophora*. Figure 1 contains important vocabulary terms referenced throughout the laboratory activity to support the teaching and learning of key ideas in endosymbiotic theory.

○ Laboratory Activity

Computational Requirements

Participants will require a computer with an Internet connection and a web browser. A text editor is also required. While any text editor can be used (e.g., Wordpad), we recommend something such as Atom (https://atom.en.softonic.com/) or BBEdit (https://www.barebones.com/products/bbedit/download.html). Finally, to visualize and manipulate the final tree, a tree viewing program is also required. We recommend FigTree (https://github.com/rambaut/figtree/releases), as it is an excellent open-source viewer that is rather intuitive and simple to use. An alternative is to use iToL (https://itol.embl.de/) (Letunic & Bork, 2021), but this interface is less intuitive and has several complicating features, though it is easily accessible online and therefore does not require any software downloads.

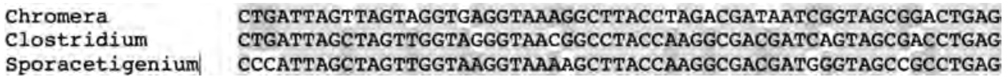
Overview

Phylogenetics is the study of evolutionary relationships between taxa. Phylogenies are evidence-based hypotheses about evolutionary relationships depicted as evolutionary trees or cladograms. One way to infer phylogenies is to compare molecular data, or DNA and protein sequences, using a variety of computer programs. These molecular sequences from various taxa are analyzed by identifying differences between the sequences through the process of alignment, and it is from the alignment of these molecular sequences that a molecular phylogeny is developed.

The first step is to align homologous sequences (i.e., sequences with a common evolutionary origin). Figure 2 depicts a small section of a DNA alignment from three different genera: *Chromera* (an alga related to the malaria parasite), and *Clostridium* and *Sporacetigenium* (two bacteria). This alignment shows the similarity between the DNA sequences of even taxa from very different lineages. Similarities between the DNA sequences are indicated in three ways in Figure 2: (1) nucleotides highlighted in light blue indicate that all three sequences have the same nucleotide in the same position, (2) nucleotides highlighted in gray indicate that two of the three sequences have the same nucleotide in the same position, and (3) nucleotides highlighted in white indicate where there is a different nucleotide in that specific position relative to the other DNA sequences in the alignment. The high degree of identity and lack of gaps in the alignment indicate that these sites are likely homologous and appropriate for molecular phylogenetic analysis. Once the sequences are aligned, phylogenetic trees can be generated using bioinformatics programs. Different programs use different algorithms to compute likely phylogenies; some programs will use maximum likelihood (ML) methods to generate these trees while others will use Bayesian (Posterior Probability) analyses (Douady et al., 2003; Svennblad et al., 2006). For the purposes of this laboratory activity, the phylogenetic tree will be constructed using a program called PhyML (Guindon et al., 2010), which uses ML methods.

When inferring deep-branching phylogenies, which compare DNA or protein sequences to analyze evolutionary relationships at high levels of biological classification such as supergroups or domains, it is important to use very slow-evolving sequences (Moreira & Philippe, 2000). Slow-evolving sequences allow for analysis of divergence between sequences that occurred deep in evolutionary time. While there are many examples of slow-evolving sequences, some of the most commonly used are ribosomal sequences, the small ribosomal subunit (SSU, also called 16S for bacteria and 18S for eukaryotes) in particular. Ribosomal sequences are extremely slow-evolving due to their conserved essential functions across all domains of life, but not so slow that no changes accrue, and thus will be useful in analyzing evolutionary relationships with respect to endosymbiosis.

This activity will determine whether the chromatophore, a chloroplast-like organelle, in the cercozoan rhizarian *Paulinella chromatophora* is related to the chloroplasts found in other eukaryotes such as archaeplastids (e.g., plants, red algae, green algae), haptophytes, cryptophytes, stramenopiles (e.g., diatoms), alveolates (e.g., dinoflagellates, apicomplexans), and a closely related cercozoan rhizarian, the chlorarachniophyte *Bigelowiella natans*. Current understanding of the tree of eukaryotes can be found in recent papers



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Chromera         CTGATTAGTTAGTAGGTGAGGTAAAGGCTTACCTAGACGATAATCGGTAGCGGACTGAG
Clostridium      CTGATTAGCTAGTTGGTAGGGTAACGGCCTACCAAGGCGACGATCAGTAGCGACCTGAG
Sporacetigenium  CCCATTAGCTAGTTGGTAAGGTAAAAGCTTACCAAGGCGACGATGGGTAGCCGCCTGAG
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Figure 2. Ribosomal DNA sequence alignment between genera: *Chromera, Clostridium,* and *Sporacetigenium*.

Table 1. List of all prokaryotic and eukaryotic organisms included in the laboratory activity. The activity uses the 16S ribosomal sequences to create a molecular phylogeny based on the plastids, although the 18S ribosomal sequences from the nuclear DNA of some species are included for the assessment activity as indicated by the asterisks. Prokaryotic species include the gram-positive and chloroflexi bacteria (the outgroup for the final tree) and the cyanobacteria (to differentiate the chromatophore in *P. chromatophora* from chloroplasts). Eukaryotic species include the Archaeplastida (plants, green algae, red algae) which have plastids related to the cyanobacteria, and an assortment of eukaryotic groups that have plastids from secondary and tertiary endosymbioses (Stramenopila, Alveolata, Rhizaria, Excavata, Haptophyta, Cryptophyta) to confirm that the chromatophore is not related to these plastids from higher level endosymbioses.

Prokaryota		**Eukaryota**	
Gram-positive bacteria	_Clostridium sp._	Archaeplastida	
	Sporacetigenium mesophilum	Streptophyta	_Arabadopsis thaliana*_
	Thermotalea metallivorans	Chlorophyta	_Micromonas sp.*_
	Geosporobacter sp.		_Chlamydomonas reinhardtii*_
Chloroflexi	_Kouleothrix aurantiaca_	Rhodophyta	_Cyanidium caldarium*_
	Nitrolancea hollandica		_Cyanidioschyzon merolae*_
	Uncultured Chloroflexi	Other eukaryotes	
Cyanobacteria	_Aphanothece sp._	Stramenopila	_Ectocarpus siliculosis*_
	Synechococcus sp.	Alveolata	_Chromera velia_
	Cyanobium sp.		_Vitrella brassicaformus_
	Prochlorococcus marinus		_Dinophysis norvegica_
	Leptolyngbya sp.	Rhizaria	_Bigelowiella natans*_
	Pseudanabaenaceae cyanobacterium		_Paulinella chromatophora*_
	Oscillatoriales cyanobacterium	Excavata	_Euglena gracilis_
	Aphanothece minutissima	Haptophyta	_Emiliania huxleyi_
	Cyanodictyon sp.	Cryptophyta	_Guillardia theta_

(Adl et al., 2019; Burki et al., 2020). Ribosomal sequences from a representative species of each of these eukaryotic lineages, including that of *Paulinella chromatophora*, will be compared with ribosomal sequences of cyanobacteria and gram-positive (monoderm) bacteria to yield insights as to the evolutionary origin of the chromatophore. These species are listed in Table 1, and their SSU sequences from bacteria and chloroplasts are in the Supplementary Material II textfile (.txt) provided with the online version of this article.

This selection of prokaryotic and eukaryotic lineages has been chosen for the following reasons. Firmicutes and chloroflexi bacterial SSU sequences were chosen as outgroups to cyanobacteria (Hug et al., 2016). A selection of cyanobacterial SSU sequences, including the representatives closely related to primary plastids were selected because they are needed to differentiate chloroplasts, which are plastids derived from a singular primary endosymbiosis of a cyanobacterium ~1 billion years ago (Falcón et al., 2010), from chromatophores. A selection of green algal, red algal, and plant primary plastid SSUs were selected as representatives of the archaeplastid lineage, the only known group with plastids most closely related to cyanobacteria. A selection of SSUs from secondary/tertiary plastids were chosen to confirm that chromatophores are not related to any higher-level endosymbioses (Table 1). Finally, we include as Supplemental Materials III textfile (.txt) provided with the online version of this article the corresponding nuclear (18S) SSUs from most eukaryotes mentioned above that can be used in an assessment activity (see below).

The following steps are designed to walk both educators and students through the process of creating a molecular phylogenetic tree that includes three iterative steps: (1) obtaining DNA sequences from the National Center for Biotechnology Information (NCBI), (2) creating a multiple sequence alignment with MUSCLE (Madeira et al., 2022), and (3) constructing a molecular phylogenetic tree from the multiple sequence alignment using PhyML. The full assignment, with directions and visuals from NCBI, MUSCLE, and PhyML, along with assessment questions, are included in the Supplementary Material I document provided with the online version of this article. Here, we summarize the overarching goals and steps for each phase of the laboratory activity.

Step One: Obtain DNA Sequences

Ribosomal sequences from representative species can be obtained from the NCBI, which is a United States government-funded national resource for molecular biology information. It provides access to many public databases, references, genomes, and several bioinformatic programs, and can be accessed here: www.ncbi.nlm.nih.gov. The representative sequences, from which specifically the SSU ribosomal sequences are included in the provided text file, are listed in Table 1. The *Paulinella chromatophora* is in bold as it is not provided and must be retrieved from an online as repository described below.

To obtain the *Paulinella chromatophora* SSU DNA sequence, students will navigate to the NCBI website (www.ncbi.nlm.nih.gov) and use the "Nucleotide" search with "Paulinella chromatophora 16S" as a query and choose the longest sequence that contains only the 16S ~1400bp. The best sequence has the GenBank accession: FJ456919.1 (https://www.ncbi.nlm.nih.gov/nuccore/FJ456919.1?report=fasta). This sequence will be added to the provided text file in FASTA format (Pearson & Lipman, 1988), which is a standard format for protein and nucleotide sequences in bioinformatic analyses. These FASTA formatted sequences are easily collated to be used in the multiple sequence alignment, the next step in the laboratory activity.

Step Two: ONE-CLICK Phylogeny

The collated FASTA sequences can be pasted as a program input for a phylogenetic pipeline at phylogeny.fr (Dereeper et al., 2008) that includes the multiple sequence alignment program MUSCLE (Edgar, 2004), which aligns SSU sequences. The alignment is then fed into the program GBLOCKS (Castresana, 2000), which trims

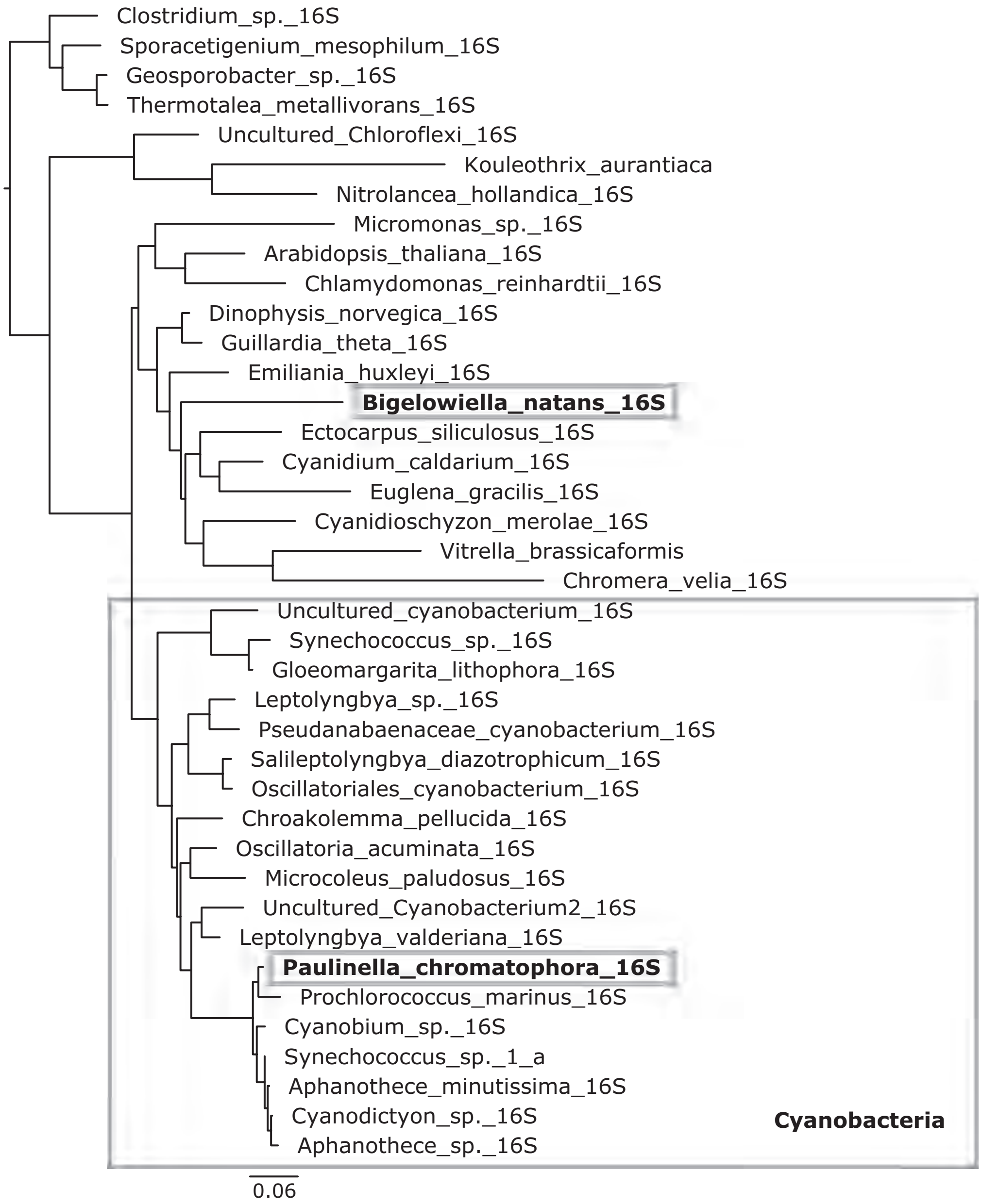

Figure 3. Final molecular phylogenetic tree that is produced by FigTree at the end of the lab activity. Observe the positions of *Paulinella chromatophora* and *Bigelowiella natans*, both cercozoan rhizarians, and the cyanobacteria in this final tree. The cercozoan rhizarians are highlighted in light blue boxes and the cyanobacteria are highlighted in a green box labeled "Cyanobacteria." The gram-positive bacteria (i.e., *Clostridium* sp., *Sporacetigenium mesophilum*, *Geosporobacter* sp., and *Thermotalea metallivorans*) are the appropriate outgroup for this tree.

poorly aligned regions. Finally, the GBLOCKS output is used to construct a phylogeny using PhyML (Guindon & Gascuel, 2003). The resulting phylogeny can be downloaded in Newick format and viewed and manipulated in FigTree (http://tree.bio.ed.ac.uk/software/figtree/) (Figure 3) or, with practice, iToL (https://itol.embl.de/) (Letunic & Bork, 2021). To answer some of the research questions, the tree will need to be rooted with the appropriate organisms as an outgroup.

Using Molecular Phylogenetic Trees to Understand Endosymbiosis

This final molecular phylogenetic tree will provide an evidence-based hypothesis for the relationship between the chromatophore of *Paulinella chromatophora* and chloroplasts found within multiple eukaryotic lineages compared with cyanobacteria and gram-positive bacteria. Once the final molecular phylogenetic tree has been constructed, students can answer the following questions:

1. Why did you choose to root the tree where you did? (Hint: what is the outgroup for this experiment?). **A: I chose to root the tree with the non-cyanobacterial bacteria (or with one of these groups) because this represents the outgroup for this experiment. The important part of the tree is within the cyanobacterial derived sequences (including plastids).**
2. In this tree what kind of group do the cyanobacteria form? What about the Archaeplastida? Explain. **A: Cyanobacteria**

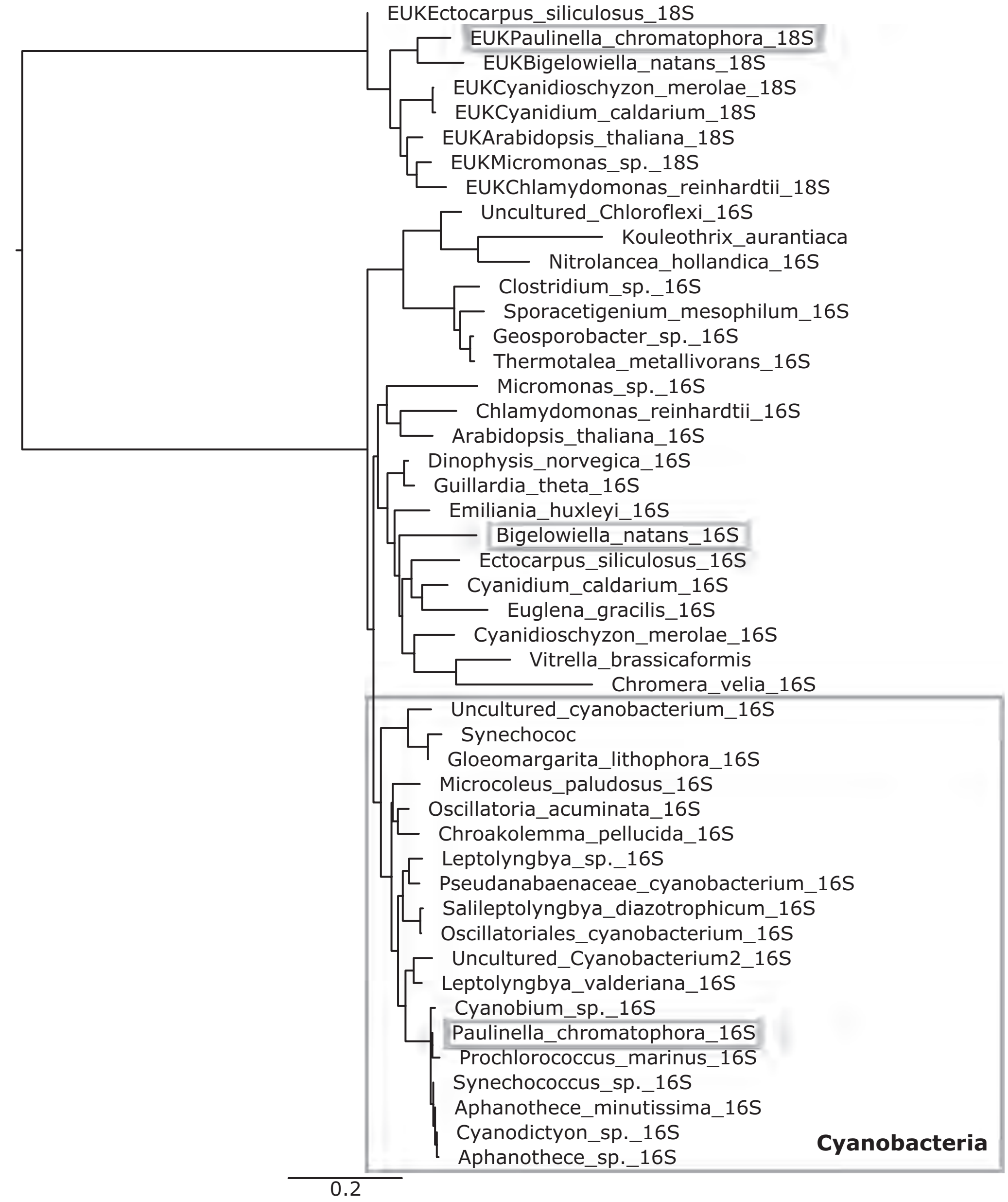

Figure 4. Molecular phylogenetic tree that is produced by FigTree with the 18S ribosomal sequences. Observe how the 16S (prokaryotic) sequences and 18S (eukaryotic) sequences form two different clades in this tree. Once again, observe the positions of *Paulinella chromatophora* and *Bigelowiella natans*, both cercozoan rhizarians, which are highlighted in blue. Make sure to observe the positions with respect to both the 16S and 18S sequences of both rhizarian species. In addition, once again observe the position of the cyanobacteria, highlighted in the green box labeled "Cyanobacteria."

form a paraphyletic group because the *Paulinella* chromatophore intervenes. Cyanobacteria and *Paulinella* forms a monophyletic group. Archaeplastida also form a polyphyletic group since the secondary/tertiary plastid 16S sequences (i.e., cryptophytes, haptophytes, stramenopiles, alveolates) branch close to rhodophyte sequences (i.e., *Cyanidioschyzon* and *Cyanidium*).

3. What organisms are most closely related to the *Paulinella* chromatophore? What conclusions can you make about the origins of the *Paulinella* chromatophore versus the chloroplasts found in other eukaryotic groups? **A: The chromatophore 16S branches within a particular group of cyanobacteria near *Prochlorococcus*. This is strongly supported. The chloroplasts appear clustering together, but not with any particular cyanobacterial group, likely due to how divergent they are after 1 billion years of evolution. We can conclude that the *Paulinella* chromatophore arose more recently than chloroplasts and derive from a cyanobacterium closely related to *Prochlorococcus*.**
4. Draw the tree (rooted appropriately) that you would expect to see if we added all of the eukaryotic cytoplasmic ribosomal sequences (i.e., the 18S ribosomal sequences) from the organisms in this study (collapse the prokaryotic groups if this makes the tree easier to draw). Use the provided 18S eukaryotic sequences to test your hypothesis. **A: This can be used as a summative assessment. Students can redo the activity and should get the tree shown in Figure 4.**

Discussion questions for students who complete the summative assessment could include: Why don't the eukaryotic cytosolic ribosomal sequences branch with their respective plastid ribosomal sequences? (A: because they are not as closely related [i.e., plastids are related to cyanobacteria]). Why is the branch leading to the cytosolic ribosomal sequences so long? (A: because eukaryotes have been evolving independently from bacteria for almost 4 billion years, so more changes have taken place). If you included archaeal 16S sequences, where do you predict they would branch? (A: eukaryotes evolved from archaea, so if archaeal 16S sequences were included, they would branch near the eukaryotic cytosolic ribosomal sequences). Does this tree change your conclusions about the origins of the *Paulinella* chromatophore? (A: No, the tree topology is almost exactly the same if the cytosolic ribosomal sequences are ignored. Therefore, conclusions about chromatophore origins would not change).

○ Discussion

Teaching Endosymbiosis as a Microbiological Macroevolutionary Process

Ziadie and Andrews (2018, 2019) identify the explicit teaching of macroevolutionary processes to be a top priority in evolution education research due to the lack of pedagogical content knowledge on the subject. Despite the calls for its inclusion in evolution education research (Catley, 2006; Padian, 2010), calls for teacher professional development materials on macroevolution (Friedrichsen et al., 2016), and its connection to evolution acceptance (Nadelson & Southerland, 2010), there has been little research on the teaching of macroevolution outside of phylogenetic biology. Endosymbiosis as a major macroevolutionary process remains untouched by evolution education research, despite research in analogous areas such as the evolution of multicellularity (Pentz et al., 2015; Ratcliff et al., 2014) and evolutionary transitions in individuality (Michod et al., 2022). Our efforts here leverage the pedagogical content knowledge on tree thinking (Baum et al., 2005; O'Hara, 1988, 1997) to teach the core principle of endosymbiosis from an evolutionary cell biological point of view. This starting point aims to make research on the teaching of endosymbiosis, and macroevolution, tractable.

Future Directions

Future research on the teaching and learning of modern endosymbiotic theory supports current efforts toward increasing attention on the role of student understanding of macroevolutionary processes on student knowledge, understanding, and acceptance of evolutionary biology. First, overarching conceptual frameworks of the core tenets of endosymbiotic theory, including current scientific research, needs to be translated into the evolution education literature for use in future evolution education studies regarding macroevolution. Second, in parallel to the development of conceptual frameworks, explicit laboratory activities centered around key concepts in endosymbiotic theory, such as endosymbiotic gene transfer, would provide instructors at the secondary and postsecondary levels of education specific activities for teaching these concepts (Friedrichsen et al., 2016). Third, future research should consider whether inclusion of endosymbiosis in the evolutionary biology curriculum leads to an increase in evolution acceptance, especially since students tend to accept microevolution rather than macroevolution (Barnes et al., 2021, 2022).

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JOSHUA S. HOSKINSON (jhoskinson@mohave.edu) is a Faculty of Life Sciences at Mohave College, Lake Havasu City, Arizona, USA. JEREMY G. WIDEMAN (jeremy.wideman@asu.edu) is an Assistant Professor at the Biodesign Center for Mechanisms of Evolution in the School of Life Sciences at Arizona State University, Tempe, Arizona, USA.

INQUIRY & INVESTIGATION

Investigating "Humanity": Reconstructing Human Evolution Using Skulls, Maps, Tools, & the History of Science

ARMIN P. MOCZEK, KIRSTIN J. MILKS,
FRANK BROWN CLOUD, LIN ANDREWS,
RAYA HAGHVERDI

Abstract

Although the central principles of evolution by natural selection can feel both abstruse and culturally fraught for learners of all ages, evolutionary logic is at the core of biological science: once students have a solid understanding of evolution, they can better understand everything else in biology. We present here a hands-on experience, coupled with intentional questioning strategies, that inspires students to use high-level evolutionary thinking and to begin asking excellent questions about what we know and how we know it. This activity leverages skull replicas of humans, chimps, and hominins to tap into young people's natural curiosity about where we come from and what it means to be a person—fundamental topics of inquiry for young people just coming into their own identities. Along the way, students are also exposed to important data and create arguments about what the archaeological record can currently tell us about the story of how people like us came to be.

Key Words: *evolution; human evolution; inquiry.*

○ Introduction

Human evolution can be a challenging topic to broach in many classrooms. In a 2016 poll reported by the National Science Board, only 52 percent of Americans were found to agree with the statement that "humans, as we know them today, descended from earlier species of animals" (National Science Board, 2018). Additionally, the teaching of human evolution has been the focus of numerous legislative bills that would restrict educational opportunities in public schools. Due to repeated controversy in courts, classrooms, and school board meetings, many biology teachers have felt hesitant to introduce the topic of human evolution, fearing local pushback and public scrutiny. However, we've had success working through the lesson described below with students of all ages who come from a wide range of political and religious backgrounds.

For this activity, students use a set of full-sized skull replicas along with a binder that has discussion prompts, graphs, and pictorial data. In our experience, the lesson design allows instructors to act as guides, directing the flow of discussion with relatively minimal prompts as students themselves generate questions and ideas. In the process, students will be engaging in authentic, collaborative scientific work—both when cooperatively investigating within small (2 to 8 person) groups and when sharing their small group's findings with the class at large—all while reinforcing general evolutionary principles and imparting some important information about human evolution, climate science, and the archaeological record.

Given that non-specialists often have difficulty applying the same type of logic to their understanding of human or non-human animals (de Waal, 1997), and the importance of high-quality evolution learning before college (Mead & Branch, 2011), we particularly wanted to design a learning experience focused on human evolution that could be highly flexible and amenable to learners of a wide range of ages, contexts, science knowledge, and life experiences. Other teams of educators have published excellent modules for using ancient skull replicas to investigate this topic, including "Mystery of the Skulls" (Yerky & Wilczynski, 2014), "Be a Paleoanthropologist for a Day" (Bayer & Luberda, 2016), and more. We've incorporated insights from their work, but we are presenting a significantly different exercise that better accomplishes our goals of student-led inquiry; authentic collaborative scientific practice; and the analysis, interpretation, and synthesis of diverse data sets. Our lesson ties the skill of argumentation from evidence to the core concept of evolution, addressing the standards shown in Table 1. We feel that our lesson has two additional important features: (a) demonstrating that many of the traits that we intuitively feel make us "most human" emerged long ago in beings whom we might not even recognize as human, and (b) demonstrating the ways in which our understanding of the world shifts as we gain access to more information, especially when this information spans many different types of data.

In conjunction with the National Center for Science Education (NCSE), a version of this activity has been tested in over 200 classrooms, including at many schools in the politically divided American Midwest and in college courses at Indiana University. With some modifications, we've also conducted this activity at retirement homes, at public science fairs, and for people who are incarcerated at the Monroe (Indiana) County Jail. In the process, we've worked with people from elementary-school age to post-retirement

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Table 1. National standards addressed by this lesson.

<table>
<tr><th colspan="2">Next Generation Science Standards</th></tr>
<tr><td>Science and Engineering Practices:
• 1: Posing questions
• 4: Analyzing and interpreting data
• 7: Engaging in argumentation from evidence
• 8: Obtaining, evaluating, and communicating data</td><td>Crosscutting Concepts:
• 1: Patterns
• 2: Cause and effect
• 6: Structure and function
• 7: Stability and change</td></tr>
<tr><td colspan="2">Supported NGSS standards:
• HS-LS4-1: Communicating scientific information that common ancestry and biological evolution are supported by multiple lines of empirical evidence.
• HS-LS4-5: Evaluating the evidence supporting claims that changes in environmental conditions may result in (1) increases in the number of individuals of some species, (2) the emergence of new species over time, and (3) the extinction of other species.
• HS-ESS3-1: Construct an explanation based on evidence for how the availability of natural resources, occurrence of natural hazards, and changes in climate have influenced human activity.</td></tr>
<tr><th colspan="2">AP Biology</th></tr>
<tr><td>Science Practices:
• 1: Using representations and models to communicate scientific phenomena and solve scientific problems
• 5: Performing data analysis and evaluation of evidence
• 7: Connecting and relating knowledge across various scales, concepts and representations in and across domains</td><td>AP Biology Big Ideas:
• 1: Evolution/Natural selection</td></tr>
<tr><th colspan="2">Vision and Change</th></tr>
<tr><td>Core Competencies:
• 1: Apply the process of science
• 4: Tap into the interdisciplinary nature of science</td><td>Core Concepts:
• 1: Evolution</td></tr>
</table>

age, including (especially in the jail classes) people who have not previously had much success in traditional academic settings, and who have professed a wide range of non-scientific conspiratorial beliefs. However, the hands-on, learner-led, qualitative-inquiry-based nature of this activity gives people an opportunity to authentically engage with difficult ideas that they might find threatening if encountered in a lecture-based setting (see Figure 1). We've discovered that when students are given the opportunity to direct the flow of their inquiry and act as spokespeople to share their burgeoning expertise, they are more likely to approach ideologically sensitive data with an open mind and perhaps even integrate newfound knowledge with their pre-existing beliefs.

We want to stress that you do not need to be an expert in human evolution or archaeology to guide students through this activity. Our online resources, hosted on NCSE's website (NCSE, n.d.), include answers to some frequently asked questions, but we've also found that in most sessions of this activity, students will generate questions that no one yet knows the answers to. This topic is an area of active research by professional scientists across the globe: future discoveries made during your students' lifetimes are likely to clarify some of our theories, and may force us to revise others. This is, after all, an essential tenant of scientific research. As students progress through this activity, they will be encouraged to ask "How do we know what we know?" "How confident can we be?" and "What critical information is missing?" In the notes below, we've included italicized notes on the sort of answers that we might give in response to these questions, but the answers your students generate will likely differ. Throughout the exercise, please encourage students to articulate the reasoning behind their ideas.

By the end of the exercise, your students will have gained a better understanding of how to engage in collaborative scientific pursuits, both in terms of working with a small team and in communicating their findings to others. They will have practiced applying evolutionary logic: that over long periods of time, natural selection can increase the frequency of traits that are beneficial within a particular environment, where each individual's environment consists of the climate, nearby plants and animals, as well as potential allies, enemies, or mates from among their own species. We also hope that your students will begin to formulate answers to the following specific questions:

- What did our ancestors look like?
- Where did they live?
- Which human traits evolved first, and which followed?
- When and where did changes occur? Under what circumstances? Why?
- Who among our ancestors seems "human" to you, who does not, and why?

Figure 1. This lesson is engaging in a variety of settings, from science classrooms to retirement homes. Photos courtesy of Armin Moczek.

○ The Activity

This lesson can be conducted in class periods ranging from 45 minutes to 70 minutes in length, although it is easier to guide students through the entire experience when you have access to the longer end of that range. You will need to prepare the binders in advance, five copies of the file found in the supplemental materials featuring 13 black and white pages and 2 color pages. You will also need a set of skull replicas: five contemporary chimpanzee skulls, five contemporary *Homo sapiens* skulls, and one skull each for five ancient hominins: *Ardipithecus ramidus, Australopithecus afarensis, Homo habilis, H. erectus,* and *H. neanderthalensis*. We've included a purchasing guide on the NCSE website. As of 2023, this set costs approximately $4,000, but because the materials are portable and the lesson requires only a single day of instructional time, it's quite feasible to share a set among an entire school district/department or even multiple districts, or to request to share with (other) institutions of higher education. Distinct sets of materials held at both Indiana University Bloomington and the WonderLab Museum of Science, Health, and Technology are available for loan and have been used widely around our state.

If it is impossible for your district to jointly purchase or borrow this set of skull replicas, or if you are working with remote students, the activity can be done with digital tools available at the National Center for Science Education's website that allow students to see rotated and magnified skulls. However, we have found that the visceral, hands-on impact of working with full-scale skull replicas dramatically increases the memorability of and engagement with the activity. Especially when working with students who are dubious about the validity of this branch of scientific research, it is crucial to provide resources that will allow the students to draw their own insights from authentic skull casts that they can hold and inspect in the real world. A superb hypothesis-testing activity by Price [2012], in which students graph changes in fetal chimpanzee skulls and then compare the adult skulls of other species to their graph, could serve as an excellent follow-up to the lesson described here. We also note that Price's lesson works well with paper pictures of all the skulls rather than casts/models.

Preparation

Before the activity, as outlined in Figure 2, prepare the room with five stations that divide your students into equal-sized groups, between two and eight students each. At each station, provide at least one copy of the printed binder with discussion prompts and data, plus a set of three skull replicas: one contemporary human, one contemporary chimpanzee, and one of the five ancient hominins. We often choose to "nickname" the skulls with students rather than use the scientific nomenclature because many students may already have preconceived notions about *Homo habilis*, for instance, that might inhibit them from engaging as inquisitively as with a skull named "HH." Finally, you may want to have students add traits sequentially to an initial model, resulting in the figure shown in Figure 3; both the blank and completed model are available in the supplemental materials accompanying the online version of this paper.

In a brief introduction, remind students of the general principles of evolution and speciation. You may choose to gradually zoom in on a phylogenetic tree to indicate the split between the ancestors of contemporary chimpanzees and the ancestors of contemporary *Homo sapiens*, diverging from our last common shared ancestor six million years ago (Prado-Martinez et al., 2013). Remind students that contemporary chimpanzees are also a product of these six million years of evolutionary change—no contemporary organism exactly replicates our ancestors as though frozen in time. But because we believe that the ancestral lineage of contemporary chimpanzees continued to live in environments that at least partly resembled the likely habitats of our last common shared ancestor, we can use the morphological features of a contemporary chimpanzee skull as an initial reference for many of the changes that occurred during human evolution.

Then present students with the overarching goals for today's activity, in which they will attempt to formulate answers to the overarching questions articulated above. Also remind students that as they work through the activity, they should bear in mind these core scientific principles:

- How do we know what we know?
- How confident can we be in these results?
- What critical information is missing?

Step 1: Observing the skulls
- Describe brain volume, tooth size, mouth protrusion, brow ridges, prominence of cheek bones, and the location of the *foramen magnum*, the hole in the back of the skull.

Step 2: Ages and geographic ranges
- Where do most species appear to have originated?
- When did upright posture first arise in the human lineage?

Step 3: Skeletal anatomy
- How has body size changed over evolutionary time?
- When did ancient hominins appear to have developed proportions similar to contemporary humans (even if they were still smaller in overall size)?
- When did ancient hominins develop brain volumes in the range of contemporary humans?

Step 4: Observations pertaining to diet and feeding habits
- What conclusions can be drawn from available data?

Step 5: Artifacts found with ancient hominin remains
- What might each artifact have been used for?
- How difficult might these artifacts have been to make?
- What might it mean for an ancient hominin to have created these types of things?
- What creations might be less likely to be found by contemporary archaeologists?

Step 6: Historic climate change
- What was the climate was like during the time period(s) when each ancient hominin species was living?
- What might have been the consequences for each ancient hominin (who may have experienced changes in vegetation, desertification, food availability, etc.)?
- What sorts of traits might help an animal—including ancient hominins—survive during periods of change and instability?

Lesson synthesis
- Where does "being human" begin?
- How recent are these discoveries?

Figure 2. Lesson overview.

The subsequent data and prompts are presented in order in the student binders (see Figure 4); we discuss each in more detail below and include sample teacher responses in italics.

Step One: Observing the Skulls

Encourage each team, working within their small group, to compare their unique set of three skulls. Provide enough time for each student to have a chance to hold and manipulate all three skulls at their station. Explain that the ancient hominin skulls are color-coded, with the brown regions indicating portions of the skull that were actually recovered by scientists, and the gray regions indicating missing fragments of the skull that had to be reconstructed (based on expected symmetry between the right and left sides, or in some cases based on the morphology of skull fragments found from other individuals of the same species who appear to have lived at similar times, with the exception of AA, in which the color-coding is reversed). When comparing the skulls, each team should pay special attention the characteristics listed in the binder: brain volume, tooth size, mouth protrusion, brow ridges, prominence of cheek bones, and the location of the *foramen magnum*, the hole in the back of the skull.

After a few minutes of inspection and discussion within their small groups (we typically allow no more than 5 minutes for this first small group discussion when leading this activity within a 45-minute class period, approximately 7 minutes for longer classes), you'll call on small groups in turn to explain some of their findings to the whole class. For a 70- to 90-minute class, you should invite each group to present their findings to their classmates, but for a 45-minute class, you'll want to have only two or three groups present their findings during this step, preferably from ancient hominins that span a wide range of time (e.g., Ardi, HH, and Eric). Encourage each small group's spokesperson to let everyone know which ancient hominin their team is investigating, explain a few similarities and differences among their set of skulls (e.g., brain volume, mouth protrusion, brow ridges, tooth size) and perhaps give a speculative guess as to which contemporary skull (human or chimpanzee) the ancient hominin skull seems more similar to.

Then, as an entire class, discuss what the location of the foramen magnum, a prominent hole in the back of each skull, might tell you about an animal's posture and style of locomotion. *This hole should be underneath the brain case for an animal that walks upright and balances its head on top of its spine, and more toward the back of the skull for an animal that walks on all fours and holds its head in horizontal extension of its spine.*

Step Two: Ages and Geographic Ranges

In Step Two of the binder, students have a chart that lists the geographic ranges where similar archaeological specimens (presumed to be from the same species of hominin over time) have been found, as well as the range in ages for these archaeological specimens. Because the archaeological record is inherently incomplete, we cannot be certain when each species arose or went extinct (Du et al., 2020); for consistency's sake, we have used the time and geographical range estimates from the Smithsonian Museum of Natural History (Smithsonian, 2022). The estimates for the timing of arrival of anatomically modern *Homo sapiens* to various geographical regions

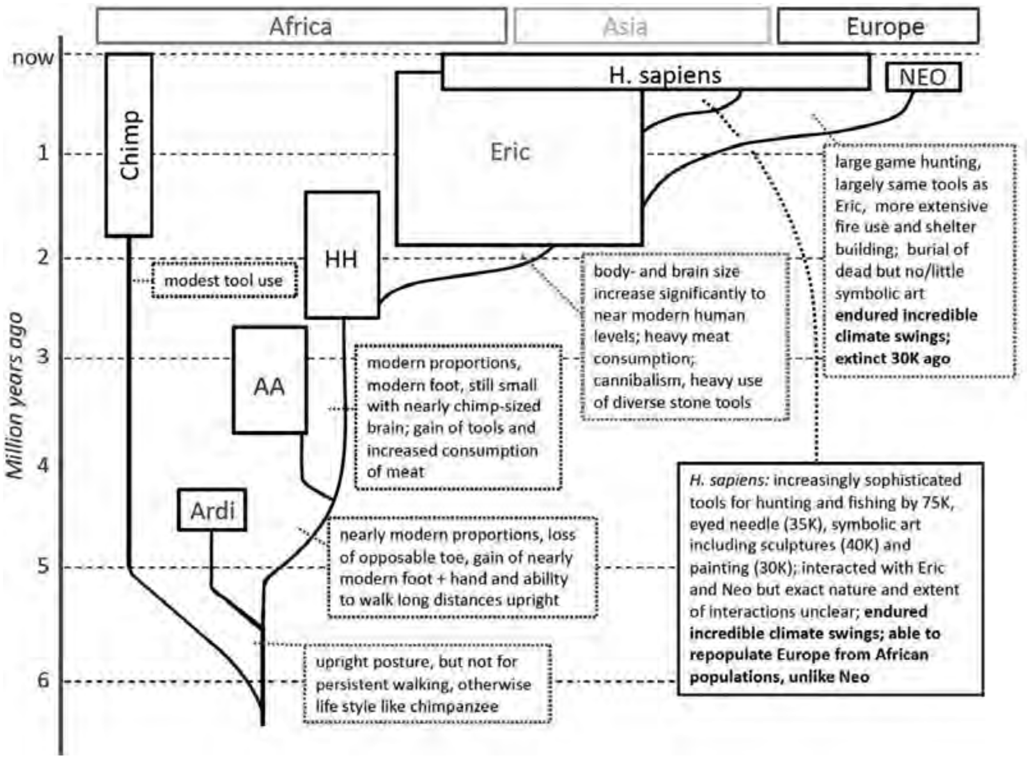


Figure 3. Hominin tree annotated with information highlighted in this lesson. Herein, "Ardi" stands for *Ardipithecus ramidus*, "AA" stands for *Australopithecus afarensis*, "HH" stands for *Homo habilis*, "Eric" stands for *Homo erectus*, "Neo" stands for *Homo neanderthalensis*, "Chimp" stands for *Pan troglodytes*, and "H. sapiens" for *Homo sapiens*.

were compiled from several sources (Skoglund & Reich, 2016; Clarkson et al., 2017; Hershkovitz et al., 2018). These estimates are by no means definitive; for example, the age of stone tools found in Shangchen, China, suggest that *Homo habilis* populations may have reached East Asia (Zhu et al., 2018), whereas the qualitative data we are presenting students mirrors the Smithsonian summary and indicates a geographical range isolated to Africa for this species. Our goal here is not to oversimplify, but rather to give students access to a good working model that many scientists would currently agree on, so that students can focus on the most salient aspects of this evolutionary story.

Each team of students should identify the ancient hominin whose skull they have been inspecting from among these data, and discuss what the geographical ranges of the archaeological finds might indicate. Each team should also double-check the location of the foramen magnum of their ancient hominin, comparing it with both the contemporary chimpanzee and contemporary human skulls.

Then, as a class, draw the age ranges onto a timeline on the board where everyone can see. (Our online materials include a reference timeline that also indicates the oldest fossil evidence for skulls that we can definitively categorize as ancestors to contemporary chimpanzees.) Then ask each team to tell the entire class about the location of the foramen magnum on their ancient hominin skull. As a class, discuss the following questions:

- Where do most species appear to have originated?
- When did upright posture first arise in the human lineage?

Whereas more recent fossils have been found in Africa, Asia, and Europe, older fossils have been found exclusively in East Africa, suggesting that East Africa may be the geographic center of origin for hominin evolution. The oldest hominin fossil found to date, that of "Ardi," already possesses a foramen magnum positioned for upright posture.

If time permits (for hour-long class periods or longer), you should also discuss:

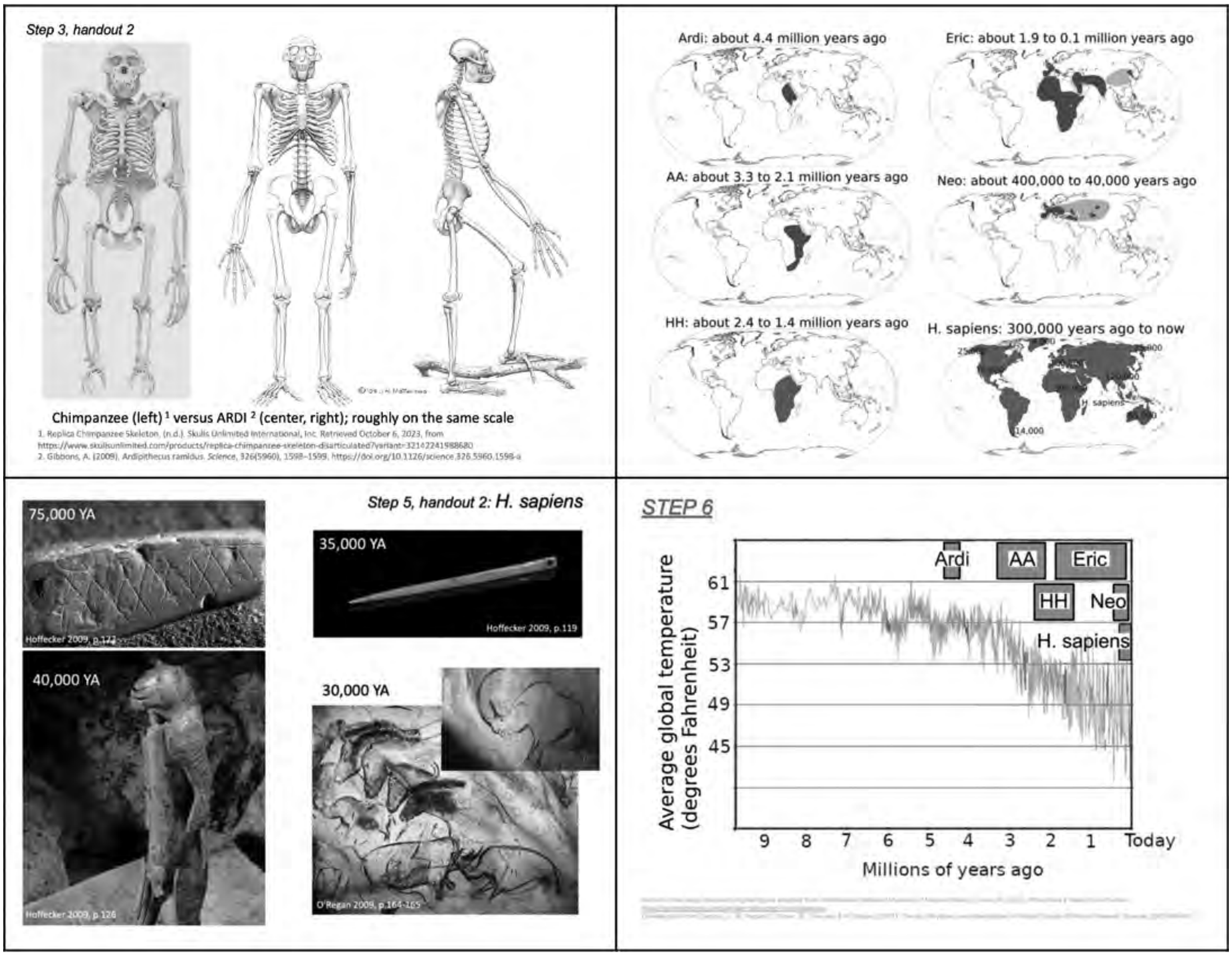
- If we didn't have these archaeological data, how might we collect data from modern-day humans to estimate where humans first originated?

Geneticists believe that we should find the greatest genetic diversity between people near the ancestral ranges of our species, because subgroups that departed this region may have experienced population bottlenecks (Cann et al., 1987). Linguists believe that we should find the greatest diversity of phonemes among languages near the ancestral ranges where human language first originated, as unique vocal sounds (such as clicks, glottal stops, or even the rolled "R" sounds that some students might struggle to pronounce in language classes) may have been dropped over time (Atkinson, 2011).

Step Three: Skeletal Anatomy

On the Step Three pages of their binders, students will find estimates for the height, weight, and brain size of each ancient hominin, as well as images of their entire skeletons (for each hominin where an entire fossilized skeleton has been found). Within their small groups, students should discuss how body size changed over evolutionary time, when ancient hominins appear to have developed proportions similar to contemporary humans (even if they were still smaller in overall size), and when ancient hominins developed brain volumes in the range of contemporary humans.

As a class, invite the small groups to share a few of their insights. Together, discuss what we can speculate based on the proportions



Step 3, handout 2

Chimpanzee (left)[1] versus ARDI[2] (center, right); roughly on the same scale

1. Replica Chimpanzee Skeleton. (n.d.). Skulls Unlimited International, Inc. Retrieved October 6, 2023, from https://www.skullsunlimited.com/products/replica-chimpanzee-skeleton-disarticulated?variant=32142241988680
2. Gibbons, A. (2009). Ardipithecus ramidus. Science, 326(5960), 1598–1599. https://doi.org/10.1126/science.326.5960.1598-a

Step 5, handout 2: *H. sapiens*

STEP 6

Figure 4. Sample binder pages from this lesson. The complete set is available in the supplemental materials accompanying the online version of this article.

and hand and foot shape of "Ardi," especially when compared with modern day chimpanzees and humans

Upright posture, as indicated by the position of the foramen magnum, appears to have arisen before persistent walking (Lovejoy et al., 2009). "Ardi" may have had an upright posture, yet several features of their hand- and foot-shape resemble those of contemporary arboreal (tree-living) primates, including feet with opposable toes that appear more suitable for gripping tree limbs than for long-distance walking across hard ground (Prang et al., 2021).

Step Four: Observations Pertaining To Diet And Feeding Habits

As a class, show the Step Four set of slides with five observations about diet and read these aloud. Lead students in a discussion about what conclusions we might draw from each of these observations.

1. *Contemporary chimpanzees and "Ardi" likely had similar diets. (Chimpanzees are omnivores and while meat does not make up a large portion of their diet, they will sometimes eat other mammals.)*
2. *HH likely used tools and ate meat (Pante et al., 2018); more ancient ancestors may not have.*
3. *ERIC likely ate meat, used tools, and used fire (Gowlett, 2016). There was reduced emphasis on chewing as a form of "cooking." (Your students may be interested to learn that chewing, which increases the amount of nutrition that can be extracted from food by beginning its breakdown before it reaches the digestive tract, seems to be an evolutionary innovation that was temporally linked with a rapid increase in brain volume among the earliest mammals (Rowe et al., 2011). Fire complemented chewing leading to even more efficient nutrient extraction, likely precipitating the further increase in brain volume among ancient hominins.)*
4. *NEO likely ate meat from animals much larger than themselves, perhaps indicating cooperative hunting (Smith, 2015).*
5. *NEO likely practiced cannibalism (Rougier et al., 2016).*

Step Five: Artifacts Found With Ancient Hominin Remains

On the Step 5 pages of their binders, students will find pictures of various artifacts that have been found in association with ancient hominin remains. In their small groups, students should compare these artifacts, discussing what each might have been used for and how difficult they may have been to make. Also consider what it might mean for an ancient hominin to have created these types of things.

As a whole class, students should also speculate about the sorts of creations that would be less likely to be found by contemporary archaeologists.

Wooden tools, skin bags, organic glues, and seaweed nets are much more likely to decompose before forming fossils. Rocks and hard clay endure most easily, although occasionally even footprints can form fossils. Some cave paintings have survived until the present day, but the ones we've found recently began to fade as soon as they were exposed to humid air from the surrounding environment or human exhalations. Although we haven't yet found compelling evidence that any hominins other than Homo sapiens *made symbolic art, we aren't sure that they didn't.*

Step 6: Historic Climate Change

On the Step 6 pages of their binders, students have graphs of average global temperatures spanning several timescales: 500 million years (Voosen, 2019), 10 million years (Westerhold, 2020), and 24,000 years (Marcott & Shakun, 2021). These temperature data are necessarily approximate, and even so, a global average temperature may not reflect the local temperature of any particular habitat. Additionally, there is no direct way to measure ancient temperature: instead, scientists have analyzed the ratio of atmospheric chemical isotopes that have been preserved trapped in ice (for roughly ten-thousand-year time scales) or the fossils of ancient aquatic lifeforms (for million- and billion-year time scales). While we cannot know for certain that the absolute temperatures presented here are accurate, it is likely that the variations we see reflect real changes in the past climate, and these variations have often been extreme.

Although only the 10-million-year time span has direct bearing on this lesson, we feel strongly that students benefit from exposure to all three, time permitting. The longest timescale helps students appreciate the wide range of temperatures that have existed on our planet, the way positive feedback loops have caused switchlike behavior in global climate, and also the dire ramifications, such as mass extinctions (Sepkoski, 1996), typically associated with dramatic changes in temperature. The shortest timescale helps students appreciate the atypical climate stability that our ancestors experienced during entire history of civilization, and indicates the sudden shift that began shortly after modern industrialization and its attendant release of ancient carbon into the atmosphere (many students may have seen this graph before, but we feel that this is important for students to see again because the 10-million-year data primarily indicates a steady *decrease* in global temperatures). In classes with more advanced students or longer class periods, we often pause here to emphasize the shifting time scale(s) at which this lesson's data sets have originated, connecting the scales of the graphs in Step 6 back to the data table of Step 2 and/or the *y*-axis of Figure 3.

Next, students will find a magnified view of the past 10 million years of the global climate data. Ask them to consider what the climate was like during the time periods when each ancient hominin species was living. What might have been the consequences for each ancient hominin (who may have experienced changes in vegetation, desertification, food availability, etc.)?

As an entire class, invite students to speculate about what sorts of traits might help an animal—including ancient hominins—survive during periods of change and instability.

Perhaps instability would favor adaptability, flexible lifestyles, and learning instead of instinctual behaviors. Students might be aware that intelligent, highly adaptive species such as crows and raccoons are thriving in urban areas of the contemporary world, despite many other species being forced to the margins of expanded human populations. The use of ancient climate data to model the likely migration patterns of ancient hominins is an active field of research (Beverly, 2023).

○ Ending the Lesson

The final 5–10 minutes of your class period should be devoted to synthesis. Place human and chimpanzee skulls next to each other and reiterate that although chimpanzees are not our ancestors—contemporary chimpanzees have been evolving to better fit their environments for as long as we have—as our closest living relatives, chimpanzees provide a good reference for comparison. Then invite your student groups to place their ancient hominin skulls on a timeline at the front of the room, so they can see the relationships between each fossil.

During this discussion, you should provide your students with some important information about each of the ancient hominins that they've been investigating.

ARDI: "Ardi" stands for *Ardipithecus ramidus* and is the most ancient hominin for which we have found fossil evidence, dating from approximately 4.4 MYA (million years ago) in East Africa. Fossils of Ardi were first reported in 1994, and the first complete analysis of its skeleton was published in 2009. Ardi possessed climbing hands and feet that resemble those of contemporary arboreal primates, and yet also had an upright posture. Our current data suggests that Ardi is not our direct ancestor, but instead a representative of a separate hominin lineage that went extinct.

AA: AA stands for *Australopithecus afarensis*, and we have found fossils of AA dating from 3.85 to 2.95 MYA in Eastern Africa. Following their first discovery in 1974, more than 300 skeletons of AA have been found. AA walked upright and had hands and feet similar to those of modern humans. Like Ardi, we believe that AA was not our direct ancestor, but rather a representative of a hominin lineage that went extinct.

HH: HH stands for *Homo habilis*, and we have found fossils of HH dating from 2.4 to 1.4 MYA in Eastern and Southern Africa. The first HH fossils were discovered in 1960. HH was a prolific producer of stone tools, and these tools were presumably responsible for the scrape marks present on fossilized animal bones often found near fossilized HH remains. HH had similar relative proportions, hand shape, and foot shape compared to contemporary humans. HH is believed to be the most ancient of our own direct ancestors for whom we have found fossils, which is why scientists have given them the same genus name *Homo*.

ERIC: Eric stands for *Homo erectus*, and we have found fossils of Eric dating from 1.89 MYA to 110,000 years ago. The first Eric fossils were discovered in 1891. Eric was the first of our direct ancestors to have brains and bodies nearly as large as contemporary humans, and over time some groups of *Homo erectus* migrated from Africa into Europe and Asia, where they established significant subpopulations.

NEO: Neo stands for *Homo neanderthalensis*, and we have found fossils of Neo dating from 400,000 to 40,000 years ago. The first Neo fossils were discovered in 1829. In Europe, the isolated subpopulation of *Homo erectus* gave rise to the Neo lineage, while *Homo erectus* remaining in Africa gave rise to the *Homo sapiens* lineage (i.e., our own) beginning 300,000 years ago. As an ice age waned, approximately 50,000 years ago, some groups of *Homo sapiens* migrated from Africa into Europe, and there was a significant overlap between *Homo sapiens* and *Homo neanderthalensis* in Europe for approximately 10,000 years until *Homo neanderthalensis* went extinct. As best we can tell, Eric, Neo, and ancient *Homo sapiens* all crafted tools, used fire, built shelters, hunted cooperatively, buried their dead, and practiced cannibalism.

While viewing the ancient hominin skulls on a timeline from most ancient to most recent, students should consider who counts as human to them, and why? Does being human begin with upright posture (Ardi), long distance walking (AA), complex tool crafting (HH), art and burial practices (Eric), metaphorical language (our only proof is for *Homo sapiens*, but we should recognize that the oldest preserved writing is only 5,000 years old, and metaphorical language use among hominins may be much older), or something else? These questions cannot be definitively answered but are instead intended to stimulate discussion.

Also, note that HH, AA, and Ardi were first discovered during the lifetimes of current scientists, and new fossils are found every year. We don't know what new discoveries will be made during our students' lifetimes.

Lastly, there is a chance that some students may ask about the scientific fraud known as "Piltdown Man," in which a research team intentionally assembled a contemporary human skull with an orangutan jaw and chimpanzee teeth, then used a rock tumbler and chemical treatments to give their creation the illusion of antiquity. We've chosen not to include this example in the main body of our lesson, choosing instead to provide an unfabricated data set to build confidence in students about our current scientific understandings.

It's true that students benefit from a discussion of the ways that our understanding of fossils has changed over time. For instance, current high-school students might be aware of museum displays of dinosaur skeletons that assembled the bones in inaccurate postures, such as the upright T-Rex; in recent years, many curators have been painstakingly dissolving glue from bones in order to display postures more accurately. Similarly, students may have seen recent newspaper articles about how cave paintings that scientists originally assumed were created by *Homo sapiens* have been re-identified as the work of *Homo neanderthalensis*. These are good-faith errors and come from the natural process of scientific models shifting as we discover additional data.

But the "Piltdown Man" story is different. Unfortunately, numerous scientists throughout history (and still today) have intentionally fabricated data. Each such case might have a unique story—the perpetrators might be motivated by financial gain, career aspirations, nationalistic pride, or even racism, as in the case of the "Piltdown Man" fraud—but the harms caused are depressingly similar. Fabricated data stalls scientific progress and often damages the public at large (e.g., the anti-vaccine movement, climate-change denial, not to mention a variety of psychology findings that have been widely reported in the popular press before the underlying studies were retracted) (Lewandowsky et al., 2013). The perpetrators of the "Piltdown Man" fraud were inspired by their pre-existing belief that human evolution must have begun with large brains and that Europe was the likely site of human origins. This 1912 fraud was intended to mislead the public, but we now have a preponderance of real evidence allowing us to address the questions of where human ancestors first lived and when our various traits arose.

○ Conclusion

While working through this lesson, students will inevitably ask many questions. In our experience, we are able to answer only a fraction of these: some questions that students generate will be philosophical (such as the question of which ancient hominins we might consider to be human), some questions will have answers that depend on data that no archaeologists have yet uncovered, and some questions will have answers present in the scientific literature that we don't remember in the moment or have not yet read. Within this qualitative exercise, such unanswered questions are perfectly acceptable. Students should be encouraged to think about what sort of experiments might help them discover answers to their questions, and to recognize that the future of science includes room for their own curiosity. In our experience, in addition to imparting important facts and encouraging high-level evolutionary thinking, the best aspect of this exercise is that the physical presence and hands-on manipulation of these skull casts helps that curiosity bloom.

Both the students and teachers we've worked with have found this lesson engaging and meaningful. Here are four comments from members of Kirstin's 2016 AP Biology class:

- "It was so engaging and intriguing. Making and using data to solve which trait came first was super interesting and I learned a lot. The real data and skulls made it hands on and interactive."
- "I really enjoyed the presentation and how it was organized—how we were asked to draw our own conclusions from given data and therefore better understand how scientists discover the things we know and hypothesize about evolution."
- "I was completely fascinated by the lesson. The lesson got me excited about evolution and the ways we can study it. I actually had my mom pull me out of lunch [at school] so we could grab coffee and talk about it."
- "I usually don't like sitting and just listening because it ends up making me sleepy, but I really liked the presentation yesterday. It's not really something I believe in, but I'm open to learning new things."

One teacher in the NCSE pilot of this lesson says, "I've found that using 3D replicas of fossils is much more impactful on student learning than simply having students look at pictures. Giving students the opportunity to handle the fossils, measure their dimensions, and observe them closely created an engaging learning environment in which they could more easily make comparisons and come up with their own questions. When students were able to compare the skull and anatomical structure of extinct hominid species with the structure of a modern human skull, the relatedness between humans and ancient hominids became irrefutable. Students who were skeptical about human evolution before the lesson became much more confident in the fact of shared ancestry."

We're hopeful this lesson will help you and your students to dig into human evolution to foster authentic scientific inquiry in high-interest, humane, collaborative ways. Equally importantly, we hope this work will inspire you to build your own lessons that leverage fun and wonder to grow learners' understandings of the natural world and its history.

Supplemental Materials

Available on the *American Biology Teacher* website:

- A digital copy of the complete student binder
- Complete annotated tree summarizing the lesson (Figure 3), plus a blank template

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ARMIN P. MOCZEK (armin@iu.edu) is the Class of 1955 Professor in the Department of Biology, Indiana University Bloomington. KIRSTIN J. MILKS (kmilks@mccsc.edu) teaches AP Biology at Bloomington High School South in Bloomington, Indiana. FRANK BROWN CLOUD (fbc@iu.edu) is a public scholar and writer based in Bloomington, Indiana. LIN ANDREWS (andrews@ncse.ngo) is Director of Teacher Support at the National Center for Science Education in Oakland, California. RAYA HAGHVERDI is a graduate student at the University of Pittsburgh and a former AP Biology student at Bloomington High School South.

INQUIRY & INVESTIGATION

Promoting Metacognitive Awareness and Self-Regulation of Intuitive Thinking in Evolution Education

TIM HARTELT, HELGE MARTENS

Abstract

Students' intuitive thinking often proves helpful in different contexts, such as everyday life, but can be an obstacle to learning about evolution. Thus, enhancing students' evolutionary understanding is often challenging, with intuitive conceptions of evolution still existing after instruction. Consequently, it is necessary to address students' intuitive conceptions explicitly. Thus, we present two metaconceptual learning activities that make students metacognitively aware of their intuitive conceptions and enable them to self-regulate these in the context of evolution: (a) a self-assessment of one's conceptions and (b) instruction on the context-dependency of conceptions. Both activities have been found more effective in enhancing students' evolutionary understanding than traditional instruction focusing solely on scientific conceptions and are, thus, recommended to supplement evolution instruction.

Key Words: *evolution; natural selection; student conceptions; metacognition; self-regulated learning.*

○ Introduction

Evolution is the unifying theme in biology (Dobzhansky, 1973) and is considered one of the "overarching core concepts" (American Association for the Advancement of Science, 2009, p. 9) of biology. Further, natural selection, in particular, is described as one of the "disciplinary core ideas" (NGSS Lead States, 2013, p. 75; see also College Board, 2019). Students should be enabled to "construct explanations […] of natural selection and evolution" (NGSS Lead States, 2013, p. 57), for example, by referring to scientific concepts such as variation, heritability, and differential survival/reproduction (College Board, 2019; Gregory, 2009). However, understanding and explaining evolution has proved challenging for students at different levels (Bishop & Anderson, 1990; Hartelt et al., 2022; Kuschmierz et al., 2020). One reason is that students' intuitive thinking influences their reasoning about evolution, and particularly, natural selection (Barnes et al., 2017; Coley & Tanner, 2015; Richard et al., 2017). Programs such as AP Biology state that it is important that students use precise language and avoid drawing on intuitive thinking when explaining evolutionary changes (College Board, 2019).

In the literature, various terms are used to describe student conceptions that are not in line with scientific conceptions, such as "misconceptions," "alternative conceptions," "preconceptions," or "intuitive conceptions" (see also Maskiewicz & Lineback, 2013). To describe the origin of many student conceptions of evolution and value students' conceptions, the terms "intuitive conceptions/ideas/thinking" will be used in this paper. Many of students' conceptions of evolution originate in cognitive biases/construals, in other words, "intuitive, often implicit, way[s] of thinking about the world" (Coley et al., 2017, p. 2). An intuitive way of thinking about the world might be "a set of assumptions, a type of explanation, or a predisposition to a particular type of reasoning" (Coley et al., 2017, p. 2). While these intuitive ways of thinking are valuable in many contexts (e.g., in everyday life), they can also hinder the acquisition of scientific knowledge, especially conceptual knowledge about evolution (González Galli et al., 2020; Shtulman, 2017; Shtulman & Calabi, 2012).

Three intuitive ways of thinking are particularly relevant in evolution education (Coley & Tanner, 2015; Gregory, 2009; Shtulman, 2006): teleology, anthropomorphism, and essentialism. A teleological bias (i.e., reasoning based on the assumption of a goal, purpose, or function) can lead to a goal-directed, purposeful understanding of evolution. An anthropomorphic bias (i.e., reasoning about non-human species or processes by analogy to humans) can lead to an understanding that species modify their characteristics intentionally during evolution. An essentialist bias (i.e., reasoning based on the assumption that group members share an immutable essence) can lead to neglecting or underestimating within-species variation, and consequently, to the assumption that species change as a whole from generation to generation.

These intuitive ways of thinking are pervasive, persistent, and context-dependent (Hartelt & Martens, 2024a): pervasive because they are core features of human cognition and exist across cultures, ages, and educational backgrounds (Coley et al., 2017; Kelemen, 1999; Medin & Atran, 2004); persistent because they often persist despite formal education, are resistant to change, and often coexist with scientific conceptions after instruction (Kelemen et al., 2013; Opfer et al., 2012; Shtulman & Valcarcel, 2012); and context-dependent because they prove helpful in some contexts but problematic in others (Coley & Tanner, 2012; González Galli et al.,

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2020; Shtulman & Calabi, 2012). In particular, it can be differentiated between everyday vs. scientific context, different scientific contexts, and different social contexts. For example, it may be appropriate to explain human actions in an everyday context in a teleological, goal-directed way but not evolutionary changes (González Galli & Meinardi, 2011; Kampourakis, 2014, 2020). Further, an essentialist bias, and thus, focusing on the similarities of a group, may be helpful when making predictions about an organism's ontogenetic development or when classifying organisms but not when explaining evolutionary changes due to natural selection (Shtulman & Calabi, 2012). Regarding the social context, it depends on the prior knowledge of the addressee how they may interpret an evolutionary explanation. For example, a speaker may have an appropriate understanding of evolution and use anthropomorphic language metaphorically, but a non-expert may interpret this anthropomorphic language inappropriately literally, and thus, intuitive conceptions may be reinforced in the addressee (Betz et al., 2019; Kallery & Psillos, 2004; Tempelmann et al., 2024; Thulin & Pramling, 2009).

Taking these considerations into account, it seems neither possible nor preferable to eliminate students' intuitive conceptions since these are resistant and also often appropriate. However, intuitive conceptions can be an obstacle in learning evolution and, thus, need to be addressed. Traditional instruction that focuses on teaching scientific concepts has been shown to have limited success in developing an appropriate understanding of evolution and reducing students' intuitive conceptions (Legare et al., 2018; Shtulman & Calabi, 2013; Tibell & Harms, 2017). When learning scientific concepts, students often do not see that these conflict with their intuitive conceptions, of which they are generally not metacognitively aware (Wingert et al., 2022). In consequence, they often integrate scientific concepts into their existing intuitive thinking, resulting in a coexistence of intuitive and scientific conceptions (Opfer et al., 2012).

Researchers and educators have suggested that instructional approaches based on self-regulated learning and metacognition can enhance students' conceptual knowledge about evolution (González Galli et al., 2020; Perez et al., 2022; Shtulman, 2022). When these instructional approaches focus on students' intuitive conceptions on a metacognitive level, they can also be described as "metaconceptual" (Yürük et al., 2009). A body of work provides evidence that metaconceptual instruction supports students' acquisition of conceptual knowledge and is superior to traditional ways of teaching where students' conceptions are not explicitly addressed (Kirbulut, 2012; Sabancı et al., 2020; Wiser & Amin, 2001; Yürük, 2007; Yürük et al., 2009, 2011). In particular, explicitly addressing students' intuitive thinking can promote their conceptual knowledge about evolution (Perez et al., 2022; Pickett et al., 2022; Wingert et al., 2022).

This paper aims to present metaconceptual learning materials for evolution education that attempt to make students metacognitively aware of their intuitive conceptions and enable them to self-regulate these in a context-dependent way: (a) a self-assessment of one's conceptions and (b) instruction on the context-dependency of conceptions (see Supplemental Material files 1–5a, b, which can be found in the online version of this article). The self-assessment can make students metacognitively aware of their conceptions as they engage in evaluating their current intuitive and scientific conceptions (Hartelt & Martens, 2024a, 2025; see also Andrade, 2019). The instruction on the context-dependency of conceptions can support students in becoming aware of the demands of different contexts and self-regulating their intuitive thinking depending on the context (Hartelt & Martens, 2024a; see also González Galli et al., 2020). In a recent study conducted by the authors of this article, evidence for the effectiveness of these specific instructional approaches in enhancing students' conceptual knowledge about evolution has been provided (Hartelt & Martens, 2024a; see section "empirical evidence for the effectiveness of the presented metaconceptual approaches").

○ Description of the Metaconceptual Learning Materials

General Remarks

The metaconceptual learning materials have been developed for and evaluated with upper secondary education students (class levels 10–13). However, the metaconceptual learning materials may also be adapted by educators for lower secondary or post-secondary education. Learning materials for two metaconceptual instructional approaches have been designed: (a) a self-assessment of one's conceptions and (b) instruction on the context-dependency of conceptions. The progress of one instructional sequence using the metaconceptual learning materials is suggested in Figure 1. However, the materials may be used by teachers differently and at different phases of evolution instruction. For example, the self-assessment may be used at the beginning of evolution instruction to make students aware of their preexisting conceptions (preferably if they already have some basic understanding of natural selection), in the middle of evolution instruction when students already should have developed a more thorough understanding of evolution, at the end of evolution instruction to let students self-evaluate their learning outcome, or at all of these phases to make the learning development visible to the students. For teaching the scientific concepts of natural selection prior to an instructional sequence using the presented instructional approaches (see Figure 1), suitable learning materials have already been published (e.g., Hartelt & Martens, 2024b; Jördens et al., 2018; Mohammadi et al., 2020).

The metaconceptual learning materials (see Supplemental Material files 2a, b and 4a, b) can either be distributed digitally or printed out. If printed out, the printing of Supplemental Material file 2a, b should be done in color, as the colors are essential in the self-assessment activity, and pens in different colors (matching the colors of the different concepts in the self-assessment) should be available to the students.

Metaconceptual Learning Material (a): Self-Assessment of One's Conceptions

In the introductory phase (Supplemental Material file 1a, b), the question is raised as to how cheetahs' ability to run fast developed during evolution (for a similar question to provoke students' conceptions of evolution, see Bishop & Anderson, 1990). To activate students' prior knowledge and allow a subsequent self-assessment, students are then asked to explain the evolutionary changes in the speed of cheetahs in written form (for the worksheet, see Supplemental Material file 2a, b, p. 1). For example, one student answered this question in a recent study conducted by us in the following way (see also Hartelt & Martens, 2024a; underlying concepts added in brackets):

> *Cheetahs became faster and faster because the other animals also improved more and more, and the cheetah, as an end consumer,*

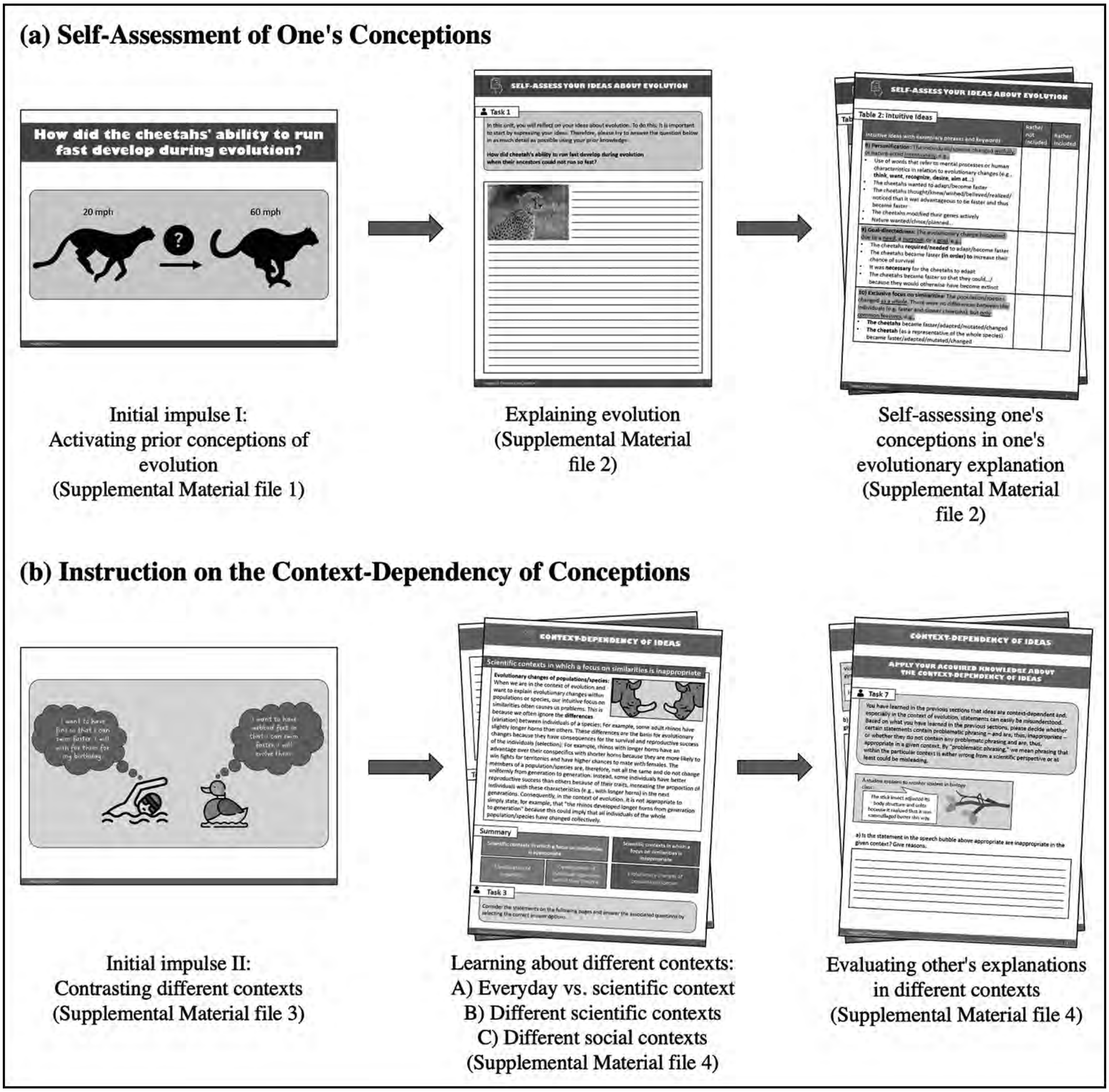


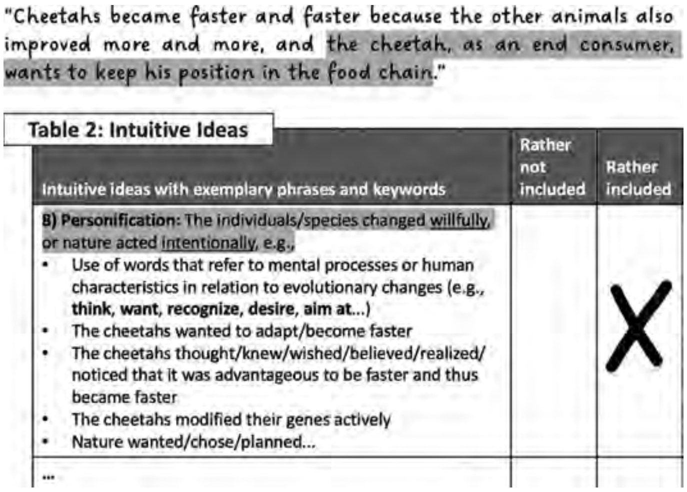
Figure 1. Suggestion for an instructional sequence using the metaconceptual learning materials (a) and (b).

Note. Figure development by the authors. Single parts of this figure are based on icons from Flaticon.com.

> *wants to keep its position in the food chain [anthropomorphism]. [...] Therefore, during evolution, it was essential for survival to be able to run short but incredibly fast, which is why only these cheetahs [variation] and their children survived because of this selection advantage [differential survival/reproduction].*

As this quote illustrates, students often have coexisting intuitive and scientific conceptions of evolution. This student, for example, expressed in their explanation of evolutionary changes an intuitive conception based on anthropomorphism and the scientific concepts of variation and differential survival/reproduction. It is important that students express their various conceptions in this task because the constructed explanation is the object of the following self-assessment.

For conducting the self-assessment itself (for the worksheets, see Supplemental Material file 2a, b, p. 2–6), students receive a list of seven scientific concepts of natural selection (e.g., variation, inheritance, differential survival/reproduction, etc.; Nehm et al., 2010) and a list of three intuitive ways of thinking (teleology, anthropomorphism, and essentialism; please note that we avoided these technical terms throughout the learning materials and used substitutes instead, e.g., "goal-directedness" for teleology). Exemplary phrases and key terms are provided in the lists for each scientific and intuitive conception to support the self-assessment. The students are asked to examine their prior explanation of evolutionary changes by color-coding in their explanation and checking off in the lists intuitive and scientific conceptions used. Taking up the previous



"Cheetahs became faster and faster because the other animals also improved more and more, and the cheetah, as an end consumer, wants to keep his position in the food chain."

Table 2: Intuitive Ideas

Intuitive ideas with exemplary phrases and keywords	Rather not included	Rather included
B) Personification: The individuals/species changed willfully, or nature acted intentionally, e.g., • Use of words that refer to mental processes or human characteristics in relation to evolutionary changes (e.g., **think, want, recognize, desire, aim at...**) • The cheetahs wanted to adapt/become faster • The cheetahs thought/knew/wished/believed/realized/ noticed that it was advantageous to be faster and thus became faster • The cheetahs modified their genes actively • Nature wanted/chose/planned...		X
...		

Figure 2. Example for the self-assessment regarding anthropomorphism/personification.

student's explanation, the student may self-assess their phrase "the cheetah as an end consumer wants to keep his position in the food chain" as anthropomorphic (respectively personifying), color the phrase in the respective color for anthropomorphism, tick off the box for anthropomorphism in the criteria list (see also Figure 2), and continue similarly for the other intuitive and scientific conceptions that are asked to be self-assessed. Depending on students' prior knowledge and self-assessment experience, or when teachers notice that students struggle with self-assessing their conceptions, teachers may consider going through the list of intuitive and scientific conceptions and assessing the exemplary explanation presented above together with the students before students individually conduct the self-assessment. Further, teachers may decide to reduce the number of scientific concepts that should be self-assessed to the most fundamental concepts of natural selection (Nehm et al., 2010): variation, inheritance, and differential survival/reproduction (number one to three in Table 1 in the worksheets; see Supplemental Material file 2a, b). To enhance the accuracy of the self-assessments, teachers may also encourage students to exchange their completed self-assessments with their peers and provide and receive feedback (see also the respective task in the worksheets), and/or teachers may collect students' self-assessments and provide feedback themselves. Based on the self-assessment (and potential feedback), students should set goals regarding future explanations of evolutionary changes as part of self-regulated learning. In total, working on this metaconceptual learning material should take around 40 minutes.

Metaconceptual Learning Material (b): Instruction on the Context-Dependency of Conceptions

To introduce the issue of the context-dependency of conceptions, the teacher shows a presentation slide (Supplemental Material file 3a, b). In this slide, anthropomorphic statements in two different contexts (everyday life context and evolutionary context) are shown. While the statement in the context of everyday life ("I want to have fins so that I can swim faster. I will wish for them for my birthday.") can be considered appropriate, a similar statement in the context of evolution ("I want to have webbed feet so that I can swim faster. I will evolve them.") would be inappropriate as organisms cannot willfully change their traits during evolution. Teachers can ask the students to compare and contrast the two statements. Based on initial students' responses, for example, that animals do not evolve new traits intentionally but that humans can produce and use objects intentionally, teachers can systematize the responses regarding what both statements share (a personifying view focusing on intentionality), and what differs between both statements (the context: everyday life vs. scientific context). Based on that, teachers can discuss with their students why one statement may be appropriate and the other not. Through the following instruction, students should become aware of the context-dependency of conceptions. To reach this goal, students read informational texts and work on different tasks (for the worksheets, see Supplemental Material file 4a, b; for sample solutions, see Supplemental Material file 5a, b). The learning materials focus on the following differentiations between contexts: (A) everyday life vs. scientific context, (B) different scientific contexts, and (C) different social contexts. Regarding (A) everyday life vs. scientific context, students are asked to explain why teleology and anthropomorphism may be appropriate in everyday life but not in the context of evolution by contrasting statements in both contexts. Regarding (B) different scientific contexts, students are asked to decide for different statements whether these are appropriate or not. This should be done based on provided information in which contexts an essentialist bias can be helpful (e.g., ontogenetic development and classification of organisms) and in which it could be problematic (e.g., evolution through natural selection). Regarding (C) different social contexts, students receive information on why it is important to consider the addressee of an explanation and their prior knowledge. They are asked to explain how a given statement about evolutionary changes may be misunderstood by someone with little prior knowledge and how these misunderstandings could be avoided. In a concluding activity, students decide and justify whether different statements regarding evolutionary changes are appropriate in a certain context. Discussions with a partner and other small tasks complement the worksheets. Teachers are free to decide whether and at which points they would like to review the tasks with the whole class. A discussion with the whole class can be very productive, especially for the evaluation of the various statements as "appropriate" or "inappropriate" as these evaluations can be discussed in a nuanced way in class. Further, teachers may decide to substitute some of the informational texts or tasks with presentations by themselves or with further class discussions. Without adjustments, working on this metaconceptual learning material should take around 70 minutes.

○ Assessment

To assess the outcome of the metaconceptual instructional approaches, students' ability to explain evolutionary changes can be assessed by using open response tasks, for example, "How would a biologist explain how the ability to dive for long periods evolved in seals when their ancestors could not dive for such long periods?" (see also Nehm et al., 2012). The quality of students' explanations can be assessed by the degree to which students use appropriate scientific conceptions and inappropriate intuitive conceptions. Teachers can use the listed conceptions in the self-assessment sheet to categorize students' intuitive and scientific conceptions. Depending on students' prior knowledge and educational level, it may also be sufficient to focus only on the intuitive conceptions and the three most fundamental scientific concepts of natural selection (variation, inheritance, and differential survival/reproduction) instead of all seven listed scientific concepts. Depending on the educational level, thresholds for good explanations may also vary. For second-semester biology college

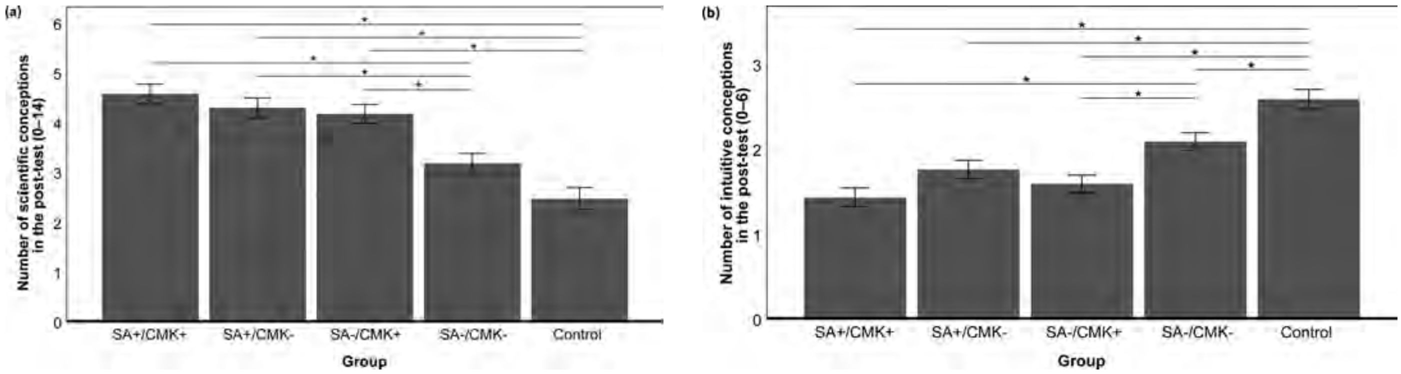


Figure 3. Students' use of intuitive and scientific conceptions across two evolutionary explanations in the post-test after receiving instruction with/without the metaconceptual learning materials. **(a)** Number of scientific conceptions (0–14). (**b)** Number of intuitive conceptions (0–6). Figure based on Hartelt & Martens (2024a). CC BY 4.0.

Note. Mean scores are displayed in the figure, and error bars represent standard errors; * = $p < .05$. SA = self-assessment of one's conceptions; CMK = instruction on the context-dependency of conceptions; plus sign (+) = the group received the respective intervention; minus sign (-) = the group did not receive the respective intervention but traditional instruction focusing on the scientific concepts of evolution; Control = group that did not receive any instruction on evolution or another topic at all.

majors, Nehm and Reilly (2007) suggested that students using four or more scientific concepts of natural selection (out of seven) should receive a "passing score," regardless of the number of inappropriate conceptions. If students use no inappropriate conceptions, even one scientific concept should be enough for a "passing score" (for details, see Nehm & Reilly, 2007). However, teachers may use considerably different thresholds based on their learners' characteristics.

The proposed summative assessment could also be transformed into a formative self-assessment if another self-assessment follows this task. For this, the exemplary phrases of the self-assessment worksheet (regarding cheetahs' speed) would have to be adjusted to the assessment task, e.g., seals' diving ability. This way, students could become metacognitively aware of and monitor their progress and changes in their conceptions, which could promote their self-regulated learning.

○ Empirical Evidence for the Effectiveness of the Presented Metaconceptual Approaches

The effectiveness of the metaconceptual approaches presented in this paper has been investigated in an experimental intervention study with $N = 730$ upper secondary school students. The methods and results of this study are described in detail in Hartelt & Martens (2024a, c) and readers are encouraged to consult these papers for detailed information beyond the main information provided in this section. Using a 2x2 factorial, pre-post-follow-up-test design, students were randomly assigned to four different groups that received either one/both metaconceptual instructions or traditional instruction focusing solely on the scientific concepts of evolution. A further fifth group received no instruction at all between the pre- and post-test, thus, differing from the other groups that received traditional instruction focusing on scientific concepts if they did not receive metaconceptual instruction. The effectiveness of the interventions was measured in terms of intuitive and scientific conceptions used by the students in two evolutionary explanations (contexts: evolutionary gain of a trait in a familiar species; evolutionary loss of a trait in an unfamiliar species). The questions prompting students to provide evolutionary explanations were phrased similarly to the question proposed as an assessment tool (see section "assessment"; see also Nehm et al., 2012). The questions included different species at the various measurement points (e.g., for the gain of a trait in a familiar species: anteaters' tongue, seals' diving ability, and hyenas' dentition) to reduce fatigue effects. Explaining the evolutionary changes of the species used as examples across the measurement points has been found equally difficult in a pre-study. The students' conceptions (for each evolutionary explanation: three intuitive conceptions and seven scientific conceptions) were coded by two experienced raters (κ = .843; almost perfect interrater reliability; Landis & Koch, 1977) based on the coding guide of Nehm et al. (2010). Considering the students' conceptions across both evolutionary explanations, it was found that students who conducted the self-assessment used more scientific conceptions compared with the students who did not conduct the self-assessment, while the use of intuitive conceptions did not differ between the groups. The instruction on the context-dependency of conceptions resulted in a higher use of scientific conceptions and a lower use of intuitive conceptions compared with the traditional instruction focusing solely on the scientific concepts of evolution (for the detailed results, see Figure 3). Moreover, a combination of both metaconceptual instructional approaches has proven to be especially successful in enhancing students' conceptual knowledge of evolution in the long term (for a detailed description and analysis of the follow-up test results, see Hartelt & Martens, 2024a). A further important finding was that making students metaconceptually aware of their intuitive conceptions led to more accurate beliefs about their abilities (for a detailed description and analysis of the intervention effects on students' self-efficacy and self-efficacy bias, see Hartelt & Martens, 2024c). Together, these findings provide strong arguments for implementing these metaconceptual instructional approaches in evolution instruction.

○ Conclusion

The metaconceptual learning materials presented in this paper represent a practical approach to address students' intuitive thinking in evolution education as the materials (see the various Supplemental Material files) are ready to use for implementation by teachers but can also be adapted by teachers for diverse student groups. The learning materials provide students with the opportunity to distinguish between intuitive and scientific conceptions, to become metacognitively aware of their conceptions, to self-regulate their conceptions depending on the context (e.g., inhibit intuitive conceptions when they are not appropriate in the context of evolution), and, in consequence, develop a profound understanding of evolution respectively natural selection. As there is robust empirical evidence for the effectiveness of the presented metaconceptual learning materials (Hartelt & Martens, 2024a), teachers can expect more scientific conceptions and less inappropriate intuitive conceptions of evolution—compared with non-metaconceptual instruction focusing solely on the teaching scientific conceptions—when implementing the metaconceptual learning materials in their evolution instruction. Further, we recommend including additional metaconceptual learning activities following the presented activities, e.g., by letting students identify explanations based on intuitive thinking in authentic media contents or encouraging classroom discussion on a metaconceptual level when a student again draws on intuitive thinking.

It has been demanded that teachers should be supported in "diagnosing and responding to students' problems in mastering new concepts" (National Research Council, 2012, p. 312) and that it should be investigated "what instructional intervention [. . .] can move students along a path from their initial understanding to the desired outcome" (National Research Council, 2012, p. 313). With the metaconceptual learning materials presented, we have addressed these issues, as they have been investigated for their effectiveness in enhancing students' understanding by addressing their intuitive preconceptions (Hartelt & Martens, 2024a), and provide students with the opportunity to diagnose their conceptions themselves and learn how to self-regulate their intuitive conceptions in the context of evolution.

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TIM HARTELT (hartelt@uni-kassel.de) is working as a researcher and doctoral candidate at the Department for Biology Education at the University of Kassel. HELGE MARTENS (martens@uni-kassel.de), PhD in biology education, is professor for biology education at the University of Kassel.

ABT AUTHORS & PHOTOGRAPHERS

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- Reviews and updates of recent advances in the life sciences in the form of an "Instant Update" that brings readers up-to-date in a specific area
- Imaginative views of the future of biology education and suggestions for adjusting to changes in schools, classrooms, and student populations
- Other timely, relevant, and interesting content such as discussions of the role of the Next Generation Science Standards in biology teaching, considerations of the nature of science with implications for the classroom, considerations of the continuum of biology instruction from K–12 to post-secondary teaching environments, or contributions that consider the likely/ideal future of science and biology instruction

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continuation

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William McComas, Editor-in-Chief
ABTEditor@nabt.org

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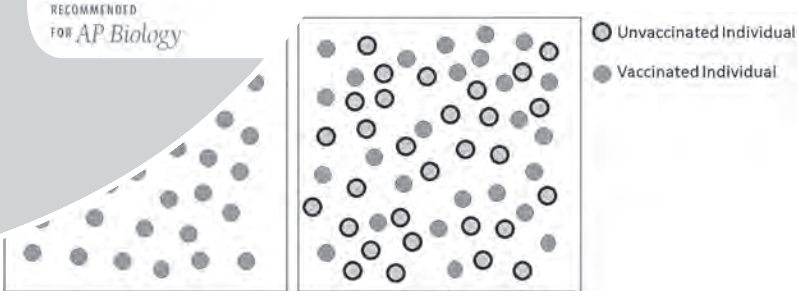
INQUIRY & INVESTIGATION

Modeling Herd Immunity: An Introductory Course Activity

RECOMMENDED FOR AP Biology



AMBER M. EADE, CORTNEY DEAN,
STACY L. HRIZO

Abstract

Teaching epidemiology, particularly more advanced concepts such as vaccine efficacy and herd immunity, can be very challenging in science classes geared for non-science majors. Nevertheless, as became quite evident during the recent COVID-19 pandemic, a thorough understanding of such topics is highly important to all individuals due to its role in informing future decision-making related to public health. One method for easing comprehension of difficult science content is to employ hands-on activities that engage students in critical thinking while visually demonstrating difficult concepts. While introductory activities illustrating the spread of disease through unprotected populations already exist, those that model the more advanced topics of vaccination and herd immunity are limited. In addressing this gap, the present manuscript describes an activity that expands on prior exercises through the addition of common biological buffers (MOPS and HEPES) mimicking vaccination status. Further, detailed exploration of buffer concentrations and interaction conditions during the development of this activity underscores potential avenues for class discussion of real-world outcomes. This includes the concept that exposure to a pathogen does not invariably result in illness, and vaccination does not always guarantee immunity against infection. Overall, the resultant activity creates avenues to enrich student comprehension of epidemiology, providing valuable insights into disease transmission, effectiveness of vaccination, and the dynamics of herd immunity.

Key Words: *Epidemiology; herd immunity; vaccination; infection model.*

○ Introduction

Pathogens require the availability of susceptible hosts to successfully reproduce and spread. Interestingly, there is an inherent dichotomy present when pathogens infect their host targets. When infecting a host, pathogens enhance their own transmission potential by spreading to additional new hosts. Simultaneously, the host organisms immune response triggers the development of a natural immunity against the pathogen. The resulting immune response ultimately diminishes the pathogens ability to reinfect the original host and limits its future transmission to other individuals. As the overall immunity against a pathogen subsequently increases within a population, the occurrence of the pathogen tends to decline. When a significant portion of the population becomes immune to a pathogen, its propagation becomes restricted and, in some instances, diseases can become eradicated. This fundamental concept, known as herd immunity, is vitally important in protecting vulnerable populations, such as those with compromised immune systems, from diseases that may produce life-threatening outcomes (Metcalf et al., 2015). The medical community has taken advantage of the concept of herd immunity using vaccinations. Instead of relying on natural immunity acquisition, which carries risks of the potential negative effects of illness due to infection with the pathogen, individuals can receive a vaccination that will prompt their immune systems to develop resistance against a specific pathogen without the untoward consequences. If a substantial portion of the population receives vaccinations, the transmission of the targeted pathogen will slow and may cease altogether, just as would occur with natural immunity.

Given the role of vaccination in the management of many modern-day diseases, the ability to demonstrate the impact of this procedure has become of interest. Moreover, discussions regarding why some individuals still experience unexpected outcomes, such as becoming ill even following vaccination, are important lessons in understanding the limitations of vaccines. Teaching epidemiology of this nature in the undergraduate student setting holds significant importance due to its role in informing future decision-making related to both public health concerns and personal healthcare choices. This became increasingly apparent during the COVID-19 pandemic, where the spread of pathogens and use of vaccines became regular discussion points throughout the world. As students may find the biological concepts underlying pathogen transmission and vaccine effectiveness difficult, employing engaging activities that visually demonstrate concepts can add contextual cues that make material easier to understand. This is especially advantageous in classes attended by non-majors, who may possess limited familiarity or interest in the subject matter. Many introductory activities showcasing how pathogens propagate within a population are available for use in non-majors' biology courses. However, activities that effectively model more advanced topics such as vaccine efficacy and herd immunity are limited.

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○ Activity Overview

Previously utilized laboratory activities demonstrate the spread of viral disease through an unprotected population, effectively conveying fundamental principles of epidemiology. Such activities utilize water-filled vials to simulate "uninfected" individuals while one "infected" vial contains sodium hydroxide (NaOH). Through a series of manual fluid exchanges, representing physical interaction between members of a community, NaOH is transmitted through the vials, thereby "infecting" other members of the population. Toward the conclusion of the activity, the addition of a phenolphthalein indicator solution to each vial exposes any changes in the fluid pH caused by the movement of NaOH during fluid swaps. Phenolphthalein is a weak acid that will change its color from clear to pink as it detects increases in pH in the range of approximately 8.2–9.8. The resulting color can range from light pink for slight increases in pH to hot pink for larger increases in pH. When added to each vial at the end of the class activity, phenolphthalein causes the vials exposed to the NaOH turn a bright pink color, while the unexposed vials remain clear. This procedure effectively identifies participants whose vials became "infected" with NaOH during the activity. The color change caused by the pH indicator creates excitement in the room as students reveal the outcome of their interactions with classmates.

Activities of this nature are both enjoyable and instructive, effectively illustrating how infections can disseminate through community interactions in an engaging manner. However, these activities do not address the concept of herd immunity and broader impacts of vaccination on pathogen transmission. A more recently developed activity employed the use of a Tris buffer to mimic the effect of vaccination, effectively expanding on commonly used laboratory activities that modeled disease transmission (Schwingel, 2018). This builds on the initial demonstration by introducing "protected" members to the population and adds in a discussion of the overall effects of herd immunity. As described below, we have since expanded on this activity further, exploring different concentrations of additional buffer options and exchange conditions in a way that provides additional discussion of real-world outcomes. Specifically, recognition of the potential outcomes resulting from different combinations of fluid interactions during this activity prompts student discussion of why some members of a community do not become ill following exposure to a pathogen whereas others who hope to be protected via vaccination can still become infected. This is an important discussion due to the variable nature of herd immunity following vaccination for various pathogens (Fine, P.E., 1993). The level of immunization required to achieve herd immunity can differ greatly based on the pathogen at hand. Herd immunity against measles, for example, requires 95% of the population to be vaccinated, whereas the vaccination threshold for polio herd immunity is 80% of the population (Plans-Rubió, 2021; Lai et al., 2022). Thus, through this exercise students may not only gain an understanding of the concept of heard immunity, but also develop more in-depth appreciation of the potential for vaccine limitations and breakthrough infections.

○ Updated Protocol Development and Testing

Development of the present activity involved evaluating the outcomes (i.e., infected vs uninfected) created through a variety of possible combinations of fluid exchanges that could be encountered during an in-class activity. Lab activities modeling pathogen transmission have students exchange fluids with either 3 or 4 partners. Due to the high number of samples that could be achieved through larger numbers of fluid exchanges, development of the present protocol started with only 3 exchanges. By adding the use of a buffer to mimic effects of vaccination, the types of fluid included in the exchanges increased from 2 in the original activity to 3 in the updated version (NaOH – infected, H20 – uninfected/unvaccinated, and Buffer – vaccinated).

Several considerations were made in determining which of all possible fluid exchange combinations required examination. In designing the fluid swaps for our study, several combinations of fluid did not require testing due to having no potential for an effect or being duplicative of another swap combination. For example, as the activity is designed to begin with only 1 vial of NaOH (*infected*) and all fluid exchanges are novel, there was no potential for any exchange combination to include more than 1 instance of full-strength NaOH. Similarly, as the testing outcome for a vial containing only a buffer or water alone would already be known (i.e., negative), there was no need to test outcomes of exchanges that contained all the same substance or any combination of only water and buffer. Combinations of water and NaOH are already known to produce a positive outcome from the traditional lab activity, thus also do not require testing. Finally, since the protocol involves moving ½ of the vials' contents during each exchange, it was not necessary to test combinations in which the initial content and fluid 1 content would be reversed. Thus, if the initial vial contained NaOH and the first exchange was water, it was not necessary to test an initial vial of water with a first exchange of NaOH. These considerations drastically limited the number of fluid combinations that required examination. The remaining fluid swap combinations that were utilized for this study are illustrated in Table 1 and further described below.

The determination of which combinations to use in the activity was based on allowing validation of expected real-world outcomes for end-of-activity discussions regarding how various interactions can prevent vs promote infection status. Specifically, three outcomes were important to maintain viability of the activity: (1) the original "infected" NaOH vial could not become "uninfected" following dilution with water and/or buffer, (2) the full-strength buffer vials should convey some level of protection to the population, and (3) the water vials must illustrate potential effects of exposure to uninfected and infected members of the population. The 12 fluid exchange combinations presented in Table 1 allowed for the testing of these outcomes.

To examine the effects of commonly used biological buffers acting as vaccines, the first attempt at introducing buffers included 0.1 M MOPS (4-(3-sulfonatopropyl)morpholin-4-ium) and 0.1 M HEPES (N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid). As described below, these concentrations were adjusted following initial outcomes that were inconsistent with the goals of the activity. Overall, it is imperative to realize that the goal of this activity is to provide meaningful population-based outcomes related to initial infection, potential of infection transmission, and potential for protection of the population due to vaccination. As real-world scenarios do allow some vaccinated individuals to become infected as well as unvaccinated individuals to avoid infection, it is not critical to analyze the outcome of every individual interaction. Instead, end results related to the goals of the activity were most important to examine. Thus, it was more imperative to ensure that initially

Table 1. Fluid Exchange Combinations Tested.

Exchange Combinations					
Vial #	**Initial Contents**	**Fluid 1**	**Fluid 2**	**Fluid 3**	**Interaction Representation**
1	NaOH	Water	Water	Buffer	Infected individual interacting with 2 uninfected and 1 vaccinated individual
2	NaOH	Water	Buffer	Buffer	Infected individual interacting with 1 uninfected and 2 vaccinated individuals
3	NaOH	Buffer	Buffer	Buffer	Infected individual interacting with 3 vaccinated individuals
4	NaOH	Water	Water	Water	Infected individual interacting with 3 uninfected individuals
5	Water	Water	Buffer	NaOH-D	Uninfected individual interacting with 1 uninfected, 1 vaccinated, and one carrier individual
6	Water	Buffer	Buffer	NaOH-D	Uninfected individual interacting with 2 vaccinated, and one carrier individuals
7	Water	Water	Buffer	NaOH-S	Uninfected individual interacting with 1 uninfected, 1 vaccinated, and 1exposed individual
8	Water	Buffer	Buffer	NaOH-S	Uninfected individual interacting with 2 vaccinated, and 1exposed individual
9	Water	Buffer	NaOH-D	NaOH-S	Uninfected individual interacting with, 1 vaccinated, 1 carrier, and 1exposed individual
10	Buffer	Water	Water	NaOH-D	Vaccinated individual interacting with 2 uninfected and 1 carrier individual
11	Buffer	Water	NaOH-D	NaOH-S	Vaccinated individual interacting with 1 uninfected, 1 carrier, and 1 exposed individual
12	Buffer	NaOH	NaOH-D	NaOH-S	Vaccinated individual interacting with 1 infected, 1 carrier, and 1 exposed individual

Note. NaOH-D = Carriers. Partial infection (1/2 strength due to 1 dilution) that may still transmit infection. NaOH-S = Exposed. Low-level of exposure (1/4 strength due to two dilutions) that may be less likely to transmit infection status.

infected vials remain infected, as the transmission of vaccination from one individual to another is unrealistic, than it was to prevent vaccinated vials from becoming infected, which is a possible real-world outcome. As such, the expected outcomes of the resulting 12 potential fluid exchange combinations that were evaluated are presented in Table 2 alongside the observed outcomes following testing with different buffer concentrations. Although not all expected outcomes were observed, the last-attempt outcomes are consistent with the goals of the study, provide realistic examples of what can occur in real-world scenarios, and prompt discussions regarding expectations vs reality with respect to vaccination efficacy.

With respect to the first testing requirement noted above, the maintenance of infection following dilution was examined using combinations of NaOH with water and/or buffer (Table 1, Vials 1–4, and 12). The positive result at all concentrations of MOPS and HEPES in Vial 4 (Table 2) illustrate the effect of NaOH cannot be simply diluted out with 3 water exchanges. However, once buffer was added, it was determined that the initial concentration of MOPS (0.1 M) was too strong and negated the effects of initial infection in each instance (Table 2, vials 1–3). As the goal of the activity was not to allow the effects of vaccination to be transferred to an initially infected individual, these results illustrated the need to decrease the concentration if using MOPS as a buffer option. Thus, our last-attempt trials occurred using 0.085M MOPS.

Notably, adding water to NaOH during this activity does result in dilution of the initial infected vial. Resulting effects of "partial" transmission of infection in later exchanges required consideration. Therefore, exchanges in later vials are noted in Table 1 as full strength (NaOH), partial dilution due to 1 prior water exchange (NaOH-D), and a second dilution due to 2 prior water exchanges (NaOH-S). As exchanges use half of the fluid from the original vial, a single dilution cuts the NaOH concentration by half, whereas a second swap then decreases it to approximately one quarter of its original concentration. For discussion purposes, it is possible to consider these different levels of "exposure" in real-world terms of potential for an infection to be transmitted. For example, transmission can occur not only with those who are infected and symptomatic, but also through exposure from carriers who are asymptomatic or early in the stages of infection but still producing infectious particles. Given the exercise starts with only 1 infected vial and the first fluid exchange immediately dilutes that concentration by half, full-strength NaOH can exist only in the initial contents of the vial or as the first fluid swap. Similarly, as it takes 2 water swaps to reach the NaOH-S status, this exchange can occur only as the third exchange (i.e., Fluid 3 of Table 1). It was unnecessary to test the effects of diluting the infection with buffer, as the effect of initial infection is anticipated to outweigh buffer in all instances. Thus, if the outcome of NaOH is not negated by three full-strength buffer exchanges,

Table 2. Infection Outcome following 3 Fluid Exchanges.

Vial #	Expected Outcomes	First-Attempt Outcomes		Last-Attempt Outcomes	
		0.1 M MOPS	0.1 M HEPES	0.085 M MOPS	0.2 M HEPES
1	Initial Infection Status Persists (+)	–[1]	+	+	+
2	Initial Infection Status Persists (+)	–[1]	+	+	+
3	Initial Infection Status Persists (+)	–[1]	+	+	+
4	Initial Infection Status Persists (+)	+	+	+	+
5	Acquisition of Infection (+)	–	+	+	+
6	Possible Acquisition of Exposure (+) or Protection via Herd Immunity (–)	–	–	–	–
7	No Transmission of Infection (–)	–	–	–	–
8	No Transmission of Infection (–)	–	–	–	–
9	Acquisition of Infection (+)	–	+	+	+
10	No Transmission of Infection (–)	–	+[2]	–	–
11	No Transmission of Infection (–)	–	+[3]	+[3]	+[3]
12	No Transmission of Infection (–)	+[3]	+[3]	+[3]	+[3]

+ = color change = *infected*

– = no color change = *uninfected*

[1] = Buffer inappropriately transmitted protection

[2] = Buffer ineffective against secondary transmissions

[3] = Buffer ineffective against multiple exposures

such as that outlined in Vial 3 (Table 2), then there is no reason to believe that it would be altered by diluted levels of buffer.

To test the second requirement of initially vaccinated (i.e., buffer) vials conveying protection when exposed to the pathogen in most instances, it was important to test the range at which the pathogen could be experienced (i.e., partial exposure through diluted levels of NaOH vs combinatorial exposure with full and diluted NaOH). Additionally, as vials 6, 8, and 9 started with water and experienced a first swap with a buffer, the outcome for these vials would be the same as for vials that started with buffer and initially swapped with water. This is confirmed by the fact that vials 9 and 11, which started with opposite contents, have identical outcomes (see Table 2). The outcomes of these vials can be simultaneously discussed as relates to individuals who started vaccinated and encounter multiple exposures to infected individuals vs. those who were not vaccinated but encounter a mixture of vaccinated and infected individuals.

Looking at Table 2 outcomes for Vials 6 and 8–11, it is evident that, even with a dilution from a first exchange with water, the 0.1 M MOPS was successful against effects of NaOH-D and NaOH-S either presented independently or combined. However, this concentration was ineffective with repetitive transmission including both full and partial infection (Vial 12). Initial concentration of 0.1 M HEPES, on the other hand, was too weak and allowed transmission of infection status following a single dose of NaOH-D (Vial 10) unless additional buffer exposure occurred, preventing dilution of the initial buffer concentration (Vials 6 & 8). As it is possible for some individuals who are vaccinated to still end up positive for the pathogen, the results of the vaccine not preventing transmission at the most extreme levels of what could be modeled with this activity, such as what was observed in Vial 12, were not concerning. However, it was important for vaccination to provide some level of protection to the population. Thus, the 0.1 M HEPES not protecting against even partial infection transmission was problematic. This led to the recognition that the HEPES concentration needed to be adjusted for future use. Increasing the HEPES concentration to 0.2 M effectively returned the buffer to being effective against partial transmission (Vial 10) while still allowing those with multiple exposures to become infected (Vials 11 & 12). While these results do not ensure that all "vaccinated" vials maintain protection against infection, they do allow an appropriate combination of infection versus protection for discussion purposes.

Finally, for those vials that started with water and maintained a first swap with water (vials 5 & 7), appropriate results for the level of infection exposure were desired. Thus, it was deemed acceptable for a vial that swapped with buffer and the lowest level of infection (i.e., NaOH-S) to maintain an uninfected status (Table 2, Vial 7), while a larger level of infection (i.e., NaOH-D) should produce infection (Table 2, Vial 5). The initial level of MOPS used was too strong to allow the appropriate level of infection status to be observed, but decreasing to the 0.085 M level resolved this issue.

As can be seen in the "Last-Attempt Outcomes" columns of Table 2, the concentrations of 0.085 M MOPS and 0.2 M HEPES met all testing requirements by (1) allowing initial infection to remain present even after multiple interactions with vaccinated vials, (2) allowing vaccination to convey a partial level of protection, and (3) illustrating appropriate combinations of interactions in the unvaccinated population. Identical outcomes were achieved in three separate lab trials.

Following determination of the appropriate buffer concentrations, a prior lab activity that illustrated infection without the use of vaccines was modified to include the new vaccination component. Protocols were designed to allow for groups of 24 and 40, modeling the sizes of typical undergraduate non-majors biology classes. The resultant activity includes 3 'rounds' each consisting of a different rate of vaccination (0%, 25%, and 75%). The protocol was first tested in the lab and subsequently in student groups consisting of either biology majors or non-majors. In the lab setting, protocols were tested and revealed infection rates of 20–33% when unvaccinated, 7.5–17% with 25% vaccination, and 0% when 75% of the vials were vaccinated. Thus, an appropriate decrease in infection rate was clearly demonstrated as vaccination rate increased. While formal student feedback data following the activity was not collected, students were reported to appear receptive to the new protocol and remained engaged during the activity.

As expected, due to the fact that students get to choose who to exchange fluid with during the activity (*see Lesson Guide below*), each attempt at this activity with different student populations produced slightly different results. However, the overall ability to illustrate the concepts of pathogen transmission and vaccine effects are consistently evident. When testing the protocol with student groups, the percent of individuals infected at the end of the exercise appropriately decreased as a result of vaccination. Unvaccinated rounds produced a 28–40% infection rate, whereas the infection rate decreased to 10–25% when 25% of the population was vaccinated and even further to only 3–5% infected when 75% of the population had been vaccinated. These results are very similar to what was achieved in the lab setting and illustrate that no matter what order the students opt to exchange fluids, the overall outcome appropriately models the intended scenario of herd immunity.

Following determination of the appropriate buffers and resulting protocol for a set of 3 exchanges, increasing to 4 fluid exchanges using the same overall protocol was also attempted. The increase in fluid exchanges resulted in too many opportunities for initially infected NaOH vials to encounter buffer solutions at the 75% vaccination rate. This resulted in the NaOH vials testing negative when exposed to the phenolphthalein indicator. As such, this violated the first goal of the activity, which was to be able to maintain a positive outcome in vials that began as "infected." With the increased buffer exchanges, the result of 4 swaps effectively led to the transmission of vaccination status, which is unrealistic in the human population. As such, we opted to limit the activity to only 3 rounds of fluid swaps.

○ Lesson Guide

What follows is a brief description of the resulting lab activity, including overall lesson plan for introducing the activity and discussing results. The ultimate objective of this activity is to demonstrate the concept of herd immunity using a hands-on simulation that showcases how disease transmission can be affected by varying vaccination rates within a population. To accomplish this task, students must first be introduced to the basic concepts of epidemiology and investigate disease transmission in unvaccinated individuals. Therefore, this is a three-part lesson that includes an introduction in addition to a multi-step activity (see Figure 1). Prior to engaging students in the hands-on activity, a brief lecture and discussion occur focusing on factors contributing to disease transmission, terminology pertinent to transmission rates, the role of organizations in tracking diseases, and how vaccinations assist in limiting transmission opportunities. Subsequently, the hands-on activity is introduced and faculty lead students step-by-step through the process, ensuring uniform progression and preventing any student from falling behind or moving too far ahead of the group. To pique interest and keep students engaged with the subject matter, the initial explanation of the activity includes informing students that the exercise they are about to complete simulates the transmission of a hypothetical sexually transmitted infection.

To ensure recognition of the distinct differences in transmission rates in vaccinated versus unvaccinated populations, this activity consists of two distinct observation rounds (see Figure 1): the first models general disease transmission with an unvaccinated population and the second demonstrates transmission within populations with an approximately 25% and then 75% vaccination rate. Optionally, if time allows, this protocol can be further adapted to include additional rounds showcasing transmission rates for other levels of vaccination (i.e., 50%). In each round, students engage in simulated transmission by exchanging fluid from vials containing water (representing healthy fluid), sodium hydroxide (representing the infection), or a buffer solution (mimicking vaccination). Students complete 3 rounds of fluid exchange with different partners, after which their infection status is revealed via use of a Phenolphthalein pH indicator. Throughout the activity, students document each of their own transmission exchanges. Once all exchanges have occurred, students collectively create a data spreadsheet that contains all information on the order of fluid exchanges as well as the results provided by the pH indicator. This information allows students to calculate the transmission rate and engage in discussions

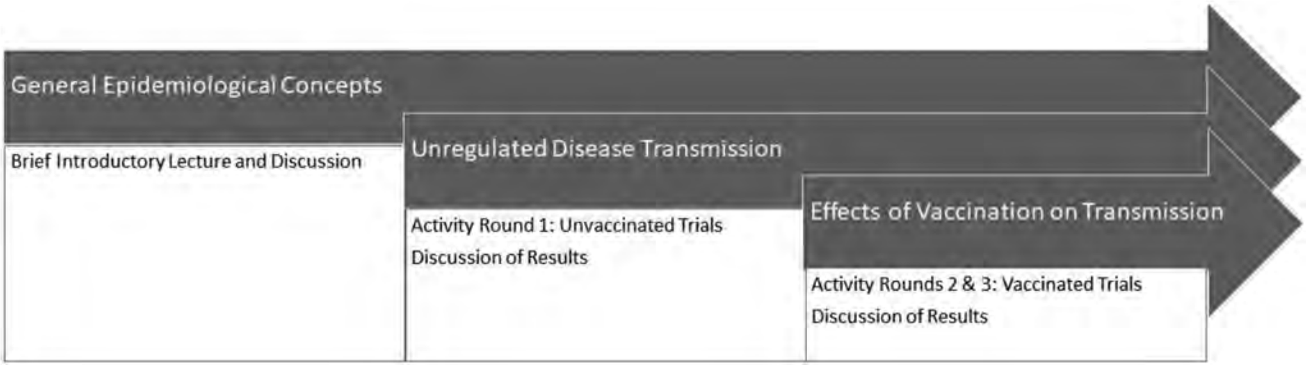

Figure 1. Activity Layout.

The suggested activity plan includes progression through a three-part progression lesson in which the hands on activities build on knowledge gained in the prior steps.

regarding disease propagation across human populations experiencing varying vaccination levels.

Through completion of this modified lab activity, students can explore the concept of herd immunity and relate it to the transmission of diseases throughout the human population. Students gain experience working with pairs during fluid exchange and working in large groups during the compilation of final data sheets and tabulation of results. Additionally, students are prompted to discuss not only the overall effects of different vaccination rates on the end result of population infection, but the potential outcomes for individuals who are vaccinated vs unvaccinated when they come in contact with an infected individual. Advanced concepts such as active infection, secondary transmission, and breakthrough infections may also be discussed in context of possible fluid exchange outcomes. Overall, it is expected that this activity will enhance opportunities for exploration of epidemiology-based topics in a classroom setting.

○ Supplemental Materials

The following appendices contain step-by-step instructions for instructors and students and are available as Supplemental Material with the online version of this article:

- Appendix 1: Instructor Activity Guide
- Appendix 2: Student Worksheet

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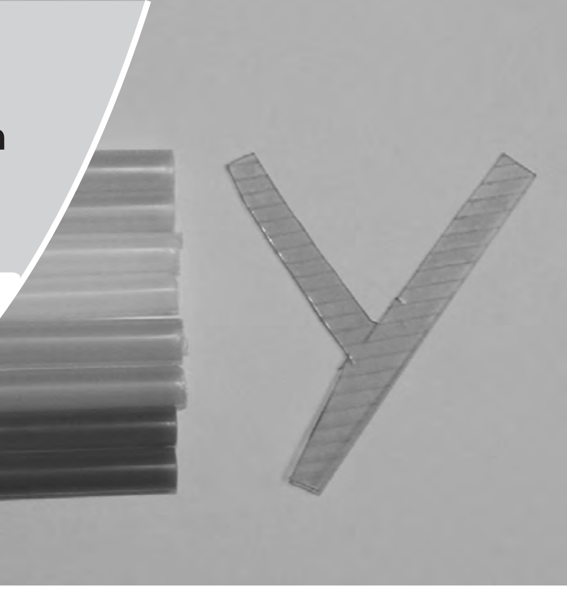
AMBER M. EADE (amber.eade@sru.edu) is an Associate Professor in the Department of Biology at Slippery Rock University. CORTNEY DEAN was an undergraduate student in the Department of Biology at Slippery Rock University. STACY HRIZO (stacy.hrizo@sru.edu) is a Professor in the Department of Biology at Slippery Rock University. Correspondence regarding this article should be addressed to Dr. Stacy Hrizo, 1 Morrow Way, Slippery Rock, PA 16057.

TIPS, TRICKS & TECHNIQUES

Building Phylogenetic Trees with Drinking Straws



MARCOS MÉNDEZ

Abstract

Tree-thinking is a required skill for evolutionary literacy, but misconceptions are frequent in interpreting phylogenetic trees. Here, an easy, cheap way of building phylogenetic trees with drinking straws is suggested. It can be used to illustrate how to build phylogenetic trees from root to tips, to rotate nodes and to collapse branches.

Key Words: *evolution; phylogenetic trees; misconceptions; hands-on learning.*

Tree-thinking, the ability to conceptualize evolution in terms of phylogenetic trees (Baum & Offner, 2008), is a skill needed to increase evolutionary literacy. However, misconceptions about how to read and interpret phylogenetic trees are common (Meir et al., 2007; Gregory, 2008; Staton, 2015; Kummer et al., 2016). These misconceptions are usually addressed by means of activities that involve building phylogenetic trees with different kinds of data (Campo et al., 2009; Rau, 2012; David, 2018; Punyasettro & Yasri, 2021), and plotting phylogenetic trees by means of drawings (Bilardello & Valdés, 1998; Kozlowski, 2010; Dees & Momsen, 2016) or computers (Perry et al., 2008; Schneider et al., 2012; Zhang, 2012; Duffus, 2019). Here, a way of building phylogenetic trees by hand is suggested, using actual physical branches, a relatively little explored approach (Halverson, 2010). Physical phylogenetic trees facilitate the acquisition of topological skills—node rotation, branch regrafting—key to tree-thinking, that can be challenging when performed mentally or using drawings.

> *Physical phylogenetic trees facilitate the acquisition of topological skills—node rotation, branch regrafting—key to tree-thinking, that can be challenging when performed mentally or using drawings.*

Physical phylogenetic trees can be built in an easy, cheap way using drinking straws. All that is needed are straws of different colors, plasticine, and a folder made of hard plastic (Figure 1). Either plastic or carboard straws can be used, but the latter are more environmentally friendly. Tree branches are made with straws trimmed to a length of either 5 or 10 cm (Figure 1). Plasticine is plugged at each end of the straws (Figure 1) to help with fitting the connectors. Several kinds of connectors can be cut from the plastic folder, using scissors or a cutter, with a width that fits the diameter of the straws (Figure 1 and Figure S1 in Supplementary Material provided with the online version of this article). Rectangular trees are assembled by joining the straws using T or L connectors, while diagonal trees are assembled using Y connectors (Figure 2). I connectors allow students to expand the length of a branch (Figure 2). This basic design can be modified at will. Possibilities are almost endless; only a few are listed here. First, pieces of paper with drawings of different organisms (e.g., caminalcules: Sokal, 1983; Gendron, 2000) can be glued at the tips of the tree using "lollypop" connectors (Figure 1). Second, straws of different colors can be used to flag different clades in the tree. Third, straws can be combined with pipe cleaners (Halverson, 2010) or coated with sandpaper of different grit, such that visually impaired students can touch the trees and figure out not only the shape but also the different clades that branch along the tree.

Building physical phylogenetic trees can be included in the teaching of tree-thinking in at least five ways. First, these trees can be easily assembled by the teacher or the students during lectures to illustrate different topologies of trees, such as symmetric and asymmetric branching. Second, building physical phylogenetic trees can be easily included in hands-on activities designed to allow students getting familiar with establishing phylogenetic relationships between organisms, such as caminalcules (Gendron, 2000). These hands-on activities can reinforce tree-thinking and tree-building skills (Schramm et al., 2019).

Third, phylogenetic trees built with drinking straws can help students in dispelling several usual misconceptions (Gregory, 2008) (Figure 3 and Further Misconceptions in Supplementary Material provided with the online version of this article). For example, several misconceptions about phylogenetic trees identified by

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Figure 1. Materials needed to build phylogenetic trees with drinking straws. From left to right, drinking straws of different colors, trimmed to a length of 10 cm, straws with plugs of plasticine at the ends, one of them with a fold, and L, T, I, Y, psi, and lollypop connectors. Connectors are cut with scissors from a folder of hard plastic, visible at the right of the figure.

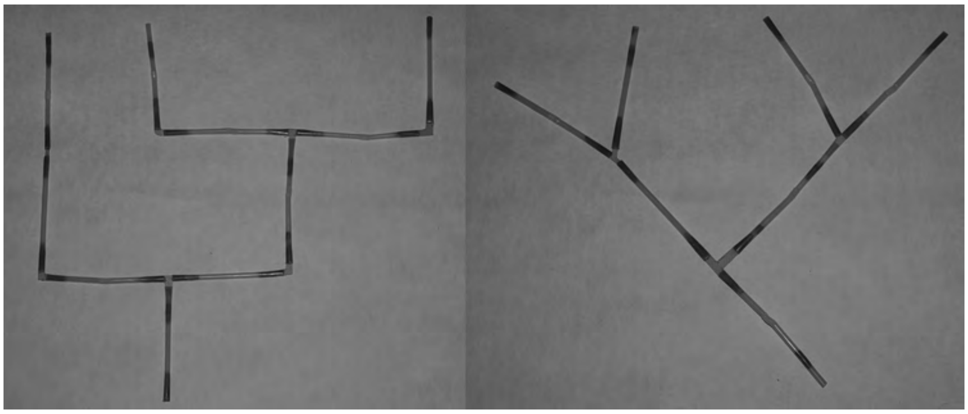

Figure 2. Left, a rectangular tree with three branches joined with T and L connectors; the leftmost branch is extended using an I connector. Right, a tree in diagonal version, using Y connectors.

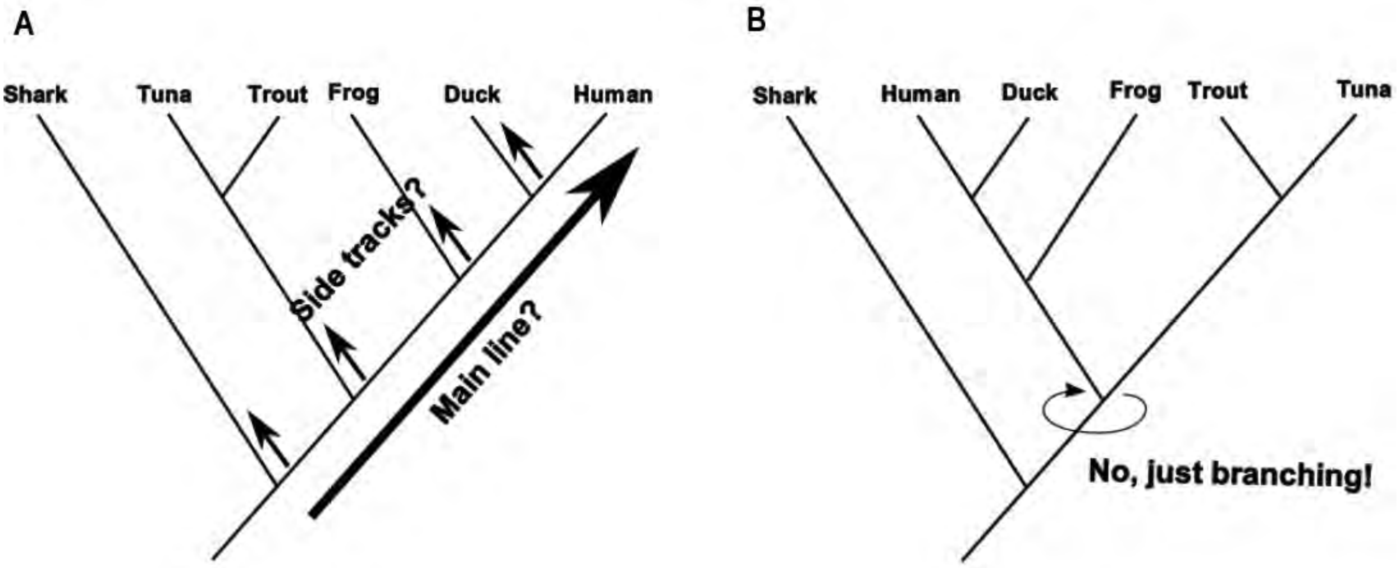

Figure 3. A: a tree showing the apparent main line of evolution toward a target, humans, while the rest of the tree is interpreted as side tracks away from that target. B: a simple rotation shows that this main line is only a product of the arrangement of the branches; actually, the tree is showing branching only from a common ancestor and no evolution toward a target.

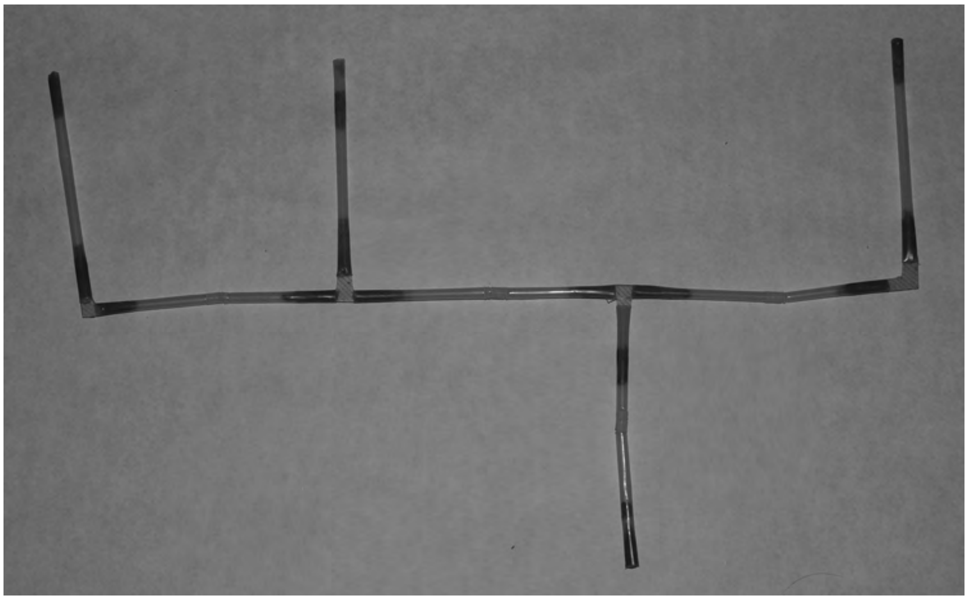

Figure 4. A tree with a polytomy, created with an inverted T connector.

Gregory (2008) can be dispelled by rotating branches around a node. Here, the misconception "main line and side tracks" is illustrated, as an on-class exercise. In asymmetrically branching trees, students tend to see one "main track," showing the "progress toward a target," while the rest of the branches are side tracks (Figure 3A). Rotation of nodes in a physical tree built with drinking straws graphically makes the point that such main track is only apparent, not real. The exercise has four steps. In step 1, show your students the phylogenetic tree on Figure 3A. In step 2, provide material to build trees with drinking straws and ask the students to build the tree, in which the misconception is apparent. In step 3, ask your students to rotate nodes of the tree so that they turn it into the tree in which the apparent track is not present. A potential solution is given in Figure 3B. In step 4, discuss how the apparent pattern in the left tree disappears without actually changing the tree, in other words, the phylogenetic relationships between species in the tree. This in-class exercise can be easily expanded to solve other misconceptions included in Gregory (2008) such as "reading across the tips" and "sibling vs. ancestor" (Further Misconceptions in Supplementary Material provided with the online version of this article).

Fourth, building physical trees helps realizing that trees are read from bottom to top, while drawing trees on a paper does not convey as easily the way in which a tree adds branches. Fifth, these trees support teaching how differences in topology involve branch regrafting, as well as how to collapse branches in order to create consensus trees with polytomies (Regrafting Branches in Supplementary Material provided with the online version of this article), both of which are more difficult with pipe cleaners. Trees with polytomies can be built simply using inverted T connectors (Figure 4), or by means of Ψ (psi) connectors (Figure 1). These suggestions can be easily developed by readers to fit their needs when teaching tree-thinking at primary, secondary, or college levels.

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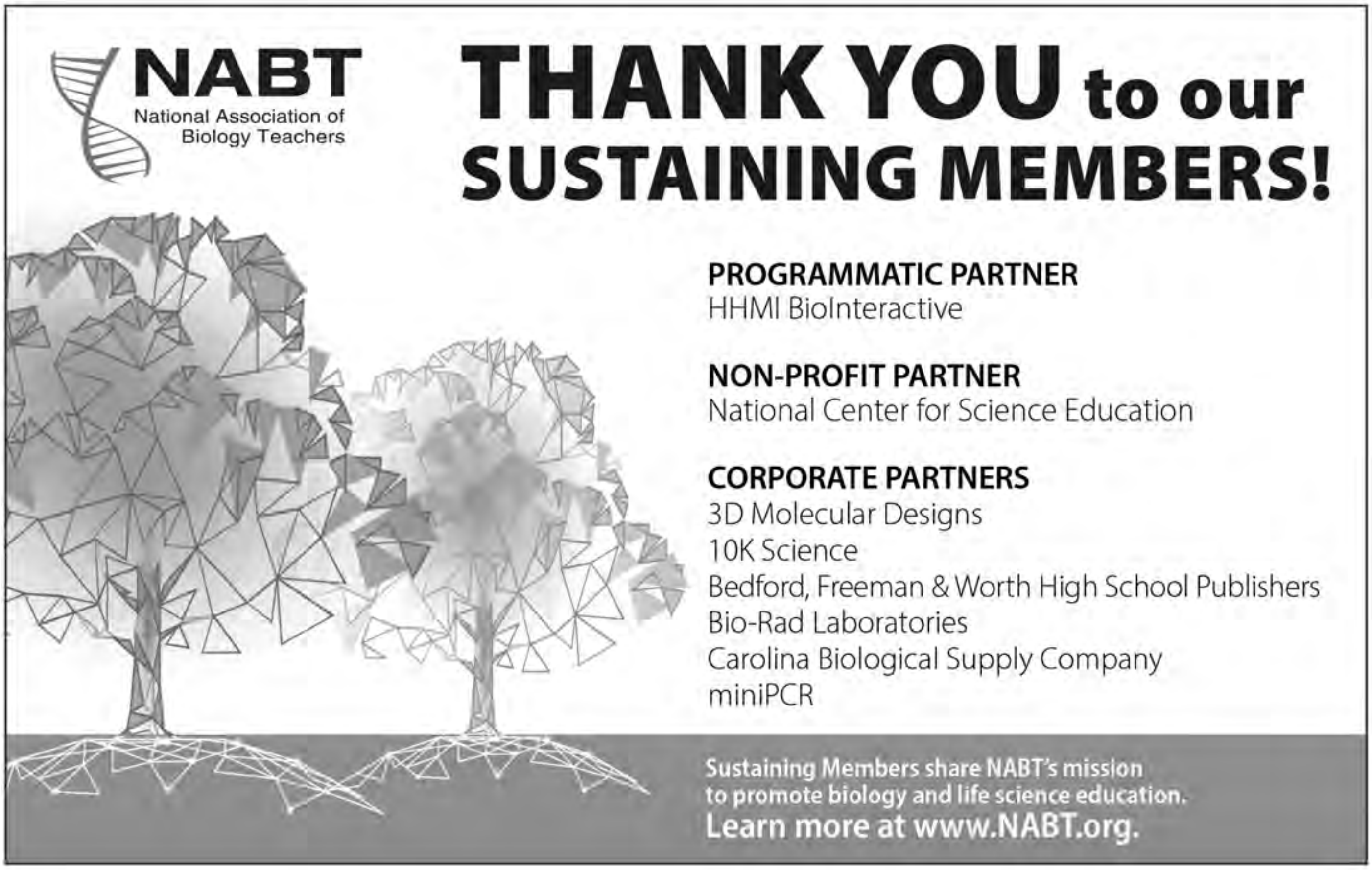
MARCOS MÉNDEZ (marcos.mendez@urjc.es) is a full professor at the Area of Biodiversity and Conservation in the Evolutionary Ecology Research Group at the Universidad Rey Juan Carlos in Madrid, Spain.



Undermining Scientific Progress: The Exclusion of Evolutionary Theory from Science Education in India

HESLLEY MACHADO SILVA

Abstract

The removal of the theory of biological evolution from school curricula in India represents a concern for the integrity of education and the country's progress. It compromises its ability to cope with climate change, undermines vital areas such as agriculture and health, and undermines its scientific and technological potential. Demonstrating the negative impacts is crucial to reversing this decision in India and setting a global example against religious influences on evidence-based education. Reconciling religious beliefs and accepting the theory of evolution is possible. Protecting the integrity of science education is essential to meet the challenges of the modern world. It is also important to consider that other countries, such as the USA and Brazil, have had their science and biological evolution education threatened by various forms of interference, therefore what is happening in India should serve as a warning and prepare reactions to this type of threat.

Key Words: *teaching of evolution; religion; curriculum; Darwin; biology.*

○ Introduction

It is challenging to measure how scientific development in various areas would have been without the knowledge derived from evolutionary theory. Some historical clues can be observed, such as what happened during Stalin's rule in the former Soviet Union (Stanchevici, 2017). During that period, the scientist and politically influential Figure Lysenko (Lecourt, 2017) persuaded the highest leader of the Politburo to deny the knowledge originating from the works of Mendel (Radick, 2023) and Darwin (Rindos, 2013) for Soviet agriculture. Instead, he advocated the belief that it was possible to shape the characteristics of plants through some form of environmentally directed human pressure. The result was the persecution, imprisonment, and death of prominent scientists, such as Vavilov (Vavilov, 1922), who based their plant science on well-founded evidence and theories, leading to unprecedented famine due to the loss of entire crops.

One might imagine that this kind of obscurity belonged to the past. Denying something scientifically proven, such as the Darwinian theory? That there would be no room for such denialism or pseudoscience in the early 21st century, with so many technological advances in various fields. These technological advancements, such as increased agricultural (Denison et al., 2003; Rindos, 2013) and livestock (Wright, 1978) productivity, vaccine (Gupta, 2024) and antibiotic production (Méthot, 2015), among other achievements, are intrinsically related to the understanding of evolutionary processes. One would expect that biological evolutionary theory would be preserved, especially in the formal school context.

However, the world in the third decade of the 21st century faces new challenges in communication in general, and especially in the dissemination of scientific knowledge (Genot et al., 2021). Instead of celebrating science for developing vaccines at record speed, allowing a return to normal life after long social isolations, the COVID-19 pandemic is now the target of falsehoods about its risks and conspiracy theories (Cinelli et al., 2020). These misconceptions resonate in vaccination rates in various countries, not only related to SARS-CoV-2 (Fridman et al., 2021). Today, incredibly, in countries such as Brazil and the USA, where the anti-vaccine movement thrives, science and biology teachers often have to explain to their students how a vaccine works, the value of a vaccine, to students who read unfounded information on their social networks, media (Silva, 2023) disseminated by all kinds of professionals, including doctors, in this process of misinformation (Islam et al., 2020).

Regarding evolutionary theory, it is no different; threats to its teaching occur in various parts of the world, once again, notably in the USA and Brazil (Wiles, 2011). This threat is evident and based on an unnecessary and counterproductive conflict with religion (Silva, 2022). This conflict occurs more specifically with dogmatic religious groups that aim to exclude the teaching of evolutionary theory or insist that it be taught on equal terms with the creationist view or its supposedly scientific variant (Berkman et al., 2008), intelligent design (Silva et al., 2010; Wexler, 2010). These two countries, with their various Christian denominations, more or less resistant to Darwinian theory, have managed to thwart these attempts, especially in public education (Alters et al., 2001; Branch et al., 2010). However, countries such as Turkey and South Korea, with different religious, economic, cultural, and political patterns,

The American Biology Teacher, Vol. 87, No. 2, pp. 132–138, ISSN 0002-7685, electronic ISSN 1938-4211. © 2025 by National Association of Biology Teachers. All rights reserved. Please direct all requests for permission to photocopy or reproduce article content through the University of California Press's Reprints and Permissions web page, https://online.ucpress.edu/journals/pages/reprintspermissions. DOI: https://doi.org/10.1525/abt.2025.87.2.132.

have experienced movements of partial or complete exclusion of evolutionary theory at various educational levels (Silva, 2017).

To illustrate the risks of this kind of trend, which is not limited to these countries, not confined to a particular religion, and not restricted to an ideology, attention should be paid to what recently happened in Indian education, the world's largest democracy, with all its peculiarities (Hardgrave Jr, 1993). One must be attentive to what occurred in this country, considering all possible repercussions, so that other peoples, nations, and educational systems can guard against the consequences of this type of unwarranted interference.

India, which in the third decade of the 21st century is among the leading players in the global economy (Jain et al., 2013) and the cutting edge of technology (Jadhav et al., 2023; Upendra et al., 2020), also holds the title of the world's most populous nation (Hertog et al., 2023). With remarkable advances in the economy and digital communication (Jordan et al., 2023), India has achieved remarkable feats, such as landing a probe in a lunar region unexplored by other nations or space agencies (Goswami, 2020). However, this outstanding scenario coexists with worrying setbacks in education (Dash, 2000).

The country recently announced curriculum changes that exclude important scientific topics from school textbooks, such as the periodic table and Darwin's theory of evolution, a measure that will affect around 134 million young students aged 11 to 18, sparking outrage among scientists and teachers (Lewis, 2023). More than 4,500 scientists, teachers, and science communicators came together in an appeal for the restoration of content on evolution in Kolkata, India (Breakthrough Science Society; Samanta, 2023; Lewis, 2023). In the meantime, there is a need to understand the reasons behind this decision and its implications in the context of the country. It is worrying that this kind of setback, which is already occurring in other countries with the influence of movements such as creationism (and intelligent design) opposed to the teaching of evolutionary theory (Silva, 2017), reaches a country of great global and regional importance.

These recent educational changes in India, particularly the removal of subjects such as evolution and the periodic table, reflect a complex interplay of factors influenced by religious, cultural, and political dynamics. Driven by the Rashtriya Swayamsevak Sangh (RSS) (Sharda, 2018), an organization with ties to the ruling Bharatiya Janata Party, the alterations in the curriculum align with a broader societal shift away from perceived external threats to Hinduism. Some religious groups in India have taken anti-evolution stances, viewing it as conflicting with creation stories (Brown, 2021). This move signals a departure from rational thinking and Western ideas, with historical narratives and, notably, scientific content becoming targets. The lack of transparency in the decision-making process and the absence of engagement with educators and parents raise concerns about the potential long-term impacts on scientific literacy and intellectual curiosity among students (and teachers) in India.

Although India has vast potential for the future, the issue of science education is crucial, and it is essential to reflect on how this measure contradicts India's achievements and aspirations to be a leader in global scientific development. In this context, it is relevant to investigate whether the action against science education is predominantly related to religious issues (Khalsa et al., 2022) or whether it also reflects negligence in relation to the country's educational development (Jayapalan, 2005). The influence of religion and politics has been previously cited concerning erratic actions in the teaching of evolution. However, India's standing in the PISA international performance test implies a further dimension of negligence in this domain (Lall et al., 2005). Despite endeavors to enhance scientific development, India appears to struggle in effectively channeling its resources toward this goal. The consequence is evident in its ranking at the bottom of this examination (Kumar et al., 2021), a situation potentially exacerbated by the removal of fundamental subjects from its school curriculum.

The difficulty of realizing the importance of evolutionary theory for science and national development can be challenging, but it is important to list the potential negative impacts in the short, medium, and long term that India will face in the face of this measure.

○ Short Term

The removal of the subject of biological evolution from the school curriculum in India, promoted by the Indian government, will have significant and immediate impacts on the educational system and the development of the country's students. The decision to exclude evolutionary theory, even in the short term, has several worrying implications:

1. *Serious Gap in Science Education:* The removal of biological evolution from the school curriculum will leave a significant gap in the science education of Indian students. Biology, as a discipline, is intrinsically connected to the theory of evolution, which serves as a central axis for understanding all topics related to life and biological diversity (Sadava et al., 2009).
2. *Lack of Integration of Biological Topics:* The theory of evolution acts as an integrating link in biological studies, connecting diverse concepts such as genetics, ecology, anatomy, and physiology (Helfman et al., 2009). Removing this central pillar will undermine students' ability to understand biology holistically, and how it is fundamental to humanity's progress (Meagher, 1999). Theodosius Dobzhansky's phrase, "*Nothing in Biology makes sense except in the light of Evolution*" (Dobzhansky, 1973), sums up the critical importance of this theory. Removing Darwin from the classroom denies students the opportunity to understand the principles that underpin all modern biology.
3. *Loss of the History of Science and Scientific Method:* Darwinian theory is not only a scientific concept, but also a striking example of the scientific method in action (Ayala, 2009). The detailed observation, data collection, rigorous analysis, and reasoned conclusions that led to the formulation of Darwin's theory are essential to understanding the scientific process (Feibleman et al., 1959), as well as being considered a historical and even philosophical milestone (Dewey, 2007) in science. To deprive students of this perspective is to damage their appreciation of the construction of scientific knowledge and the unfolding of a theory in related areas.
4. *Harm to Inspiration and Religious Tolerance:* Charles Darwin's life serves as an inspiration to many students and future researchers around the world (Desmond et al., 1992). His biography highlights that religion and science are not incompatible, showing that it is possible to maintain religious beliefs while adhering to fundamental scientific

principles (Ayala, 2007). In the scientist's own words: "*It seems absurd to me to doubt that a man can be a fervent theist and an evolutionist.*"[1] In India, where religious beliefs play a prominent role in society, this lesson is of great relevance.

The abrupt removal of biological evolution from the Indian school curriculum in the short term creates a deficiency in science education that undermines understanding of biology and undermines students' ability to develop a comprehensive view of science. Furthermore, this action represents a setback in recognizing Darwin's contribution to science and the need to teach sound scientific methods. It is therefore important to consider the negative impacts that this measure will have on the next generation of Indian scientists and citizens and to re-evaluate its implementation.

○ Medium Term

The removal of the subject of biological evolution from the school curriculum in India, in the medium term, will have profound and challenging consequences for the scientific fields and for the training of new professionals in the country. Several effects will become evident as the students who have been deprived of this knowledge progress through their academic and professional journeys:

1. *Challenges for science undergraduates:* In science degrees, especially in the biological and health fields, students will arrive without a solid knowledge of the theory of evolution (Dagher et al., 1997). This will raise serious questions about how these courses will work on fundamental subjects that have been deemed dispensable by the Indian education authorities.
2. *Lack of connection between biology and health:* Biology and health are intrinsically linked, and the theory of evolution provides an essential basis for understanding how diseases evolve and how organisms respond (and undergo selection) to them (Moalem, 2007). The absence of this knowledge can hinder the connection between these fields, which is detrimental to the training of doctors and other health professionals.
3. *Difficulties in Understanding Bacterial Resistance:* A practical example of the value of knowledge about biological evolution is understanding bacterial resistance to antibiotics (Mancuso et al., 2021). Without a basis in natural selection and evolution, students may struggle to understand how bacteria develop resistance to antibiotics over time (Dykhuizen, 1990).
   These insights are essential to guide future Indian scientists (and those from any part of the world) in the development of new antibiotics, taking into account potential bacterial resistance. Likewise, upcoming medical professionals will comprehend the importance of judiciously prescribing these medications, avoiding indiscriminate use, as witnessed in various countries during the COVID-19 pandemic, notably in Brazil (Silva, 2021), which posed risks of new strains of antibiotic-resistant bacteria. Furthermore, aspiring scientists must address the issue of resistance when considering the production of novel vaccines to confront this evolutionary phenomenon of microorganism resistance. This is essential for medical practice and public health (Aslam et al., 2018).
4. *Impact on the training of scientists:* The lack of teaching on the theory of evolution and fundamental concepts such as the periodic table will compromise the training of a new generation of scientists (Sheldrake, 2005). The development of solid scientific skills and a comprehensive understanding of the natural world are essential for scientific and technological advancement, and this depends on India having scientists with a solid knowledge base.
5. *Damage to Research Capacity:* Scientific research depends on understanding the fundamental principles that govern the natural world (Lenski, 2015). The removal of evolutionary theory from the Indian education system poses a profound threat to the future scientific endeavors of the country. First, a solid understanding of evolution serves as the cornerstone for various scientific disciplines, providing a framework for comprehending the interconnectedness of life forms, biological processes, and ecological systems (Pásztor et al., 2016; Stauffer, 1957). Without this foundational knowledge, aspiring Indian scientists may find themselves inadequately equipped to explore the intricacies of genetics, ecology, and other pivotal domains that rely on evolutionary principles. Second, innovation in scientific research often thrives on the ability to draw connections across diverse fields of study. The exclusion of evolutionary theory disrupts this interdisciplinary harmony, hindering the capacity of future scientists to synthesize insights from biology, medicine, and environmental science (Kartman, 1967; Watts et al., 2019). In an era where groundbreaking discoveries emerge at the crossroads of different scientific realms, the absence of evolutionary knowledge could impede the holistic perspective necessary for pioneering research. Therefore, the limitation of knowledge about evolution not only jeopardizes the individual scientific pursuits of aspiring researchers but also undermines the collective potential of the scientific community in India to contribute meaningfully to the global advancement of knowledge.

The removal of the theory of evolution from the Indian school curriculum in the medium term will have substantial negative impacts on the training of professionals in the scientific and health fields. It will be crucial for educational institutions and the Indian government to consider the damage this measure could cause and seek ways to reintroduce and strengthen the teaching of biological evolution to ensure a more solid future for science and education in the country.

○ Long Term

The government of India's long-term withdrawal from the subject of biological evolution has profound and lasting implications for the country, affecting crucial areas such as agriculture, livestock, health, and its claim to be a global reference in these areas.

1. *Impact on Agriculture and Livestock:* Agriculture and livestock are the backbone of the Indian economy (Cagliarini et al., 2011; Pandey, 1995), and understanding evolution plays a key role in these areas. Natural selection and genetic adaptation are essential concepts for improving productivity, pest resistance, and the development of crop varieties resistant to adverse conditions (Mehrotra, 1989).

The absence of this knowledge could jeopardize India's ability to feed its growing and enormous population.

2. *Historical damage to agriculture:* History shows us examples of how the denial of evolutionary theory and ideological interference in science can seriously damage a country (Joravsky, 2010). The case of the Lysenko doctrine in the Soviet Union resulted in significant delays in agricultural research and widespread famine in the 20th century, which cost the lives of millions through the denial of genetics and Darwinian evolution (Lecourt, 2017). India must learn from this kind of historical mistake and recognize that sound, evidence-based science is fundamental to progress.
3. *Failure in Public Health Development:* Understanding evolution is also vital for public health. Without it, future health professionals may struggle to understand the rapid evolution of pathogens (Elena et al., 2003), such as viruses and bacteria, and drug resistance. The handling of the COVID-19 pandemic, the mutations of SARS-CoV-2, the difficulty of treatment and the need for vaccination, have shown how important it is to understand the evolution of a virus (Silva, 2021) and be prepared to deal with this type of event. This could compromise the country's ability to deal with disease outbreaks and develop effective public health strategies.
4. *Compromising the Scientific Vanguard:* The lack of a solid foundation in science and evolution could compromise India's ability to lead technological and biotechnological advances in the future. Biotechnology, in particular, is an area that relies heavily on an understanding of evolutionary principles for the development of new therapies, drugs, and technologies (V. Gupta et al., 2017).
5. *Damage to tackling climate change:* India, as the world's most populous nation, faces serious challenges related to climate change, which may be compounded in their coping by the removal of critical scientific topics from its education, such as the subject of evolution. The nation is vulnerable to extreme weather events such as intense monsoon rains and prolonged droughts, often exacerbated by global warming (Aadhar et al., 2018; Guhathakurta et al., 2011). Understanding climate and environmental sciences is fundamental to facing these threats, and understanding how evolution occurs in the face of these conditions is fundamental to reducing the effects of these changes, including the extinction of species and the abundance of pests.

The long-term removal of the theory of biological evolution from the Indian school curriculum jeopardizes not only science education, but also economic progress, food security, public health, and India's position as a leader in technological and biotechnological advances.

○ Possible Reasons

The contemporary rejection of the evolutionary theory in India is a complex phenomenon rooted in the nation's rich tapestry of religious diversity, where Hinduism, Islam, Sikhism, and various other faiths coexist (Lopez Jr, 1995). The multifaceted nature of this rejection may find its origins in the intersection of religious beliefs with the scientific narrative proposed by evolutionary theory. India, with its pluralistic society, is home to a spectrum of religious interpretations, ranging from conservative to more liberal perspectives (Singh, 2004). The rejection of evolution might be linked to a conservative strain within certain religious communities that perceive evolutionary principles as conflicting with their traditional religious tenets.

The diverse religious landscape in India introduces a challenge of navigating through contrasting theological viewpoints (Patrick, 2020), where interpretations of creation and human origins may vary significantly. The rejection of evolutionary theory might, in part, be a response to the perceived threat it poses to established religious narratives, particularly those rooted in literal interpretations of religious texts (Bowler, 2003). This rejection may be exacerbated by the presence of religious fundamentalism within certain segments of the population, where adherence to strict and dogmatic interpretations prevails (Nieminen et al., 2014). Consequently, the rejection of evolution may not be a singularly religious stance but rather a manifestation of broader socioreligious dynamics that shape attitudes toward scientific paradigms. Understanding the nuanced interplay between religious diversity and scientific acceptance is crucial for devising effective strategies to reconcile these seemingly conflicting domains within the educational landscape of India.

In the intricate tapestry of Indian society, religion plays a significant and multifaceted role. Despite the rich diversity of religious practices, Hinduism stands out as the predominant faith, influencing the cultural, social, and political fabric of the nation (Bloch et al., 2009). The heterogeneity within Hinduism itself, with its myriad shades of belief systems, further adds to the complexity of the religious landscape (Sharma, 2011). Amid this intricate scenario, the recent removal of evolutionary theory from the Indian education system introduces a unique challenge. While the decision is ostensibly linked to appeasing various religious sentiments, it is noteworthy that many scientists within the Hindu community do not view the intersection of their religious identity with scientific principles as a point of contention. In fact, a considerable number of Hindu scientists exhibit a harmonious coexistence of their faith with evolutionary theory (Breakthrough Science Society; Samanta, 2023). This nuanced perspective is exemplified by the acceptance of the Dashavatara, the ten avatars of Lord Vishnu in Hinduism, as a conceptual framework aligning with the tenets of Darwinian evolution (Palai & Mishra, 2022). Despite the prevailing religious landscape, the attitudes of Hindu scientists underscore the potential compatibility between religious beliefs and acceptance of evolutionary science, emphasizing the need for a more inclusive and informed approach to education in India.

The current decision by the Indian government to withdraw the teaching of evolutionary theory from regular education reflects a complex interplay of political considerations, religious sentiments, and societal dynamics (Laborde, 2021). In an effort to cater to and align with various religious groups within the country, the government appears to be responding to demands that perceive the teachings associated with Darwinian theory as challenging or conflicting with traditional religious beliefs (Aiyar, 2020). By removing evolution from the curriculum, the government may be attempting to appease conservative religious factions, particularly those who view the theory as a threat to their doctrinal narratives. This strategic move, while ostensibly aimed at garnering support from religious communities, raises concerns about the potential compromise of scientific integrity in the educational system. The government's decision underscores the delicate balance that political leaders often navigate between secular education and accommodating diverse religious beliefs, highlighting the need for a nuanced approach that preserves the integrity of scientific teachings while respecting the religious fabric of the nation.

Government officials, particularly those engaged in public education, must recognize that science and religion are not antagonistic, even on the matter of evolutionary theory. The religions pertinent to India hold significance beyond its borders. It is crucial to identify researchers, religious figures, and thinkers who have successfully navigated the path toward harmony among Hindus (Raman, 2012), Muslims (Kojonen, 2023), Sikhs (Jhutti-Johal, 2011), Buddhists (Jackson, 2020), and others Asian religious prevalence (Brown, 2020). By doing so, not only can the current situation in India be averted, but also the emergence and endorsement of similar detrimental interferences can be preempted.

Again, based on this type of threat to the Indian education system, and the reasons for it, one has to wonder what might happen in countries such as Brazil and the USA, which are also experiencing this type of risk. As an example, Brazil recently experienced this in the Bolsonaro government, in which there were several high-ranking members in education (Silva, 2023) and science (Burity, 2021) who openly declared themselves aligned with creationism and intelligent design (Silva, 2023b), as well as refractory to evolutionary theory. This probably resulted in a framework that supported denialism in the pandemic period, in education and in scientific research in the country at that time. As in India, the Brazilian government sought to please and align itself with the growing number of more dogmatic religious denominations (Almeida, 2019), especially in relation to customs (Kibuuka, 2020).

Conclusions

The removal of the subject of biological evolution from school curricula by the government of India represents an act of concern not only for the scientific community, but also for the integrity of education and the future of the country. This act, which allows dogmatic religious issues to interfere in the education system, raises a number of critical questions and demands a considered analysis of the resulting effects. India, a nation that seeks to excel globally in economics, technology, and scientific research, risks undermining its own scientific knowledge base by removing a topic as fundamental as biological evolution from the curriculum. Science is a search for truth based on empirical evidence, and evolution is a well-established scientific theory that is accepted by the global scientific community. To deny its inclusion in education is to deny a fundamental understanding of biology and science as a whole.

India's approach to education, allowing religious dogma to influence the curriculum, undermines the principle of the secular state and threatens the integrity of science education. This creates an environment conducive to the proliferation of misinformation and the polarization of society on fundamental scientific issues. India will only be able to face the challenges posed by climate change if it places science education as one of the country's priorities. Only with all the knowledge relating to ecology and evolution, and biology as a whole, will it be possible to mitigate this situation imposed on humanity and its population in particular. Furthermore, it is imperative that in-depth research, analysis, and discussions take place to assess the true extent of the damage caused by this inadequate intervention in Indian education.

Clearly demonstrating the negative impacts of this measure could be an important step in promoting the reversal of this decision in India and possibly serve as an example for other countries considering similar curriculum changes. However, it should be recognized that reconciliation between religious beliefs and acceptance of the theory of evolution is possible. Finally, the removal of biological evolution from the school curriculum in India represents a global challenge to the integrity of science education in a world where religious and political influences can affect evidence-based teaching. It is crucial that society, educators, and political leaders are vigilant to ensure that science education is preserved and that future generations have access to reliable, evidence-based knowledge to face the complex challenges of the modern world.

This addressed issue is relevant to educators in the United States, as well as worldwide, as it highlights a global concern regarding the teaching of evolutionary theory and its relationship with religious influences in educational systems, along with the risks associated with its exclusion from curricula. Similar issues arise in various countries, including the United States and Brazil, where creationist and intelligent design movements challenge the teaching of evolutionary theory in school curricula. By addressing the situation in India, an additional perspective is sought to be provided on the challenges faced by nations in different parts of the world. The removal of the theory of evolution from education in India serves as a striking example of the negative consequences that may arise when religious influences interfere with evidence-based teaching. Understanding these challenges is crucial to promoting global awareness of the importance of preserving the integrity of science and biology education, as well as its implications in other fields of knowledge, irrespective of religious pressures. It aims to encourage a broader dialogue on the reconciliation between religious beliefs and acceptance of evolutionary theory.

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Note

1 https://www.darwinproject.ac.uk/letter/DCP-LETT-12041.xml

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HESLLEY MACHADO SILVA (heslley@uniformg.edu.br and heslley.silva@uemg.br) holds a post-doctorate in Education and Science from the University of Minho, Portugal, and a PhD in Education from the Federal University of Minas Gerais, Brazil. He is a professor and researcher at the State University of Minas Gerais (UEMG) and the University Center of Formiga (UNIFORMG).

TIPS, TRICKS & TECHNIQUES

Plants Are Not Independent Organisms: Introducing the Role of Mycorrhizal Fungi in Plant Roots with a Safe Method for Visualization

SOON-JAE LEE

Abstract

In plant science education, students can develop their perception of plants as independent living organisms. However, the accumulated studies testify that plants are not living alone in nature. The current paradigm in plant science explains a plant as a holobiont (plant and interacting microorganisms), instead of a single organism. In the center of this new paradigm, there is a symbiosis between plant and arbuscular mycorrhizal fungi (AMF). About 80% of terrestrial plant species form mutualism with AMF that act as an extension of roots. The symbiosis is crucial for plant nutrient uptake from the soil as well as resistance against biotic and abiotic stresses. Although the symbiosis is crucial for plant physiology and ecology, the efforts for summarizing current understanding and formulating it for teaching are relatively poor. For efficient introduction in class, it is important to visualize the AMF living inside roots. However, the widely used methodology for staining requires a carcinogenic chemical. Based on the current state of knowledge in AMF biology, I introduce AMF and a safer methodology for visualizing AMF in plants. Furthermore, I provide suggestions for teachers to design class activities that could inspire students to learn and think about plant physiology and ecology together with AMF.

Key Words: *arbuscular mycorrhizal fungi; plant holobiont; symbiosis; plant physiology; ecology; primary; secondary; undergraduate.*

○ Introduction

Plants are autotrophs. The sentence in biology textbooks describes one of the fundamental features of the organism. As an autotroph, a plant can produce its source of energy using photosynthesis. The description, together with the term “producer” in ecology, has led teachers to introduce a plant as an independent organism living and functioning alone in nature. However, plants are not independent organisms in nature. Besides the carbohydrates generated by photosynthesis, a plant requires various macro- and microelements to grow and maintain its metabolism. The acquisition of various nutrients for a plant is associated with water uptake from the soil and has been known to be realized by the interaction between root and abiotic components of soil. However, recent studies revealed that the mineralization of plant-absorbable forms of nutrients as well as the absorption from soil is mediated by various soil microbes rather than roots in nature (Berlanga-Clavero et al., 2020; Vandenkoornhuyse et al., 2015). Furthermore, resistance to environmental changes, crucial for plant homeostasis, is constituted by the various microbes interacting with the plant (Berlanga-Clavero et al., 2020; Vandenkoornhuyse et al., 2015). From the below-ground rhizosphere to the above-ground phyllosphere exposed to air, plants are multi-organismal systems functioning as one unit of life through intimate and continuous inter-kingdom interactions between the plant and the resident microbes (Berlanga-Clavero et al., 2020; Lee et al., 2019; Lyu et al., 2021; Smith & Read, 2008). The symbiotic meta-organisms that may have evolved together under natural selection and form functional units are termed holobionts (from the Greek *holos*, whole or entire) (Margulis & Fester, 1991; Mindell, 1992). Plants co-evolved interacting with microbes for nutrient uptake and homeostasis maintenance, represents holobionts that are not only physically associated but also have evolved to function together during most of their lifetime (Lee et al., 2019; Margulis & Fester, 1991; Mindell, 1992). Recent advancements in next-generation sequencing have allowed the study of plants in their natural form, rather than in sterile artificial conditions, which has been previously the case. Numerous studies of microbes interacting with plants have revealed intimate functional associations between the plant and microbes (Berlanga-Clavero et al., 2020; Bordenstein & Theis, 2015; Hoysted et al., 2018; Lee et al., 2019; Lyu et al., 2021). There has been a paradigm shift in plant sciences to consider plants as holobionts, not individual organisms (Berlanga-Clavero et al., 2020; Lee et al., 2019). The plant holobiont concept is becoming a new standard to study and understand plant physiology and ecology (Figure 1). For teachers in biology, it is therefore important to introduce the concept clearly to students. Introduction and demonstration of one good example of a mutualistic symbiosis, at the core of the plant holobiont, can be helpful for teachers to introduce the concept and inspire students.

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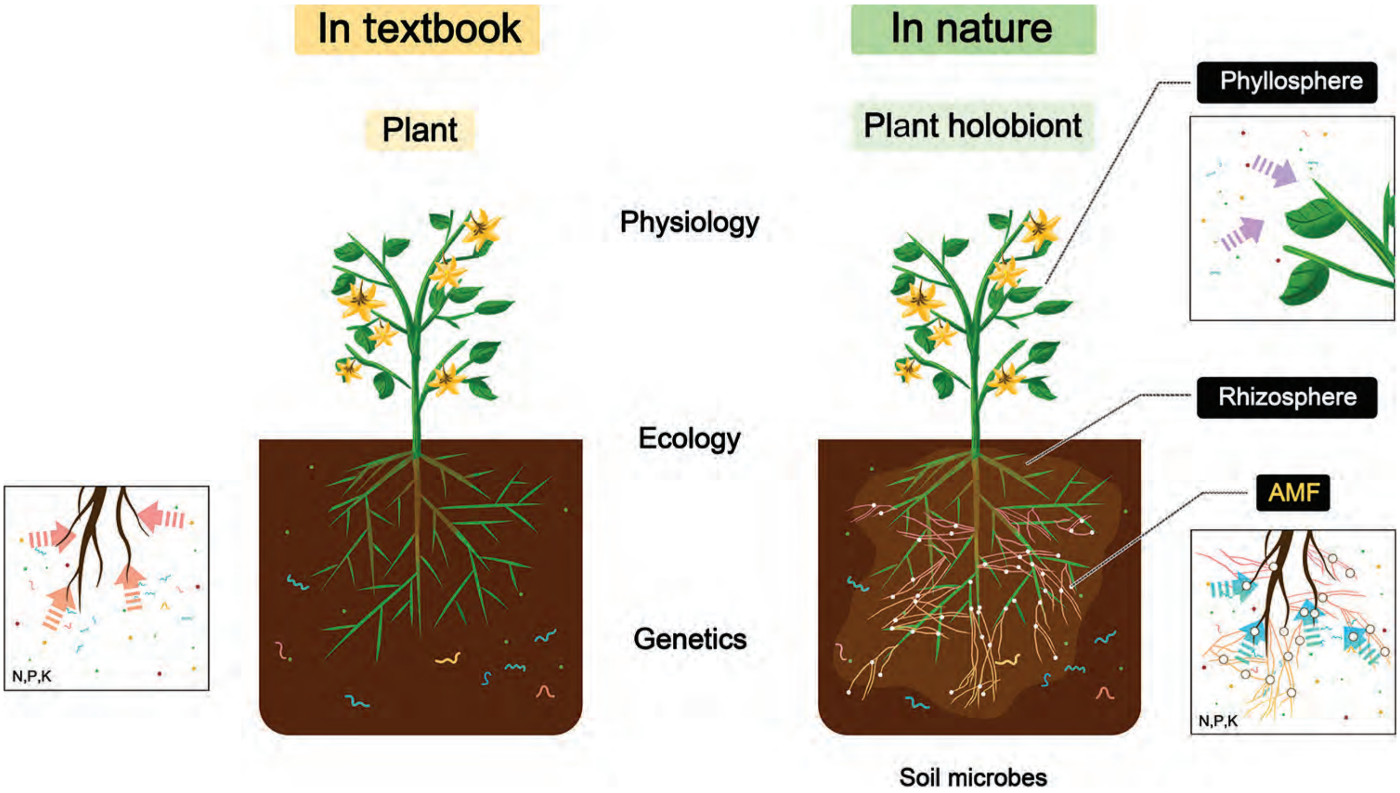

Figure 1. The plant holobiont concept in plant science. Traditionally, a unit for understanding physiology, ecology, and genetics of plants was plant itself. However, the recent paradigm of plant science considers plant holobiont as the unit. Co-evolved microorganisms of plant rhizosphere and phyllosphere form a complementary single system of life together with host plant. The plant holobiont concept can explain how plants can absorb nutrients in soil and maintain homeostasis in nature. AMF stands for arbuscular mycorrhizal fungi.

The symbiosis between plant and arbuscular mycorrhizal fungi (AMF) is one of the oldest and most successful mutualistic partnerships on Earth. The mutualism is understood to have evolved due to the limitation of plant root capacity for foraging soil nutrients, especially phosphate and nitrogen, which are crucial for plant growth (Smith & Read, 2008). Fossil records testify that the history of the symbiosis began with the first appearance of terrestrial plant ancestors about 450 million years ago (Ordovician) (Field et al., 2015). Since then, AMF have co-evolved with plants and helped plants to thrive and colonize the land. Unlike most of the other mutualisms found on Earth, there is no host-symbiont specificity in AMF-plant symbiosis. From liverworts to angiosperms, about 80% of terrestrial plant species form a symbiosis with AMF that occur in almost all terrestrial ecosystems (Field et al., 2015; Hoysted et al., 2018). All AMF share a common ancestor, forming the monophyletic fungal phylum Glomeromycota (Smith & Read, 2008). The plant-AMF symbiosis initiates with AMF colonization of plant root cortex cells. During root cell colonization, a unique morphological structure called the arbuscule (often described as "a tree-like structure" and giving the name of symbiosis) forms which functions as a hub for nutrition exchange between two symbiotic partners. After the colonization of root cells, AMF extend the mycelium toward the soil. The developed network of mycelium in soil absorbs various soil nutrients (especially phosphate and nitrogen) and transports them to the host. As a reward, AMF acquire their source of carbon in the form of sugar and lipids, and rely exclusively on plant photosynthesis for carbon. Although soil nutrition acquisition is the core function of AMF for the plant, there are other effects of AMF colonization of plants. The root colonization of AMF can improve plant resilience against abiotic (e.g., drought, flood, or salinity) as well as biotic (e.g., pathogen or herbivory attack) stresses (Begum et al., 2019; Li et al., 2019; Stratton et al., 2022). Consequently, symbiosis with AMF influences plant growth, yield (Felfoldi et al., 2022; Pena Venegas et al., 2021), as well as the interaction with other microorganisms in the environment (Real-Santillan et al., 2019), ultimately affecting the phosphate, nitrogen, and energy flux of food webs in the ecosystem (Nuccio et al., 2013; Smith & Read, 2008). The plant sequestration of recent photosynthates for plant holobiont maintenance is mainly mediated by AMF (Kaiser et al., 2015), while various soil nutrients processed by microorganisms are delivered to the plant via AMF (Nuccio et al., 2013). The symbiosis between plant and AMF forms the backbone of the plant holobiont (Lee et al., 2019). Also, it should be highlighted that AMF can form a symbiosis with more than one plant individual simultaneously. It has been shown that AMF can colonize the roots of the same plant species as well as different plant species (Allen & Allen, 1986). Therefore, it is feasible that AMF can mediate nutrition allocation between the interconnected individuals and affect the competition as well as collaboration between neighboring plants. Across the network of AMF hyphae, the absorbed nutrients from soil can be allocated from rich to poor patches (Whiteside et al., 2019). Indeed, it was demonstrated previously that below-ground AMF diversity can determine above-ground plant growth and diversity (van der Heijden et al., 1998; van der Heijden et al., 2003). The AMF-plant symbiosis is key to understanding plant physiology and to explaining the survival and reproduction of plants in the environment.

Considering the ubiquitous nature of AMF in ecosystems and their importance in plant physiology and ecology, it is crucial to introduce AMF to students studying biology. Unfortunately, an introduction to microbes without hands-on observation can be unintuitive for students as they can find it difficult to draw the organisms in their minds and consequently fail to understand how the symbiosis functions. Since AMF are fungi, it will be important for teachers to organize class activities to help students visualize plant colonization of AMF for the effective introduction of plant holobiont. However, a widely used methodology for staining AMF requires a carcinogenic chemical, Trypan blue (PubChem CID: 6296) (Gange et al., 1999). For the health of students, especially those who are not fully familiar with handling of hazardous chemicals, it is important to introduce alternative methods for achieving the same education goals. Here, I introduce a safer and simpler methodology for visualizing AMF colonization in plant roots. Furthermore, I suggest some considerations for teachers to help design class activities to introduce the plant-AMF symbiosis jointly with plant physiology and ecology.

○ A Safe Staining Method for Classroom Activity to Introduce the Plant-AMF Symbiosis

The principle of AMF staining is to distinguish the AMF structures from the plant root tissue. To achieve this, staining reagents target the fungal cell wall component, chitin. As an azo dye, the binding capacity of common ink for chitin is similar to Trypan blue (Shimizu et al., 1995), therefore ink can replace Trypan blue. Both chemicals are soluble in water and should be applied with an acidic bath. In this method, there is no need to prepare the stain solution with hydrochloric acid or lactic acid. Vinegar, which is safer and easier to apply, can be used for stain preparation. Overall the process is composed of the following three steps: (1) cleaning of tissue, (2) staining, and (3) destaining.

Sampling of plant materials for class activities

Sampling roots colonized by AMF in nature is simple, as approximately 80% of terrestrial plant species on Earth have mutualistic interaction with AMF (Smith & Read, 2008; Veiga et al., 2013). Some plant species from Brassicaceae, Caryophyllaceae, Cyperaceae, and Proteaceae families are non-mycorrhizal. They are not the primary hosts for colonization, although occasionally their roots can still be colonized by AMF if there are nearby AMF host plants. Therefore, plants from the four families are not recommended for sampling. Plants from Fabaceae and Poaceae, the two dominant plant families in both urban and rural ecosystems form symbiosis with AMF (Smith & Read, 2008). The major crops, such as rice, wheat, maize, potato, carrot, and onion can also be the good samples. The choice of plant species for root sampling will be dependent on the aim of the course or the scientific research question. It will be also interesting to introduce that the most of root crops people consume daily have AMF colonization. People are not only eating the plants, but also AMF in the root. The entire root system of the target plant should be carefully dug-up from the soil. Since there is more active AMF colonization occurring in younger and active roots, it is important to minimize the damage in the younger part of the root system. Sampled roots can be rinsed with tap water 3–4 times to remove the soil and neutralize the pH in the root surface. The washed material can be stored in a plastic bag or 50mL falcon tube with 50% ethanol in a regular fridge (4°C) for up to a year. When it is time to stain AMF, the stored samples are removed from the ethanol and rinsed several times with tap water to completely remove the ethanol. After washing, the younger part of the roots can be sampled by tweezers and a scalpel then stored in a container (2mL Eppendorf tube is recommended) for following the staining process.

Cleaning of sampled tissue

For the observation of AMF structures, it is important to make a good contrast between plant tissue and stained fungal structures. Plant roots are, in general, not white or transparent as various phytopigments in roots such as tannins exist. Also, to effectively stain AMF structures, it is important to soften the root tissue to allow penetration of the stain. For this, 10% Potassium hydroxide (KOH) (w/v) can be used. The sampled root pieces can be placed in 10% KOH solution for 20–180 minutes in a 70-90°C water bath. It is also possible to incubate the root pieces with KOH solution at room temperature over 24 hours. It is important to regularly check the condition of samples to avoid tissue damage. The fully cleaned root should be almost transparent and very soft. Carefully decant the 10% KOH solution and rinse the root with tap water several times to neutralize the pH. Depending on the plant species, the time of 10% KOH solution incubation can vary. Woody roots with more tannins should be incubated for longer with several replacements of the 10% KOH solution.

Staining

In order to stain the roots, previously described protocol (Vierheilig et al., 1998) can be further modified and used. A solution of 3-5% ink and vinegar (v/v) should be prepared. Depending on the preference and availability in the classroom, various types of ink and vinegar can be used. Considering the accessibility and handling convenience, black or blue colored pen inks can be used. The pH range variation of vinegar (pH 2–3) is an adequate pH level for staining. Therefore, the choice of vinegar is not critical for the staining. For the same reason, students can use fresh lemon juice (approximately pH 2) instead of vinegar. The cleaned root should be placed in the staining solution and incubated for 20–30 minutes in 90°C water bath. Alternatively, for few days courses, samples with staining solution can be stored in room-temperature overnight.

Destaining

Destaining is a step to adjust the contrast between stained AMF structures and non-stained plant tissue. Approximately 100mL of tap water with two droplets of vinegar can be used for rinsing the stained root. The stained roots can be placed in the destaining solution and incubated for 2 minutes at room temperature. It is not recommended to incubate in a 90°C water bath. Depending on the plant species, simple tap water without vinegar can be enough for destaining. After the destaining, roots can be stored in 50% ethanol for 2–3 months.

○ Observation of AMF Structures

The AMF structure inside the stained root can be observed with a light microscope (x200 to x400 magnification). Root pieces are placed on a slide glass and covered with a cover-slip. The cover-slip should be gently pressed with a finger to flatten the root tissue and ensure a flat surface for microscopic observation. A soft paper towel can be placed on top of the slides to avoid direct touching by the finger while pressing. For long-term storage of the slides, apply the transparent nail polish solution to seal the space between the cover-slip and slide glass.

In general, students can observe three different structures of AMF in a root as shown in Figure 2. First, students can observe fungal hyphae without septa (cell wall-like structure crossing the cytoplasm), which is a distinct characteristic of AMF compared with other fungi. However, with old mycelium, septa can be occasionally formed and observed. The second structure that can be observed is an arbuscule. This structure is formed by the mutual folding of cell membranes between the plant and AMF. It is not the result of invasive colonization with digestion of plant cells. The arbuscule is the structure for nutrient exchange between the two symbiotic partners. The last structure that can be observed is the vesicle, which is the structure used for carbon storage. Here, the AMF stores carbon in the form of lipids. These three structures are observable in x200 magnification with a light microscope. However, depending on the plant species and the age, clearer observation of the AMF structures can be made in higher magnification.

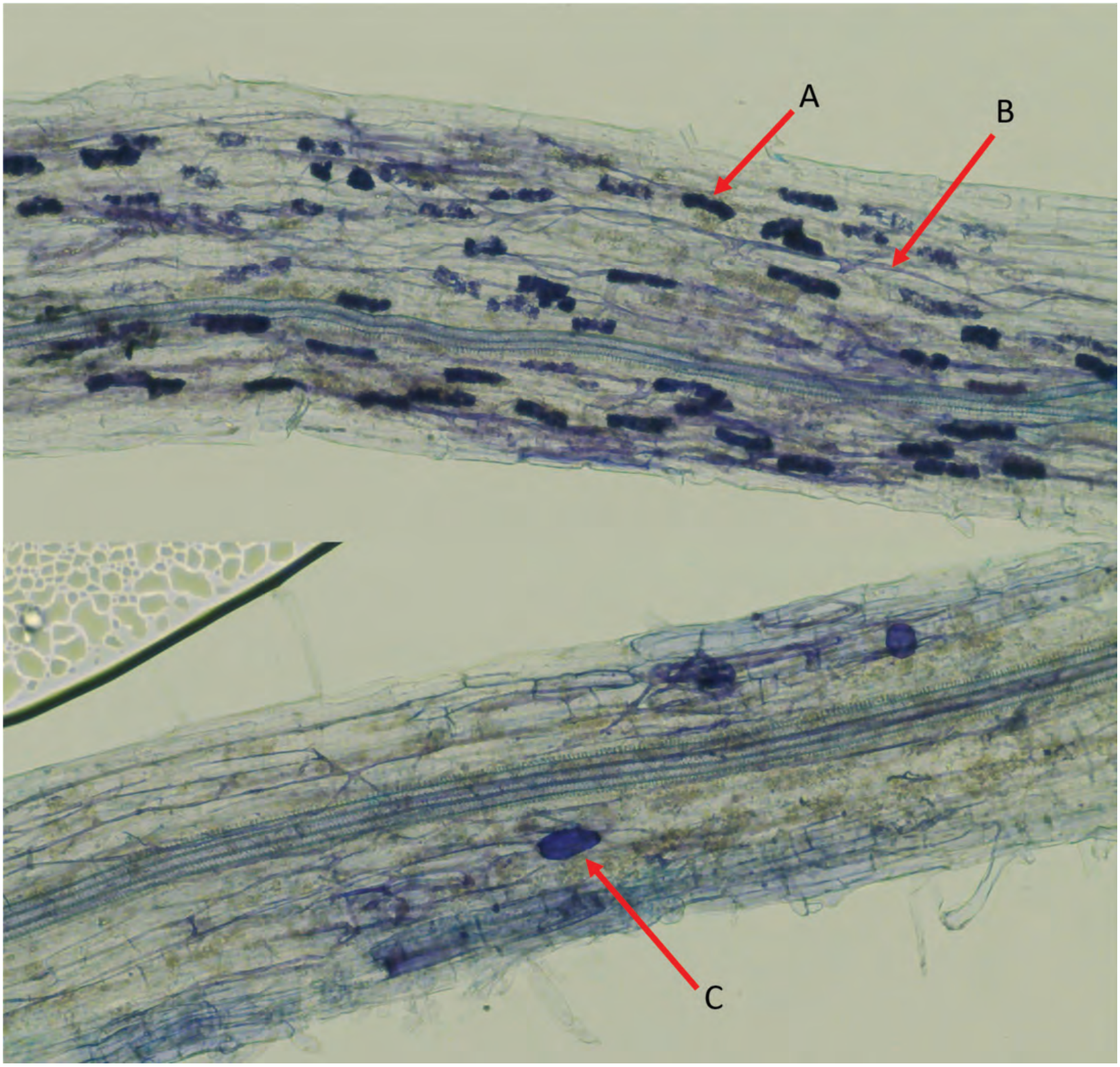

Figure 2. Stained root of *Pulsatilla alpina* with present method. Magnification x250. Fungal structure was stained in blue. The dense cluster inside of the plant cell (A) is arbuscule, the structure of nutrition exchange between plant and AMF. Intraradical mycelium without septa (B) can be observed. Carbon storage structure called vesicle (C) can be observed.

○ Consideration Points for Developing Class Activities

Plants live in symbiosis with a multitude of microorganisms in nature. In this article, I have introduced the AMF-plant symbiosis and the role of the symbiosis, a key part of the plant holobiont. Furthermore, I introduced a safe methodology for the visualization of AMF that can be used to design class activities. Teachers can show how prevalent the symbiosis between plant and AMF is in nature, while students can think and question what will be the natural form of plant roots in the soil. Here, I further suggest two teaching goals that can be designed by teachers for class activities: (1) to emphasize how ubiquitous AMF are in nature and (2) to let students realize the importance of AMF in plant physiology and the associated ecology.

For the first teaching goal, it is important to design the class activity to include field sampling. Teachers can lead students to sample as diverse plant individuals as possible, to highlight that the plant-AMF symbiosis can be observed in almost all cases. The class can be designed together with botany courses to allow students to think about different lineages of plants and their association with AMF. The AMF structures will have morphological variation among different plant species. In general, plant root cell size can vary both interspecifically and intraspecifically. Consequently, the cortex cell occupying AMF structure, such as arbuscules, can have different morphology depending on plant cell structural variation. Designing a class activity for describing morphological variation in AMF structure along different lineages of plants could be interesting. It will also be interesting to design the class to contrast the two common groups of plant, monocots and eudicots. Eudicots have taproots while monocots have fibrous root systems. Consequently, the two types of plants have different foraging strategies in the soil as well as associations with AMF. The activity will make a good transition from the first teaching goal to the second teaching goal, by linking plant physiology and ecology with AMF.

As an extension of plant roots, AMF mycelium forages the part of soil that plant roots cannot reach and absorbs various nutrients. Due to the differences in the root system, photosynthesis type, age, stage of development, and nutrition requirement, different plant species have different degrees of association with AMF (Weishampel & Bedford, 2006). Therefore, designing a class activity for tracking AMF root colonization along the different plant physiological traits will be useful for students to understand the potential role of plant-AMF symbiosis. For this, the quantitative measurement of AMF colonization will be required. The measurement can be accomplished with the introduced method of staining. Here, I am introducing the modified methods for AMF colonization measurement from a previous publication (McGonigle et al., 1990). To measure the degree of colonization, one can randomly cut and sample the 10–20 pieces of 1–3 cm sized root from the stained root system of a plant individual. One slide with aligned root pieces can be made per each plant individual to measure the AMF colonization. With a light microscope, one can make 4–5 observations along the length of each root piece. The number of observations and the total number of positive observations of AMF structures can be counted and noted for each plant individual. With the formula: (The number of positive observations for AMF structure)/(The total number of observations) x 100, AMF colonization percentage per plant can be calculated. The calculated percentage can be used for the comparison of different groups of plant morphological traits such as plant height, total leaf area, or shoot and root biomass.

It is also possible to further design the class activity for plant ecology. Together with the plant diversity study, the root colonization of AMF can be measured and compared. For example, the AMF colonization difference between dominant species of the site compared with non-dominant species can be measured. Different habitats as well as seasonal change can be further considered. It might also be interesting to design an activity to measure abiotic factors of soil together with AMF colonization of plant and plant diversity. It has been documented that soil physicochemical characteristics can affect the symbiosis between a plant and AMF and modulate the mycorrhizal benefit to the plant (Jansa et al., 2002; Pena Venegas et al., 2021). Detailed soil physicochemical characteristic investigations (for example, electric conductibility, or macro-/microelement analysis) require lots of time and resources to be realized in the classroom. However, it can be possible to check the pH of the soil by sampling a small amount of the soil from the site and by using general pH meter with soil wash. The measured pH can be further investigated together with AMF colonization and plant diversity.

○ Conclusion

To address the new paradigm in plant sciences and introduce students to the plant holobiont concept, education in plant science should be changed. The introduction of the symbiosis between AMF and plant, the backbone of the plant holobiont, can be an effective way to introduce the new concept to students. By introducing the current state of knowledge of the plant-AMF symbiosis, and combining it with a safe methodology for visualizing AMF in plants from nature, teachers can design classroom activities for introducing AMF biology jointly with other courses such as botany and ecology. Considering the importance of AMF for the physiology and ecology of plant holobiont, further efforts by teachers are required to design experiments to inspire and engage the students.

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SOON-JAE LEE (soon-jae.lee@unil.ch) is Premier Assistant in the Department of Ecology and Evolution, Biophore, University of Lausanne, Switzerland.

CLASSROOM MATERIALS & MEDIA REVIEW

JEFFREY D. SACK, DEPARTMENT EDITOR

Before Biology Video Series

The Amoeba Sisters
Found at https://www.youtube.com/playlist?list=PLwL0Myd7Dk1HxSDd1lczdBdH8orrrDhB1

Imagine that yesterday you taught a great lesson on genetics. The students were engaged and you know there was some learning that took place. When the students walk in the door today, you want to continue that learning high, so you need to find some way to pull them back in. Well, the Amoeba Sisters have such a tool that can help.

The Amoeba Sisters are real sisters (but not real amoebas) who have developed a series of educational videos and other products that focus on high school biology learning. One sister, who is a former high school biology teacher, develops the content, while the other sister does the animations. Their video series, called **Before the Bell Biology,** is a collection of short formative quizzes that review some of the major biological topics. There are currently 15 videos in the series. Some of these include: enzymes, biomolecules, cell membranes and transport, cell organelles and structures, photosynthesis, and plant structure and diversity.

The questions of each quiz appear on the screen with a 30-second timer, so students need to answer them before the screen changes to the next item. Most of the questions are multiple choice and true/false, and, even though they are not higher-order thinking items, they do review vocabulary and the main concepts of the topics. The answers and rationales for each question are given right after the timer expires, so students can check their work right away. There are bookmarks along the progress bars of the videos that allow the user to move easily from question to question without having to wait for them to advance automatically. This feature can help save time and lets teachers select only those items they want their students to answer. The music that plays during each quiz can also be turned off if students find it distracting. There is a downloadable answer page from the Amoeba Sisters' website that can be printed and used to record student responses.

The content of the quizzes focuses on the main ideas students should remember. For example, the genetics quiz asks students to remember terms such as *allele* and *gene*, as well as genotypes and runs them through some genetics problems. The cell organelles and structures quiz asks questions about functions of several organelles and also about the differences between prokaryotic and eukaryotic cells. As mentioned, the questions are mostly recall and do not require a lot of in-depth knowledge, but are great for reinforcing general concepts and terms students need to know.

Each of the videos in the series runs just under 10 minutes. In a 45-minute class period, this is a lot of time to use. Teachers should consider whether use of **Before the Bell Biology** might be better at the end of class instead of the beginning. Or, another idea might be to intersperse the questions through the lesson to check for understanding. Teachers could also assign the videos as homework, asking students to quiz themselves as review before a test.

However teachers choose to use **Before the Bell Biology**, they will have an engaging activity that will help enhance student learning. While the animations and illustrations are somewhat elementary in their appearance, the content is definitely geared toward high school students and does an good job of presenting the material on grade level. There are vocabulary terms and concepts that need to be explained to ensure they are being understood, but students should have no problem navigating their way through the videos.

Jeffrey D. Sack, Ed.D.
Science Education Consultant/Writer
Westbrook, CT 06498
sack.jeffrey@comcast.net

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Bloomington High School South, Bloomington, IN
Brentwood Academy, Brentwood, TN
Cabarrus Kannapolis Early College High School, Concord, NC
Canadian Valley Technical Center, OK
Caney Valley High School, Ramona, OK
Cardinal Gibbons High School, Raleigh, NC
Carrboro High School, Carrboro, NC
Castle Park High School, Chula Vista, CA
Cedarcrest High School, Duvall, WA
Central Carolina Technical College, Sumter, SC
Central Falls High School, Central Falls, RI
Central Magnet School, Murfreesboro, TN
Chelan High School, Chelan, WA
Chester High School, Chester, PA
Clayton High School, Clayton, MO
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
Dougherty Valley High School, San Ramon, CA
El Centro College, Dallas, TX
Emergence Academy, Springfield, MA
Emmett High School, Emmett, ID
Fairhaven High School, Fairhaven, MA
Florence Freshman Center, Florence, AL
Florida SouthWestern State College, Naples, FL
Freedom High School, Freedom, WI
Frontier Regional School, S Deerfield, MA
Georgia State University Perimeter College, Decatur, GA
Gillette College, Gillette, WY
Grandville High School, Grandville, MI
Greater Lowell Technical High School, Tyngsborough, MA
Greater New Bedford Regional Vocational Technical High School, New Bedford, MA
Greensburg Salem High School, Greensburg, PA
Hampton Roads Academy, Newport News, VA
Harmony School in Innovation, Katy, TX
Hillsboro High School, Hillsboro, OR
Hilltop High School, Chula Vista, CA
Holt High School, Holt, MI
Julia R Masterman School, Philadelphia, PA
Kenmore West High School, Buffalo, NY
Kent County High School, Worton, MD
Kettle Run High School, Nokesville, VA
Lake Metroparks, Concord, OH
Laurence Manning Academy, Manning, SC
Lexington High School, Mansfield, OH
Louisiana School for Math, Science and the Arts, Natchitoches, LA
Martin Luther College, New Ulm, MN
Mary Persons High School, Forsyth, GA
Marysville High School, Marysville, KS
McDowell Intermediate High School, Erie, PA
Metropolitan Community College, Omaha, NE
Midland Park High School, Midland Park, NJ
Mid Michigan College, Harrison, MI
Minnetonka High School, Minnetonka, MN
Morganton, NC West Mifflin Area High School, West Mifflin, PA
Moscow High School, Moscow, ID
Mount Abraham Union High School, Bristol, VT
Nassau Community College, Garden City, NY
Northampton Area High School, Northampton, PA
Northwest Mississippi Community College, Oxford, MS
Olivet Nazarene University, Bourbonnais, IL
Oologah High School, Oologah, OK
Palm Tree School, Fairfax, VA
Perkins High School, Sandusky, OH
Pike High School Freshman Center, Indianapolis, IN
Pikeview High School, Princeton, WV
Pinecrest High School, Southern Pines, NC
Putnam City High School, Oklahoma City, OK
Riverside City College, Riverside, CA
Riverside High School, Leesburg, VA
Saint Ignatius High School, Cleveland, OH
Seabury Hall, Makawao, HI
Seneca East High School, Attica, OH
Sherando High School, Winchester, VA
Sibley East Middle and High School, Arlington, MN
Skyline High School, Sammamish, WA
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
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State Library of PA, Lykens, PA
Stillwater High School, Stillwater, OK
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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
Ursuline Academy, Dedham, MA
Vincennes University, Vincennes, IN
Visitation Academy - Saint Louis, St. Louis, MO
Walters State Community College, Rutledge, TN
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

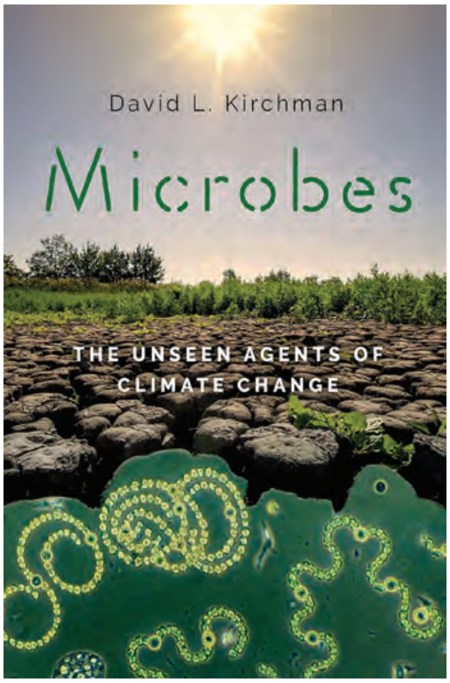
The mission of the NABT BioClub is to recruit, support, nurture, and promote students who have an interest in biological sciences for personal reasons, academic preparation, the betterment of society, and possible career opportunities by providing guidance, resources, and activities to meet these goals.

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.

BOOK REVIEWS

KIRSTIN MILKS & FRANK BROWN CLOUD, DEPARTMENT EDITORS

Microbes: The Unseen Agents of Climate Change. By David L. Kirchman. 2024. Oxford University Press Books. (ISBN 978-0197688564). Hardcover. 240 pp. $34.95.



Microbes: The Unseen Agents of Climate Change clearly steps through the role of microbes in cycling Earth's nutrients, the disruptions due to climate change, and potential solutions. This book is a must read for anyone living in our dramatically changing world. The importance of microbial processes in climate change models has only recently been acknowledged, perhaps because we've been needing a book like this one to bridge the gap between microbial ecosystems and planetary change in an approachable, concise way. Thank goodness it's here now!

David Kirchman's extensive knowledge of microbial ecology and physiology combined with his teaching prowess come together to create a powerful, concise reference text. An emeritus professor at University of Delaware, Kirchman's research focuses on microbial carbon cycling across the globe. *Microbes* eloquently summarizes his deep understanding of the history, present, and future of this field. In only 176 pages of this well-referenced book, Kirchman leads the reader through microbial physiology, biogeochemistry, and climate science in an engaging way for readers at a high school level and beyond. From van Leeuwenhoek's discovery of "tiny animalcules" under his microscopes to Father Secchi's measurements of water turbidity measurements with black and white "Secchi" discs, *Microbes* weaves the stories of scientific discovery throughout the ages into modern understandings. In intertwining these stories from different fields with the evolution of our understanding of the role of microbes over time, Kirchman teaches us not only about the science, but also the process of science.

Carbon dioxide, methane, nitrous oxide—which of these is the most damaging greenhouse gas? Where does it come from? How do cow farts, canned whip cream, and rock-eating microbes influence climate change? Kirchman steps through the different sources and sinks of carbon, sulfur, and nitrogen on land and in the ocean, then deftly connects Earth's planetary conditions throughout time with microbial processes to highlight future concerns, areas for research, and potential solutions. Kirchman presents the information in a clear, thoughtful way that makes these topics approachable. I especially appreciated how he shows the interconnectedness and complexity of Earth's different ecosystems. For example, peat bogs can release large amounts of methane and nitrous oxide, while storing some carbon. What are the costs and benefits? Should the peatlands be drained to halt these two more damaging greenhouse gases? If adding iron to ocean waters can stimulate phytoplankton growth, providing a carbon sink over time, should we dump iron in the ocean? Kirchman thoughtfully walks the reader through the pros and cons of several different scenarios for mitigating climate change using microbes.

As a biologist who is passionate about our microbial world, I greatly appreciate well-written books with microbes as the central focus. *Microbes: The Unseen Agents of Climate Change* is certainly a new favorite of mine, but my appreciation goes well beyond simply good writing about microbes. It is the kind of book we've been needing for scientists, the public, and policymakers. I can see this book being required reading for biology, sustainability, and environmental engineering classes. I will be using this as the text for my next environmental microbiology course and will use it to restructure key lectures in my general microbiology course. However, I think it's also the kind of book that high school students could pick up to help them see a future for our world and inspire them to learn more about microbes—the invisible organisms that shape our Earth.

Anne M. Estes, PhD,
Associate Professor
Towson University
Baltimore, MD
aestes@towson.edu

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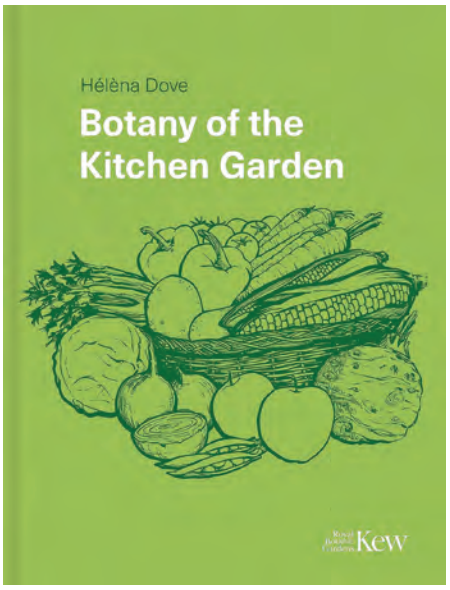
Botany of the Kitchen Garden: The Science and Horticulture of Our Favourite Crops. By Hélèna Dove. 2023. Royal Botanic Gardens, Kew. Distributed in North America by University of Chicago Press. (ISBN 978-1-84246-783-1). Hardcover. 158 pp. $30.00.



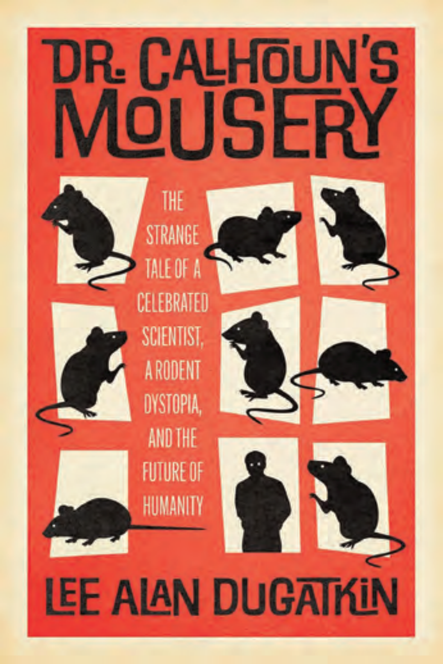
Ever wonder where tomatoes originated, or why blueberries need soil with a low pH? *Botany of the Kitchen Garden* includes a brief history of beloved plants including the climate and country of origin. By describing the climate of origin, one can understand why each plant thrives in certain growing conditions through the lens of evolutionary biology. This catalog of garden species is interspersed with additional gardening topics such as grafting and composting. While this book is not a manual about gardening, the information contained within would be particularly useful to teachers working in a school garden and planning teachable moments from the experience.

I am constantly looking to make content connections to students' lives as I teach biology, and this book will help teachers do just that. It can be used as a source about knowledge on plant adaptations and other fun facts about the fruits and vegetables students eat, as well as on the growing conditions needed for different fruits and vegetables. As an example, readers will learn why beans are legumes, information about nitrogen fixation, the botanical names of the different parts of a bean pod, and the function of the string in string beans. Botanical terminology is explained and examples are provided throughout. I'm excited that the next time my students plant bean seeds I will be able to create a lesson richer in content.

* * *

Kristin Hudlow
Centennial High School
Bakersfield, CA
Khudlow@kernhigh.org

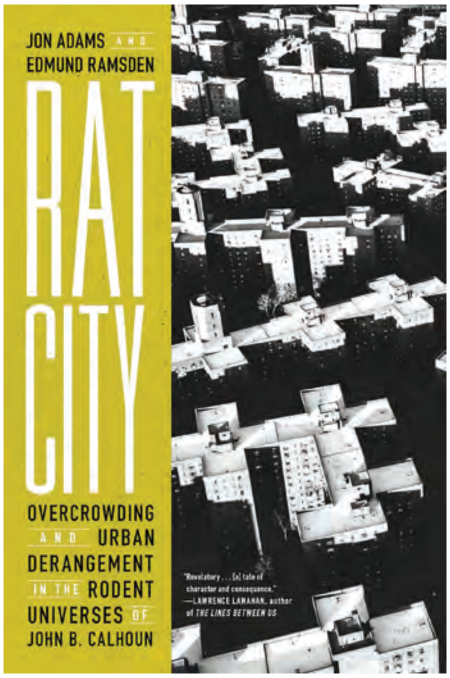
Dr. Calhoun's Mousery: The Strange Tale of a Celebrated Scientist, a Rodent Dystopia, and the Future of Humanity. By Lee Alan Dugatkin. 2024. The University of Chicago Press. (ISBN: 9780226827858). Cloth. 240 pp. $27.50. Ebook also available.



Rat City: Overcrowding and Urban Derangement in the Rodent Universes of John B. Calhoun. By Jon Adams and Edmund Ramsden. 2024. Melville House. (ISBN: 9781685890995). Hardcover. 384 pp. $32.50. Ebook also available.

About a decade ago, a friend was rehabilitating a wounded squirrel and asked for ideas: the squirrel had stopped eating. My personal expertise in squirrels extends only as far as an unbridled glee at seeing them pillage various neighbors' "squirrel-proof" birdfeeders, but I offered to help. The squirrel seemed healthy. The food seemed appropriate. We felt stumped.

On a whim, I removed all the food from the squirrel's serving bowl, tied some to a string, and dangled it awkwardly out of reach. That is, I dangled the food in a position that I naively assumed would be out of reach. Within fifteen minutes, the squirrel had found a way to climb a nearby curtain, sway it purposefully, then arch its back across empty space to nab a snack. The squirrel engaged in dramatic acrobatics to obtain the exact same food it hadn't touched in the food bowl.



I found myself thinking of this squirrel while reading *Dr. Calhoun's Mousery* and *Rat City*, a pair of recently published books that both discuss the life, experiments, and legacy of biologist John Calhoun. The most famous of Calhoun's experiments featured an indoor colony of mice. With no predation and unlimited access to food and water, Calhoun wanted to determine how crowded the seventy-square-foot enclosure would get. After several generations, the mice in this colony ceased breeding; five years later, the last denizens died of old age.

From this single finding (which was contradicted by prior results from other researchers studying overpopulation in mice, and which none of Calhoun's own future experiments ever reproduced), Calhoun extrapolated a wide range of conclusions about human behavior and the dire ramifications of humanity's increasing urbanization.

Calhoun's ideas were widely celebrated in the popular press—in the late 1960s and early 1970s, journalists were eager to promulgate sweeping theories that could explain social unrest—and authors such as Tom Wolfe and Hunter S. Thompson began to incorporate the term "behavioral sink" into their writing, the idea that an overly urban population would inevitably reach a state of terminal decrepitude.

But upon Calhoun's first formal presentation of his findings to a scientific audience, his work was panned. No effort had been made to control the rodents' olfactory environment over time (and we now know that mouse olfaction is so sensitive that the presence of a T-shirt previously worn by a male rather than a female lab technician is enough to trigger a dramatic spike in cortisol and a nearly two-fold dampening of pain response)—the mice were living in an indoor space with recycled air and impermeable floors that was never cleaned during the five-year experiment, with a visiting *Newsweek* journalist describing the "rank mouse smell" as "overpowering." There was virtually no genetic variation among the mice, heavily inbred to create a near-clonal true breeding line before the experiment began. And inside the habitat, there was no novel visual, auditory, or olfactory stimulation other than the occasional visits of human researchers, which caused the mice to swarm.

And yet, Calhoun still clung dogmatically to his initial conclusions, espousing the theory that individuals (whether inbred mice or urban-dwelling humans) who had been exposed to crowded conditions in youth were likely to become permanently deviant and adopt "unnatural" practices such as excessive self-beautification or homosexuality. (Interested readers might consult Eliot Schafer's *Queer Ducks*, which stresses that researchers of that era did not understand the frequency of homosexual behavior in nature because their internal biases caused them to assume that all mating events were between heterosexual pairs, even in species with such subtle sexual dimorphisms that it is difficult to assess biological sex in the field.) Then, near the end of his career, Calhoun became infatuated with supposedly utopian ideals such as cybernetic brain uploading and other technological replacements of our essential nature as embodied biological organisms.

Beneath the surface of either of these books, there are hints of a tragic cautionary tale that echoes Calhoun's own conclusions about rodents: an acute period of deprivation can have lifelong consequences. There was a time when Calhoun was thirty-six years old with two kids and a mortgage and impending unemployment. Up until that time, Calhoun had been an assiduous ecologist who studied birds and rodents simply to learn more about their behaviors in the wild. But that is not where the big research money lies. And so during that year, as Calhoun scrambled to find a new source of income, he began to talk about the importance of studying rodents to gain insight into our own mental health, and he never stopped. Even when the conclusions that he could draw about human behavior based on the rodent studies were tendentious at best.

And as his (tendentious) ideas were translated into the popular press, the consequences often grew even worse. Citing how many more rodents could be stored in a given space if they were stacked in isolated cages than if they were allowed to congregate, developers constructing housing for underserved human populations adopted isolation and segregation as their design goals rather than considering what type of interactions would best foster a sense of community, leading to travesties such as the Pruitt-Igoe housing complex.

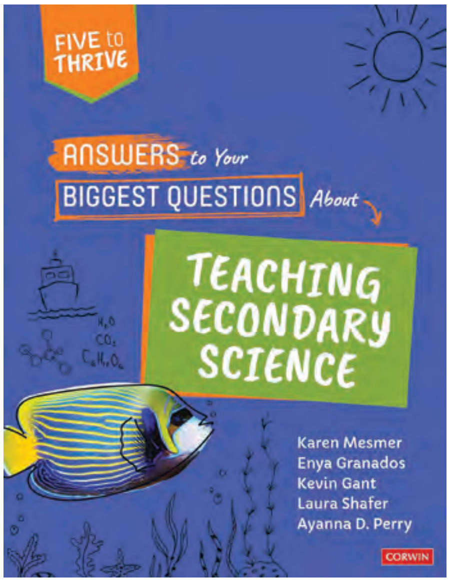
I assume that most readers, even if interested in Calhoun's life and works, will read only one of these new books. *Dr. Calhoun's Mousery* has more detail linking Calhoun's life to popular culture at the time, noting almost every moment when his ideas were alluded to in songs, television spots, magazine articles, and more. Written by a biologist, this book also amply describes Calhoun's struggles to find lab space and funding commensurate with his visions. *Rat City* has more detail about other researchers' studies that were being conducted contemporaneously with Calhoun's, and the authors strive to link Calhoun's ideas to current issues such as the shut-in phenomenon in modern Japan, the shift in funding from psychiatric care to the prison-industrial complex in America, and the dramatic increase in the use of prescription medications to address mental conditions. *Rat City* also uses a mix of past and present tense to describe past events—writing that Calhoun *is* building a mouse enclosure, to make the prose sound more immediate—that I dislike, especially since there are several sections where the verb tense careens from past to present within the space of a paragraph or even a single sentence.

To my mind, both books seem excessively laudatory of Calhoun's work, but I imagine that someone would have to believe that Calhoun was an insightful thinker in order to write such carefully researched biographies. I agree with the sentiment that we can better understand human minds through the study of other animals; we share neural architecture and our cognitive capabilities seem to exist on the same continuum. And yet, it seems odd to me that Calhoun chose to draft press releases, scientific reports, and research lectures with such strident conclusions. His private notes make clear that Calhoun was aware that the quirks of a particularly instantiated history would have a major influence on the result of experiments like his—another researcher had already shown that mice would exhibit less territoriality if a slightly larger initial seed population was used—but Calhoun still spent much of his life making grand claims extrapolated from an experimental result he'd observed … once.

* * *

Frank Brown Cloud
Ivy Tech Community College
Bloomington, IN
fcbrowncloud@protonmail.com

Answers to Your Biggest Questions About Teaching Secondary Science: Five to Thrive [series] (1st Edition). By Karen Mesmer, Enya Granados, Kevin Gant, Laura Shafer, & Ayanna D. Perry. 2024. Corwin Press, Inc. (ISBN 978-1-0719-1637-7). Paperback. 192 pp. $33.95. Ebook also available.



While sitting in my secondary science methods course last week, the topic of being prepared for full-time teaching was brought up. As all seven of us looked around, in our last semester before student teaching, an air of nervousness arose. We've been in courses for almost four years now that focused on preparing us for our future careers as educators; however, we still have questions that we can't even put into words. It is often said that you're never truly prepared to be a teacher, and as I was sitting in my class this issue became more pressing than ever. How can I get answers to questions about teaching that I might not even realize I have?

As if the universe was listening, I was offered the opportunity to review *Answers to Your Biggest Questions About Teaching Secondary Science: Five to Thrive*, by a team of educators including NABT member Enya Granados. This book is a guide focused on helping newer teachers answer big questions about teaching and managing a classroom ranging from "How do I build a positive science community?" to "Where do I go from here?" These main questions are further broken into smaller questions, similar to the process done to create a good lesson plan (p. 60). Throughout the book, resources are given in the margins to offer readers various forms of learning and ways to help answer the question of where to go from here? *Answers to Your Biggest Questions* is an essential guidebook that I think not only newer teachers should read, but also those experienced teachers who want to refine and reflect on their practice.

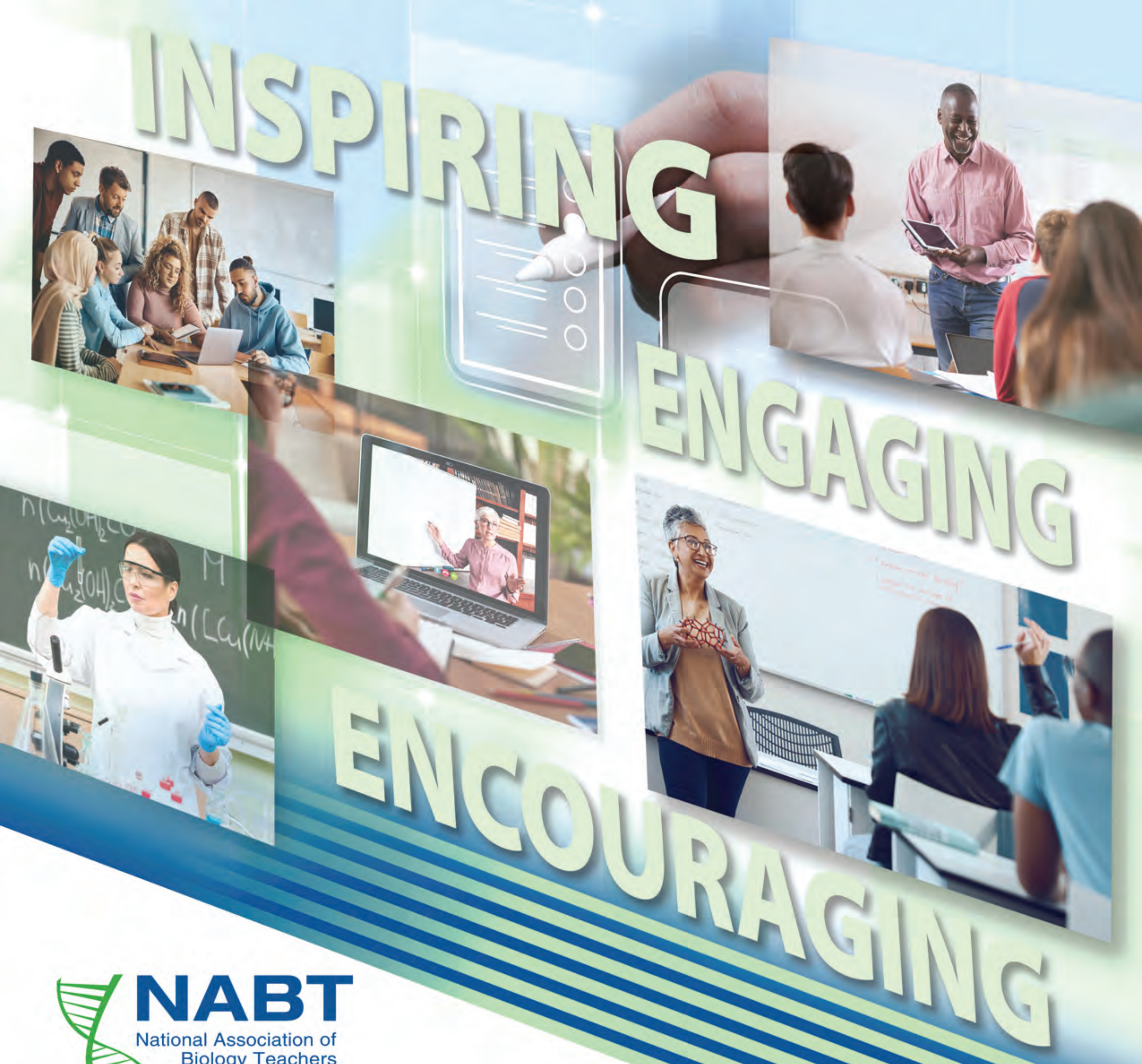
Throughout its six chapters the book examines the roles of identity, interaction, and management. The first chapter focuses on the idea of building a positive science community. This is done by consistently trying to learn about and understand a student's identity, integrating healthy conversations within the classroom, and creating relationships with caregivers. Noted in this part is how to shift a student's narrative regarding their science identity to a positive one. This chapter is for those who want to build a more equitable and supportive classroom and offers great resources on how to build cultural competency as well.

Chapter 2, the longest chapter, focuses on the broad but critical topic of classroom management. A very helpful tool within this chapter is a chart of helpful items to stock within a science classroom. Further, this section covers various areas of classroom management, from planning a unit to grouping students. I think this chapter is the most beneficial for teachers who are not yet confident in their classroom management abilities. Not only does this chapter help with various logistical aspects of a classroom, but it also offers a focus on how to incorporate the three NGSS aspects of disciplinary core ideas, science and engineering practices, and crosscutting concepts into lessons.

The next three chapters focus on engagement, discussion, and assessment. Chapter 3 utilizes the idea of project-based learning within the classroom and proper integration. Covered in this chapter are various ways to shift the role of students from learning about science to figuring out and doing science. Chapter 4 covers discussions and how to foster positive discourse within the classroom, as interaction is a main point in this book. I liked the subsections that discussed how to plan for various forms of discussion (partner, small-group, whole group), as I often struggle with not only choosing the most effective form but also how to integrate planning and timing. Chapter 5 covered the idea of assessment: planning assessment, the various forms, and using assessment as a measurement for student learning. Educators are constantly being asked about the topic of assessment and why specific assessments are used. This chapter helps build a foundational understanding of assessment and its various uses.

The last chapter focuses on growth, which has occurred for me just by reading this book. *Answers to Your Biggest Questions About Teaching Secondary Science* offers resources and ways to improve on being the best educators we can be, even if that is a role we haven't stepped into yet. This guide offers beneficial information for everyone in an easy-to-read, color-coded format, a teacher's dream! When the topic of being prepared to be an educator is brought up again, I don't think I'll feel as nervous, having read this book.

Elicia Moreno,
teacher candidate
Indiana University School of Education
Bloomington, IN
eliciamoreno25@gmail.com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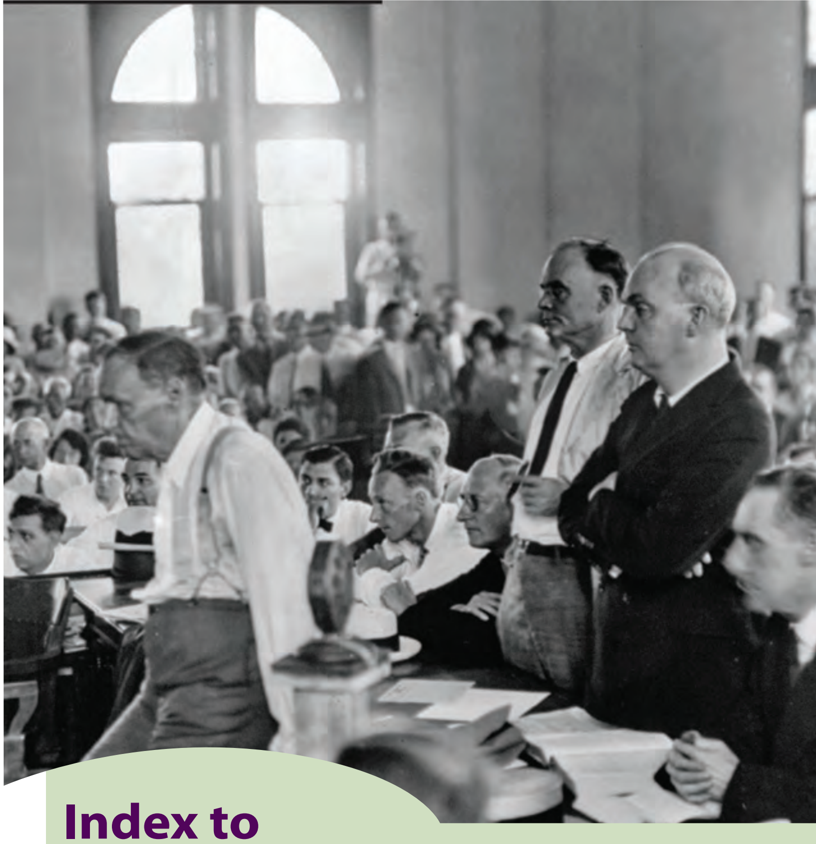

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