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About Our Cover

The Painted Snail (*Polymita picta*) is one of the most recognizable species of land snails. The inch-and-half long species is endemic to eastern Cuba. Like many species in the family Cepolidae, and in the genus *Polymita* in particular, Painted Snails display an array of stunning, contrasting colors. The range of shell color variation within Painted Snails involves the background color as well as the colors, location, and dimensions of the spiral bands that embellish the shells.

The Ryker mount in the photo, on display at the Bailey-Matthews National Shell Museum on Sanibel, Florida, parades a variety of Painted Snails from eastern Cuba, most likely from the Oriente Province. The snails were a late-1930s gift from Dr. Carlos de la Torre to Dr. William J. Clench, noted mid-Century malacologist from the Museum of Comparative Zoology at Harvard. De la Torre, himself a malacologist, was at one time President of the University of Havana and Mayor of the City of Havana.

The photo was taken by José H. Leal, Ph.D., Science Director and Curator of the Bailey-Matthews National Shell Museum in Sanibel, Florida.

Contents

Feature Articles

- Using the Discovery of the Structure of DNA to Illustrate Cultural Aspects of Science**
Drawing attention to Rosalind Franklin's role in the discovery of the structure of DNA
Peng Dai, David Rudge 256
Available online at <https://www.nabt.org/ABT-Online-Current-Issue>

Research on Learning

- Mendel or Molecules First: What is the Best Approach for Teaching General Genetics?**
A comparison of two sequential courses indicated that there were no statistically significant differences with the two approaches
Charles E. Deutch 264

Inquiry & Investigations

- Drowsy *Drosophila*: Rapid Evolution in the Face of Climate Change**
Giving students opportunities to make connections between climate change & evolution through various modes of inquiry & self-investigation
Jennifer Broo, Jessica Mahoney, Julie Bokor, Daniel Hahn 272



- Using Project-Based Learning to Teach Phylogenetic Reconstruction for Advanced Undergraduate Biology Students: Molluscan Evolution as a Case Study**
Phylogenetics plays a central role in understanding the evolution of life on Earth
Andrew A. David 278

- Using Environmental DNA to Connect Lab Science with Field Practice**
Helping students make connections between different skill sets and engaging them in a deeper level of inquiry
James McNeil, Anneke DeLuycker, Sarah Putman 285



- Following Phenotypes: An Exploration of Mendelian Genetics Using *Arabidopsis* Plants**
Arabidopsis thaliana is the ideal organism for teaching a variety of basic genetic concepts
Courtney G. Price, Emma M. Knee, Julie A. Miller, Diana Shin, James Mann, Deborah K. Crist, Erich Grotewold, Jelena Brkljacic 291

Tips, Tricks & Techniques

- Using Peer Review to Improve Lab Report Assignments**
Increasing student understanding of science as a process that includes peer review as well as the lab activity
Chelsie L. Romulo, Arzo Raoufi, Kim Largen, J. Reid Schwebach 301

- STEAM Connections: Painting with Bacteria**
Introducing students to a multidisciplinary approach to STEM by incorporating arts into the discussion of gene expression & microbiology
Regina Wu, Caren Brinkema, Michaela Peterson, Adam Waltzer, Jeanne Chowning 305

- Wright Stain and Fluorescence-Based Methods in *Tetrahymena* for Phenotypic Analysis**
Tetrahymena is an excellent model organism for investigating function, morphology, structure, phagocytosis & ciliary motion
María del Rosario Ramírez Mata, Robert M. Kao 312

Departments

- Guest Editorial** • Genomics—Past, Present, and Future: A Letter to My Daughter • Eric D. Green 253
Book Reviews • Amanda L. Glaze, Department Editor 318
Classroom Materials & Media Reviews • Remy Dou, Department Editor 323

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GUEST EDITORIAL

Eric D. Green

Genomics—Past, Present, and Future: A Letter to My Daughter

Eric D. Green, M.D., Ph.D. is the Director of the National Human Genome Research Institute (NHGRI) at the U.S. National Institutes of Health (NIH). This editorial is written as a letter to his daughter, Abbey, who will graduate from high school in June 2018.

To Abbey, My Remarkable Daughter:

In a few weeks, you will graduate from high school and begin the next phase of your life journey. Reflecting on this milestone, I am struck by how your life has paralleled spectacular advances in an area of biology that I have worked in for my entire career—genomics, the study of all the DNA of a living organism.

When you were born in 1999, I and thousands of other genomics researchers around the world were working intensely on the Human Genome Project, a 13-year odyssey to “decode” the human genome (that is, to determine the order of the roughly 3 billion “letters” in human DNA). While you were finger-painting and napping in pre-school in 2003, we completed that effort. As you moved through grade school and middle school, we worked diligently to make sense of our genome’s code by analyzing all those ordered Gs, As, Ts, and Cs—we identified the approximately 20,000 genes in the human genome; we began to identify the additional sequences that precisely turn those genes on and off in the right cells and at the right time; and we came to appreciate the way that chemical modifications of DNA influence how genomes operate (something known as epigenomics). We also made great progress in cataloging the 3–5 million “spelling differences” present in each of our genomes, and launched major studies to determine which of these differences play a role in human health and disease.

These efforts were greatly aided by one of the most stunning technological advancements seen in either of our lifetimes—no, not the “smart phone” or Instagram or self-driving cars—rather, methods to sequence or read the code within DNA. Sequencing that first human genome by the Human Genome Project cost nearly \$1 billion. However, in the time that it has taken for you to move from a pre-K student to a high school senior, scientists have developed totally new and inexpensive ways to sequence DNA. Now, a human genome can be sequenced for about \$1000, which is only slightly more expensive than the latest iPhone!

Once limited to research laboratories, DNA sequencing is now affordable for numerous applications—perhaps the most impactful of these will be in medicine. During your past few years of high school, we have witnessed some truly inspirational early successes using a patient’s genome sequence to tailor their medical care—an area known as genomic medicine. This has led to novel approaches for helping patients with rare genetic diseases and more common diseases like cancer, for performing prenatal genetic testing, and for selecting medications more precisely based on a patient’s unique genomic makeup. Just as graduating high school will mark a pivotal transition in your life, genomics is in the midst of transitioning from the research laboratory to the clinic.

But should you really care about all this genomics stuff? Is this all nothing more than your researcher dad being nerdy about his work again? Or will genomics have a meaningful role in your future and the futures of everyone else? Along with the genes that your mother and I have already given you, let me now give you some loving advice as an early graduation present—**genomics will be relevant to you.**

Obviously, genomics will be directly relevant if you become a scientist, doctor, nurse, pharmacist, or other healthcare professional, or if your career takes you into an area related to genomics (such as law, ethics, engineering, computer science, or education). More importantly, genomics will be relevant to you as patient—and as a relative or a friend of a patient. Your generation will witness genomic medicine becoming widespread and routine. That means your healthcare providers will speak the language of genomics, so you too will need to be literate in the basics of genomics to make informed healthcare decisions. Beyond medicine, DNA sequencing will be used for detecting infectious outbreaks, improving and monitoring our food, increasing the accuracy of forensic investigations (think CSI!), assessing the health of our environment, and advancing our understanding of evolution, among other applications. It will also provide a new lens through which you will be able to view aspects of human origins, your own family history, and even elements of our culture. However, this is a powerful lens—one that must be used carefully and with appropriate consideration of the important ethical and societal issues involved. In short, your generation will truly see genomics become part of everyday life, but with that will come some important challenges for health equity and social justice.

While I realize you learned a few basics about genomics in high school biology, the field is moving rapidly, and many things will change in the coming years; for that, you must commit to becoming a life-long genomics learner. The good news is that the necessary information will be but a mouse click (or two) away, with numerous online resources (for example, genome.gov and unlockinglifescode.org) already available and poised to track upcoming genomic advances and to provide tools for keeping you “genomically literate.”

More than a decade before you were born, I chose to be a genomics researcher because I believed that a more complete understanding of our DNA blueprint would improve human health. Today, I am confident that the genomic advances made by my generation will provide a foundation on which your generation will further advance genomics in previously unimaginable ways. Most importantly, I am certain that genomics will benefit your life—and, as a veteran genomics researcher, that makes me immensely proud.

Eric D. Green, M.D., Ph.D.

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Using the Discovery of the Structure of DNA to Illustrate Cultural Aspects of Science

PENG DAI, DAVID RUDGE



ABSTRACT

DNA is a central topic in biology courses because it is crucial to an understanding of modern genetics. Many instructors introduce the topic by means of a sanitized retelling of the history of the discovery of the structure of DNA by James Watson and Francis Crick. Historical research since 1968 has revealed that Rosalind Franklin's contributions were more significant than they are usually depicted. In light of this, we developed a two-class lesson plan that draws attention to Rosalind Franklin's role in the discovery and to the social and cultural aspects of science. The first class provides background information regarding what led scientists to recognize that DNA was the molecule of heredity. Students watch a documentary video that includes interviews with some of the surviving protagonists. Students (working in groups) are then asked to debate Franklin's role to refine their awareness of how social and cultural factors affected both the process of science and how it has been recounted. The second class has students work in groups to build a structural model of DNA through hands-on activities. The essay concludes by drawing attention to how the two-day lesson plan, developed for a college-level biology course, can be adapted for use in other settings.

Key Words: history of science; biology education; DNA structure; social and cultural aspects of science; gender; Rosalind Franklin.

○ Introduction

Learning the structure of DNA is considered essential to an understanding of genetics (NRC, 1996; NGSS Lead States, 2013). This being said, students often have difficulty appreciating how the structure of DNA makes many life processes possible, such as the inheritance of genetic information and the synthesis of proteins (Newman et al., 2012). Standards documents are also quite clear that students need to learn more than science content—they need to learn about issues associated with the nature of science (NOS), such as the tentative nature of scientific knowledge (Lederman, 2007).

Learning the structure of DNA is considered essential to an understanding of genetics.

One particularly promising method to help students learn science content and issues associated with the NOS is through the history of science (Matthews, 1994; Rudge & Howe, 2004). Teaching science through the use of history demystifies the process of science and provides students with a better understanding of how science relates to other aspects of the world, including social, cultural, and ethical issues (Matthews, 1994). Research suggests the use of history of biology, particularly by means of stories, is an effective way to teach students to appreciate issues associated with the NOS, e.g., the fact that scientific knowledge is socially and culturally embedded (Williams & Rudge, 2015, 2016).

Textbooks often introduce the topic of genetics to students with reference to James Watson and Francis Crick's amazing discovery of the structure of DNA in 1953. Most present this history in a sanitized way, one that plays up the genius of these two white men working in isolation, with minimal reference to the contributions of others whose work made their discovery possible. Historical scholars, in the wake of the publication in 1968 of Watson's autobiographical account, *The Double Helix*, have started to draw particular attention to the neglected role of Rosalind Franklin in these accounts (Gibbons, 2012; Glynn, 2012; Klug, 1968; Maddox, 2002; Sayre, 1975). In light of these considerations, we created a two-day lesson plan that incorporates more of the actual history of how the structure of DNA was first discovered, with emphasis on Rosalind Franklin's contributions. Our specific goals were to engage student interest, and draw their attention to the role of gender as well as other social and cultural influences in this episode. We taught NOS explicitly and reflectively (c.f. Abdel Khalick & Lederman, 2000) by making NOS issues a planned instructional activity and by giving students opportunities to reflect on these issues, in this instance by means of arguments and class discussions. Our focus on these issues served as a segway to students developing models of the structure of DNA.

○ History Behind the Discovery of the Structure of DNA

Textbooks regularly draw attention to the fact that James Watson and Francis Crick discovered the structure of the DNA molecule in 1953 and were awarded the Nobel Prize together with Maurice Wilkins in 1962. They also draw attention to how the discovery of the structure of DNA paved the way for modern genetics. While Watson and Crick are highly appraised for their work, the role of Rosalind Franklin, who produced the X-ray diffraction image of DNA (Photo 51) that made Watson and Crick's discovery possible, is often neglected (Elkin, 2003; Fausto-Sterling, 2002; Gibbons, 2012; Jungck, 1984; Klug, 1968; Maddox, 2002; Rapoport, 2002; Sayre, 1975).

In the early 1950s, it was already established that DNA (a long molecule of repeating nucleotide bases) was the genetic material. This being said, it was a complete mystery how such a simple molecule (from a chemical standpoint) could contain the information for complex proteins and accurately replicate itself. Watson and Crick (working at Cambridge University) and Wilkins and Franklin (working at Kings College), were among many scientists at the time intrigued by the mystery of figuring out the structure of DNA that gave it these properties. Stylistically, their approach to this problem could not have been more different. Watson and Crick had been hired to do different projects and collaborated together on the structure of DNA in their spare time. Wilkins and Franklin were both hired (in part) to study the structure of DNA, but did not work together, owing in part to a fundamental disagreement regarding whether Franklin had been hired to work as Wilkins assistant or, as she believed, an independent researcher in her own right. Watson and Crick devoted themselves to creating models; Wilkins and Franklin on collecting data, namely X-ray diffraction images of DNA. Franklin focused specifically on refining her technique, because she recognized the information provided by the photographs regarding how the parts of the molecule were arranged would be essential for a resolution of the problem.

In 1951, Watson attended a lecture given by Franklin on her work. She reported discovering that DNA can exist in two forms, A and B. Watson returned to Cambridge with a rather vague recollection of what Franklin had presented, further complicated by the fact that he was still a novice to X-ray crystallography and how to interpret it. One week later, based on his recollections of Franklin's presentation, Watson and Crick proposed their first model for the structure of DNA, which proved to be a complete failure. Ironically enough, Franklin was among the critics who attended this first presentation and pointed out the flaws of their model. This experience solidified her conviction that it was too soon to speculate.

In May 1952, Franklin produced the clearest picture of the B form of DNA (Photo 51). She suspected that both the A and B forms of DNA were helical, but did not want to announce this finding until she had sufficient evidence. As such, she briefly turned her attention back to the A form before announcing her decision to leave Kings College. Her decision stemmed primarily from the ongoing antagonism with Wilkins. But to make sense of why these proved sufficient to lead her to conclude she had to leave Kings, we need to consider other social and cultural factors. At the time Franklin worked in England, the field of science was heavily dominated by men, and indeed women scientists were looked down upon (Crease, 2003; Gibbons, 2012). The personnel committee ultimately accepted her resignation, but on the condition

she would finish her analysis of her DNA findings and publish her results. As a result, Wilkins took over her lab and obtained Photo 51, which he shared with Watson without her permission in January 1953. Watson and Crick were at this point in a much better position to correctly interpret the photograph and piece together what the structure of DNA must be. And in February 1953, they announced their discovery of the structure of DNA (Watson, 1968/2012). Their model of its structure so perfectly fit the experimental data that it was almost immediately accepted by the scientific community, including Franklin. But at the time she was unaware of the pivotal role her photograph had played in allowing them to build their model (Ashcroft, 2015; Elkin, 2003; Glynn, 2012; Maddox, 2002).

Watson and Crick's model of the structure of DNA has been called the most important biological discovery of the twentieth century. Only nine years later, in 1962, Watson, Crick, and Wilkins were jointly awarded the Nobel Prize. This happened after Franklin had died in complete ignorance of her contribution and at a time when the Nobel Prize committee rules prohibited the awarding of the prize posthumously. In 1968, Watson published his memoir, *The Double Helix: A Personal Account of the Discovery of the Structure of DNA*, which drew attention for the first time to the crucial role Franklin's research had played in their discovery. Additional historical scholarship since has provided additional evidence. Indeed, some have argued that equal credit should be given to Rosalind Franklin (Gibbons, 2012; Maddox, 2002; Olby, 1974; Rapoport, 2002). The lesson plan below incorporates this historical scholarship. We begin by sharing our learning objectives and how they are assessed before turning to the specific details of the lesson plan, along with recommended modifications for instructors having different needs.

○ Learning Objectives

The lesson plan contains both NOS and content learning objectives. During the first class one of our main objectives, an NOS concept included in the NGSS, is for students to be able to identify that "Science is a human endeavor" (NGSS Lead States, 2013, App. H). More explicit extensions under this category are that men and women from different social, cultural, and ethnic backgrounds work as scientists and engineers; science and engineering are influenced by society, and society is influenced by science and engineering (NGSS Lead States, 2013, App. H, p. 6). Engaging students by sharing a story based on a more accurate representation of the history of research on the structure of DNA, puts them in a position to appreciate the difficulties women scientists experienced in the socio-cultural environment that existed in England during the early 1950s. A follow-up class discussion prompts students to consider the role of gender bias in science with reference to Rosalind Franklin's experiences, and to reflect upon the extent to which gender bias might inhibit the process of science. Arguments about her contribution and the role of gender help students understand the influence of society and culture on science.

The lesson plan also has several other learning objectives with reference to the Next Generation Science Standards. The specific cross-cutting concepts, science and engineering practices, and life-sciences core idea met by this lesson plan are listed in Table 1 (NGSS Lead States, 2013, App. E, F, & G). For instance, one of the crosscutting concepts is the relationship between the structure of a molecule and

Table 1. Crosscutting Concepts, Science & Engineering Practices, and Life Sciences Core Idea in the Next Generation Science Standards covered by this class.

Crosscutting Concepts	Part of Lesson
<i>Patterns:</i> Observed patterns of forms and events guide organization and classification, and they prompt questions about relationships and the factors that influence them.	After watching the video and summarizing the main features of DNA structure, students identify patterns, including how it is composed of repeating nucleotide base pairs.
<i>Cause and effect:</i> Mechanism and explanation. Events have causes, sometimes simple, sometimes multifaceted. A major activity of science is investigating and explaining causal relationships and the mechanisms by which they are mediated. Such mechanisms can then be tested across given contexts and used to predict and explain events in new contexts.	During the students' presentation, they attempt to explain the underlying causes of the DNA structural patterns and interpret the functions associated to DNA replication process.
<i>Structure and function:</i> The way in which an object or living thing is shaped and its substructure determine many of its properties and functions.	During the students' presentation, they interpret functions associated with the process of DNA replication in terms of underlying structural patterns.
Science & Engineering Practices	Part of Lesson
Developing and using models	Students are asked to design and construct DNA models and to describe the structure of DNA.
Engaging in argument from evidence	Students are required to create arguments that include both evidence for their position and justification of their response.
Obtaining, evaluating, and communicating information	Students working in groups share and evaluate arguments with their peers.
Life Sciences Core Idea	Part of Lesson
<i>Inheritance of traits:</i> DNA carries instructions for forming species' characteristics. Each cell in an organism has the same genetic content, but genes expressed by cells can differ.	By explaining the process of DNA replication based on the DNA structure, students demonstrate their understanding that all the cells in an organism have the same genetic content.

its function. During the second day of the class, students are asked to explain the semi-conservative mode of DNA replication with reference to their understanding of DNA structure and, in particular, the fact that it exists as a double helix. Each strand of the original molecule serves as a template for a new complementary strand, thus ensuring faithful replication of the DNA molecule.

○ Assessment

We use class discussions to assess students' understanding of the issue associated with NOS, and presentations of DNA structural models to assess their learning of DNA content. During the first day, students are asked to answer and discuss the open-ended questions on Handouts 1 and 2 (Appendix), through which the instructor can identify students' understanding of the social and cultural aspect of NOS. For the second day of the lesson, the instructor can evaluate students' presentation on their DNA model using the presentation rubric (Handout 3, Appendix), which provides the instructor with information on the students' understanding of DNA content covered in this lesson.

○ DNA Structure Lesson Plan

We created our two-day lesson for use in a college introductory biology course for non-majors, but (as indicated below) we have

several suggestions for how to modify it for use in high school and other settings.

○ Materials

- One computer to play the PowerPoint and the NOVA video, "DNA—Secret of Photo 51"
- Handout 1 with discussion questions for Day 1 (Appendix)
- Handout 2 with argumentation table for Day 1 (Appendix)
- Supplies and rubric for making DNA models and giving presentations (Handout 3, Appendix)

○ Preparation

We created our lesson plan based on the history of the discovery of the structure of DNA specifically to illustrate the role of gender and other social and cultural factors in science. The class is introduced to this history by means of a 52-minute video, "DNA—Secret of Photo 51," produced for PBS (Public Broadcasting Service) and made available online by WGBH. It is currently available on both the NOVA website and YouTube. The video reviews the history of how the structure of DNA was discovered, discusses the semi-conservative nature of DNA replication, and includes extensive interviews with

surviving major participants. We have found that the video is a particularly effective way to engage students and deepen their understanding of the history behind the discovery.

○ Procedure: Day 1

During the first class the instructor presents background information about DNA by means of a PowerPoint. The presentation introduces DNA as a long chain (polymer) composed of repeating units (monomers) called nucleotides. They also learn that nucleotides are composed of three components—(1) a nucleotide base (adenine, thymine, guanine, cytosine), (2) a sugar (2'-deoxyribose), and (3) a phosphate group—and how new nucleotides are added on to the growing polymer. We also introduce Chargaff's rule (a general pattern found among DNA molecules in nature), which states: for any given molecule of DNA, the amount of adenine is always equal to the amount of thymine, and the amount of cytosine is always equal to the amount of guanine. After this we ask students to think through and share answers to the first question on Handout 1 (Appendix). We have found this assists us in assessing students' knowledge before we continue to the remainder of the lesson. We then briefly introduce James Watson, Francis Crick, Maurice Wilkins, and Rosalind Franklin. We emphasize that they are among many scientists during the 1950s who were interested in unraveling the mystery surrounding the structure of DNA. At this point, our presentation using the PowerPoint has made students aware of all of the relevant information scientists had at the time the protagonists began their research. It is presented by means of the story specifically to intrigue students into wondering how scientists were ultimately able to discover the structure of DNA.

After this background information has been shared, students are asked to read over five additional open-ended questions on Handout 1 for later class discussion. Students watch a brief video, "DNA—Secret of Photo 51," which narrates the history of the discovery of DNA structure. Students are then asked to work in groups and complete the remaining questions on Handout 1 regarding how the process of science, as illustrated in the film, is affected by culture and society. Students are then required to share their thoughts and have a class discussion on each question. For example, students discuss how Watson and Crick recognized that DNA must be a double helix. In our own class, most students agree that Rosalind Franklin played a critical role in the process of discovering the structure of DNA. They also acknowledge how the social and cultural environment in England led to the gender bias and negatively influenced female scientists working in that time period.

Students, working individually, are next asked to explore the episode from an ethical perspective by filling out Handout 2 (Appendix). Students are asked to consider both *would* and *should* Franklin have received the Nobel Prize with Watson, Crick, and Wilkins if she had lived? Students are asked to create arguments for their position that both includes evidence for their position and explains their response. In this way, the process of having students reflect on these questions improves students' critical thinking skills and helps them understand in concrete ways how society and culture influence science. Students working in groups then share and evaluate arguments with their peers. In our class, we've found students generally agree that, had she lived, Franklin *should* have received the Nobel Prize.

For instance, one of our students responded, "Rosalind Franklin should be awarded the Nobel Prize with Watson and Crick because she provided the crucial information to the last step of creating the structure of DNA." However, with regard to the other question, which asks students to speculate on what *would* have happened if Franklin was alive at the time that the Nobel Prize was awarded for the discovery, students often disagree. For example, one of our students responded, "Rosalind Franklin would have received the Nobel Prize because she would have stood up for herself and defended her research," whereas another student responded, "She would not have received it because women were still persecuted by a dominant male social environment." The first day of the lesson concludes with students discussing an open-ended question: If you were either Watson or Crick, what would you have done? The discussion will not only serve as an opportunity for students' further reflection on NOS as illustrated by the Franklin story, but will also help students to appreciate these issues are part of contemporary science as well.

○ Procedure: Day 2

The second day begins by having students summarize what they have learned in terms of the content, the main features of DNA, from the last class. We've found that our use of a story based upon Rosalind Franklin's experiences leads to greater student recall and understanding of both the structure of DNA and the details of the discovery. Students recognize that DNA is a polymer composed of nucleotides and takes the form of a double-stranded helix. They know that adenine always pairs with thymine, and guanine always pairs with cytosine in DNA. The two DNA strands run in opposite directions. Students recognize that Watson and Crick's discovery of the actual structure of DNA was made possible by crucial evidence furnished by Rosalind Franklin, and further, how her important role in the discovery was and has been diminished in the retelling of the story. The instructor then discusses the process of DNA replication, building off of the video's previous discussion of the semi-conservative mode by which replication takes place. Our goal at this point is to connect student understanding of the semi-conservative nature of replication with the structural properties of the DNA molecule that allow this process to take place.

Students are next asked to work in groups and design a structural model of DNA that incorporates the main features they just reviewed. The instructor supplies students with both a rubric (Handout 3, Appendix) and materials that allow them to create a physical model of DNA. In our introductory biology course, we provide a diversity of materials to encourage student creativity: such as two sizes of paper clips, colored pipe cleaners, styrofoam sheeting, colored papers, marshmallows, spaghetti, gummy bear, etc. The rubric contains guiding requirements designed to focus students' attention on the main features of DNA molecule. Before they start to make the DNA structural model, students are required to draw their blueprints of it on the white boards provided for each group. In particular, they need to make decisions on what materials they will use to represent each part of the molecule. As students work through their blueprints and models, the instructor walks around providing suggestions and answering questions. This hands-on activity of making DNA models can contribute to students' understanding of the abstract concepts of the structure of DNA and how this structure is related to the functions of DNA,

despite the fact that the molecule is microscopic. The class concludes with presentations of students' models. These presentations serve as an opportunity to assess students' understanding of science content.

○ Activity Modifications

The following are recommended modifications of the lesson plan for use by instructors who might need to customize depending upon their particular needs:

- *Alternative ways to use the video.* Another way to use the video would be in combination with Handout 1, i.e., pausing the film at various points to give students opportunities to think and discuss the questions on Handout 1 while viewing the video. Depending upon the objectives of the instructor, showing parts of the video, rather than the whole video might be an option.
- *Alternative ways to teach the lesson without the video.* Instructors can alternatively create a PowerPoint to present this story and illustrate different personalities of the scientists and many crucial features of the scientific process (Emani, 2010). Students could also read either Watson and Crick's original paper (2003 [1953]) or a brief summary of the paper written in a way that is grade-level appropriate.
- *Alternative ways to use the in-class DNA modeling activity.* The activity of developing a DNA structural model could be assigned as team homework to be completed outside of class using the rubric and materials students find at home.
- *Alternative ways to use the lesson to teach the NOS.* The lesson plan could be elaborated to focus on an additional NOS objective, "Imagination and creativity in scientific investigations." Creativity occurs throughout the scientific process, from coming up with new ideas and research questions to collecting, analyzing, and interpreting data (Lederman et al., 2002). During the first class, instructors could add an additional discussion question to Handout 1: "Do you think Watson and Crick used their imagination and creativity in their investigations? If yes, please explain why with examples. If not, please explain why not." The video about Watson and Crick figuring out the specific pattern about the bases A, T, C, and G bonded, constructing the DNA model, and discovering the structure of DNA provides an example of how scientists use imagination and creativity in their investigations. If time permits, instructors could additionally use a formal assessment created by the Lederman group named VNOS (Views on the Nature of Science), which includes a specific question about social and cultural influences on science (Lederman et al., 2002). The VNOS questionnaire could be used before and after the history-based class to assess change in student understanding of NOS concepts.

○ Conclusion

Our experiences using this lesson plan in class, particularly students' responses to the questions provided in Handouts 1 and 2 suggest students find issues associated with NOS, especially the social and cultural influences on science, to be particularly engaging. The fact that students were able to correctly build DNA models and make successful presentations suggests that the historical lesson plan, far from distracting students, actually enhances student understanding of the structure of DNA. Asking students to present their views by means of arguments

is one way to help them improve critical-thinking skills, as when they respond to questions and challenges from other students. We find this historical lesson plan helps students better understand not only scientific content, but also the process of science. Therefore, we recommend instructors use this lesson plan for improving students' learning of the structure of DNA as well as their understanding of social and cultural aspects of science often left out in the science classroom.

○ Resources

The NOVA video "DNA—Secret of Photo 51" is available for free online at <https://www.youtube.com/watch?v=0tmNf6ec2kU>

The argumentation worksheet was revised by authors based on V. Sampson, *Argument-driven inquiry in biology: Lab investigations for grades 9–12* (Arlington, VA: NSTA Press, 2014).

References

- Abd-El Khalick, F., & Lederman, N. (2000). The influence of history of science courses on students' views of nature of science. *Journal of Research in Science Teaching*, 37(10), 1057–1095.
- Ashcroft, F. (2015). Rosalind Franklin and Photograph 51. *The Lancet*, 386(10002), 1439–1440.
- Crease, R. P. (2003). The Rosalind Franklin question. *Physics World*, 16(3), 17.
- Elkin, L. O. (2003). Rosalind Franklin and the double helix. *Physics Today*, 56(3), 42–48.
- Emani, C. (2010). Using the "DNA story" to inculcate a scientific thought process in the classroom. *American biology Teacher*, 72(7), 410–413.
- Fausto-Sterling, A. (2002). Gender and Science in the DNA Story. *Science*, 298(5596), 1177–1178.
- Gibbons, M. (2012). Reassessing discovery: Rosalind Franklin, scientific visualization, and the structure of DNA*. *Philosophy of Science*, 79(1), 63–80.
- Glynn, J. (2012). *My Sister Rosalind Franklin*. Oxford: Oxford University Press.
- Jungck, J. R. (1984). The DNA helix, featured scientist: Rosalind Franklin: "Bold Experimentalist." *American Biology Teacher*, 46(8), 430–472.
- Klug, A. (1968). Rosalind Franklin and the discovery of DNA. *Nature*, 219, 808–844.
- Lederman, N. (2007). Nature of science: Past, present, and future. In S. K. Abell & N. Lederman (Eds.), *Handbook of Research in Science Education* (pp. 831–880). Mahwah, NJ: Erlbaum.
- Lederman, N. G., Abd-El-Khalick, F., Bell, R. L., & Schwartz, R. S. (2002). Views of nature of science questionnaire: Toward valid and meaningful assessment of learners' conceptions of nature of science. *Journal of Research in Science Teaching*, 39(6), 497–521.
- Maddox, B. (2002). *Rosalind Franklin: The dark lady of DNA (1st ed.)*. New York: HarperCollins.
- Matthews, M. (1994). *Science Teaching: The Role of History and Philosophy of Science*. New York: Routledge.
- National Research Council (NRC). (1996). *National science education standards*. Washington, DC: National Academies Press.
- NGSS Lead States. (2013). Topical arrangement of the *Next Generation Science Standards*. Retrieved from <http://www.nextgenscience.org/search-standards>
- Newman, D. L., Catavero, C. M., & Wright, L. K. (2012). Students fail to transfer knowledge of chromosome structure to topics pertaining to cell division. *CBE-Life Sciences Education*, 11(4), 425–436.
- Olby, R. C. (1974). *The path to the double helix: The discovery of DNA*. N. Chelmsford, MA: Courier Corporation.

Rapoport, S. (2002). Rosalind Franklin: Unsung Hero of the DNA Revolution. *The History Teacher*, 36(1), 116–127.

Rudge, D. W. & Howe, E. M. (2004) Incorporating history of science in the classroom. *The Science Teacher*, 71(9), 52–57.

Sayre, A. (1975). *Rosalind Franklin and DNA* (1st ed.). New York: WW Norton & Company.

Watson, J. D. (2012). *The double helix: A personal account of the discovery of the structure of DNA*. Oxford: Atheneum. (Original work published 1968.)

Watson, J. D., & Crick, F. H. (2003). A structure for deoxyribose nucleic acid, 1953. *Nature*, 421(6921), 397.

Williams C. T., & Rudge, D. W. (2015). Mendel and the nature of science. *American Biology Teacher*, 77(7), 492–499.

Williams C. T., & Rudge, D. W. (2016). Emphasizing the history of genetics in an explicit and reflective approach to the teaching of nature of science: A pilot study. *Science & Education*, 25(3–4), 407–427.

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Appendix

Handout One

Video Worksheet

We will watch the NOVA video named “DNA-Secret of Photo 51.” Please read over all of the questions and answer Question 1 in preparation for our later discussion.

1. James Watson, Francis Crick, Maurice Wilkins, and Rosalind Franklin are the scientists associated with the discovery of DNA structure. Have you ever heard of them? And, if so, from where? What do you know about them?
2. What was the social and cultural environment in England at that time that negatively influenced female scientists?
3. Rosalind Franklin, as depicted in the film, seems to have a difficult time interacting with the other scientists. To what extent would you attribute this to her individual personality, and to what extent does it reflect how women were (are) treated by male scientists? Explain your answer.
4. Describe the contribution of the following scientists in the discovery of the structure of DNA: James Watson, Francis Crick, Maurice Wilkins, and Rosalind Franklin.
5. How did Watson and Crick recognize that DNA must be a double helix? Were any values or ethics violated by these researchers? Explain your answer.
6. In what ways does the social (or institutional) structure of science, as revealed in this film, promote or inhibit scientific activity? Explain your answer.

Handout Two

Leading Question: If Rosalind Franklin had lived, would she/should she have received the Nobel Prize with Watson, Crick, and Wilkins? Work in groups, discuss with your peers, and fill in the argumentation worksheet individually, and defend your answers.

Argumentation Worksheet

Constructing and Argument		
The Leading Question	Would Franklin have received the Nobel Prize with Watson, Crick, and Wilkins if she had lived?	Should Franklin have received the Nobel Prize with Watson, Crick, and Wilkins if she had lived?
Our Claim		
Our Evidence		
Our Justification of the Evidence		

Handout Three

Three-Dimensional DNA Model and Presentation Rubric

Instructions:

- Design and use the available materials to build a three-dimensional DNA model based on what we learned about DNA structure in the last class.
- Pick supplies (two sizes of paper clips, colored pipe cleaners, Styrofoam sheeting, colored papers, marshmallow, spaghetti, gummy bear, etc.) to represent the main components of DNA including: sugar, phosphate, and bases (adenine, guanine, cytosine, or thymine).
- Use this sequence for one half of the DNA molecule: 3' CATAGTGCCA 5'
- Give a presentation, in groups of two or three, explaining the structure of DNA.

Checkout Questions:

Before you start to build the DNA model, use the white board to draw a blueprint that illustrates the structure of DNA. Discuss with your partners about how you are going to build the model and what materials you will use to represent each main molecule.

Grading Rubric:

1. The model contains three main building blocks (sugar, phosphate, base), and each molecule is represented by different materials. The four bases (A, T, G, and C) should be represented by four different colors.
_____ /1
 2. The model should match the sequence given above.
_____ /1
 3. The model should follow Chargaff's rule: adenine bonded to thymine and cytosine bonded to guanine.
_____ /1
 4. The model consists of two strands and is double-helix shaped.
_____ /1
 5. The two strands are anti-parallel directions, and the 3' and 5' ends are properly represented.
_____ /1
 6. The model is accurately labeled or a key is attached.
_____ /1
 7. The model is three-dimensional and can be physically twisted.
_____ /1
 8. The model is neat and creatively constructed.
_____ /1
 9. The presentation is clear and well organized.
_____ /1
 10. Everyone in the group contributes to the presentation.
_____ /1
- Total Points _____ /10



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Mendel or Molecules First: What is the Best Approach for Teaching General Genetics?

CHARLES E. DEUTCH

ABSTRACT

A key question in teaching a General Genetics course is whether to present the major concepts of Mendelian genetics first, or to start with the essential ideas of molecular genetics. A comparison of two sequential courses at Creighton University with similar groups of students indicated that there were no statistically significant differences in exam scores or final grades with the two approaches. It thus may be better to focus on the questions of how best to present the material in each area to contemporary students and how better to prepare them to take exams that involve different types of questions requiring analytical, numerical, and writing skills. These issues are discussed in the context of the modern biology curriculum.

Key Words: Mendelian genetics; molecular genetics; undergraduate curriculum.

○ Introduction

A course in General Genetics is an important component of most undergraduate biology curricula (Cheesman et al., 2007). General Genetics is often taken after two semesters of General Biology and two semesters of General Chemistry. The course is usually designed to cover several topics that are considered essential for any biology major, including the storage and expression of biological information, the role of gene products in determining specific structures and their functions, the origin and consequences of genetic variation, the role of natural selection in the process of evolution, and the analysis of genomes and the reconstruction of phylogenies (Brownell et al., 2014; GSA, 2015; Smith & Wood, 2016). These topics fit into several of the five core concepts and six core competencies of the *Vision and Change in Undergraduate Biology Education* proposal made by the American Association for the Advancement of Science (AAAS, 2011). Although there is little debate about the importance of these topics, there is less agreement about how best to teach them and how to assess student learning. Some have advocated the use of more active modes of teaching that go beyond lectures and forms of evaluation that are more complex than multiple-choice

tests (Lee & Jabot, 2011; Smith & Wood, 2016). However, there are difficulties in implementing these changes and some ambiguity about their success (Andrews et al., 2011; Waldrop, 2015).

A key question in teaching General Genetics is whether to teach the basic concepts of Mendelian genetics first, then follow with a discussion of molecular genetics, or vice versa. Although there has been some research on this question as it applies to middle- and high-school students (Duncan et al., 2016), there have been no systematic studies about this issue as it applies to college- or university-level students. Several textbooks are currently available for use in a General Genetics course at this level (Hartl & Ruvolo, 2012; Griffiths et al., 2015; Hartwell et al., 2015; Klug et al., 2015; Sanders & Bowman, 2015; Pierce, 2016; Snustad & Simmons, 2016; Brooker, 2017). These books vary somewhat in difficulty and content but cover the same essential topics. They are now published in both digital and print versions, and have online resources for students in addition to the written text. These textbooks all use some version of the “Mendel first” approach and include chapters on (1) the basic patterns of inheritance in eukaryotic organisms as seen in monohybrid and dihybrid crosses; (2) mitosis, meiosis, and chromosome distribution; (3) extensions of Mendelian patterns including variations in dominance relationships, lethal alleles, and sex linkage; (4) chromosome mapping in eukaryotes and chromosome structure; (5) genetic analysis of prokaryotic organisms and viruses; and (6) extranuclear or organellar inheritance. These chapters are followed by ones dealing with (7) DNA structure; (8) DNA replication and recombination; (9) transcription and RNA processing; (10) translation and protein synthesis; (11) gene regulation in prokaryotes and eukaryotes; and (12) mutation and DNA repair. Later chapters in the textbooks are more variable but commonly focus on topics such as developmental genetics, cancer, genomics, DNA technology, population genetics, and evolution. Some textbook authors do suggest ways in which the chapters could be re-ordered to cover molecular genetics before Mendelian genetics (Sanders & Bowman, 2015; Brooker, 2017). Two versions of a genetics textbook have sometimes been published in the past (for example, Russell, 2005, 2009), but this is not common now.

During the Fall semesters of the academic years 2014–2015 and 2015–2016, I taught the General Genetics lecture course (BIO 317) at Creighton University in Omaha, Nebraska, as a sabbatical replacement for the two regular geneticists who were on sequential leaves. Although they had taken somewhat different approaches to the course, they both used *Introduction to Genetic Analysis* by Griffiths et al. (2015) as the primary textbook, and this book had been ordered before I arrived. I used the tenth edition in 2014–2015 and the eleventh edition in 2015–2016. The course at Creighton has two semesters of General Biology and two semesters of General Chemistry as prerequisites. Although the class is positioned as a second-level course, many students defer taking it until their senior year because it is believed to be one of the hardest classes in the biology program there. As a result, some students take courses in Cell Structure and Function or Biochemistry before General Genetics, but others do not. There is a separate Genetics Laboratory course (BIO 318) that is taken by some students either concurrently or after the Genetics lecture course. In 2014, I followed the general organization of the textbook by Griffiths et al. and used the “Mendel first” approach. In 2015, I revised the organization and used a “molecules first” approach with the same book. This thus constituted a kind of natural experiment in which to explore the advantages and disadvantages of the two different approaches with similar groups of students.

○ Course Organization

The General Genetics class was offered each year as two separate but parallel sections with about 30 students each and met for 50 minutes three times a week. Each class was based on a PowerPoint presentation, but often included examples of relevant data or sample problems.

Creighton uses an in-class video recording system so all the PowerPoint presentations and the class audio were digitally saved for later review. A written handout was provided for each class with a summary of the lecture material, a list of specific terms to define and know, and problems from the textbook to be answered. There were no graduate assistants or separate recitation sections, but undergraduate assistants offered tutoring sessions, and I held extensive office hours/problem sessions each week. The initial enrollments were similar over the two years. In the Fall semester of 2014, there were a total of 52 students, which included no sophomores, 27 juniors, and 25 seniors. In the Fall semester of 2015, the initial enrollment was again 52 students, but included 12 sophomores, 18 juniors, and 22 seniors. This reflected a difference in the advising process in which some Biology majors were encouraged to take the class sooner. The sequences of topics for the two versions of the course are shown in Table 1.

Some topics were covered in a single class period, but others took a period and half or two periods. The schedule was adjusted during the semester, and some topics that were initially included, such as Developmental Genetics, were dropped as I learned more about the department’s curriculum.

The courses were similar in that each had four or five in-class exams consisting of two parts. Part I was a set of 30 multiple-choice questions worth 2 points each for a total of 60 points. These questions were a combination of relatively simple genetic problems and those that tested student comprehension of the basic genetic facts or concepts. Part II consisted of four genetic problems or short-answer questions worth 10 points each for a total of 40 points. The genetic problems were more complex than those in the multiple-choice section and often involved multistep calculations. The short-answer questions were usually introduced with a figure similar to one used in the textbook or in the PowerPoint presentations given in class.

Table 1. Course sequences in General Genetics (BIO 317) at Creighton University.

Fall 2014—“Mendel first”	Fall 2015—“molecules first”
The genetic system	The genetic system and genetic analysis
Inheritance of single genes in eukaryotes	DNA structure and organization into chromosomes
Variations in single gene inheritance	DNA replication and transmission in prokaryotes
Inheritance of independently assorting genes	DNA replication and transmission in eukaryotes
Inheritance of organelle genes	Transcription in prokaryotes
Gene interactions	Transcription and RNA processing in eukaryotes
Inheritance of linked genes and recombination	Protein synthesis
Mapping genes on eukaryotic chromosomes	Cloning and sequencing of DNA
Mapping genes in bacteria	Transcriptomes and proteomes
DNA structure and chromosome organization	Inheritance of single genes in eukaryotes
DNA replication	Sex linkage and human pedigree analysis
Transcription	Dihybrid crosses and independent assortment
RNA processing	Variations in Mendelian patterns
Protein synthesis	Polygenic traits and epistatic interactions
Regulation of gene expression in bacteria	Organelle genetics
Regulation of gene expression in eukaryotes	Inheritance of linked genes and recombination

(continued)

Table 1. Continued

Fall 2014—"Mendel first"	Fall 2015—"molecules first"
Transposable elements	Mapping genes on eukaryotic chromosomes
Mutation	Mapping genes in bacteria
DNA Repair	Molecular mechanisms of recombination
Recombination and recombinational repair	Regulation of gene expression in bacteria
Large-scale chromosomal changes	Regulation of gene expression in eukaryotes
Population genetics	Transposable elements
Inheritance of complex traits	Large-scale chromosomal changes

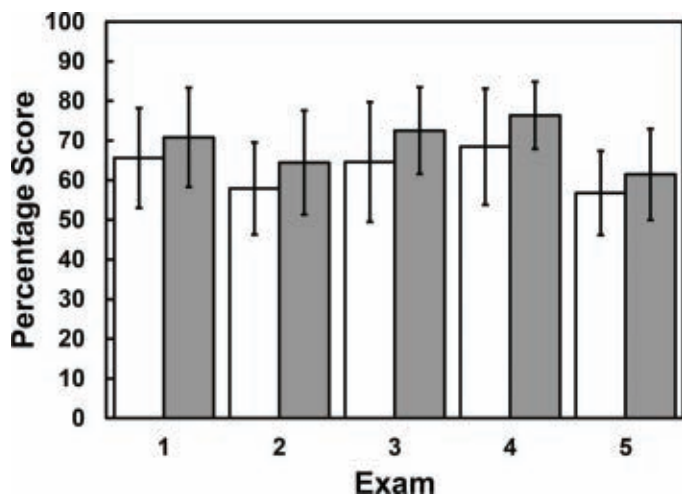


Figure 1. Mean percentage scores on the five exams in General Genetics (± 1 SD) for the Fall semester of 2014. The open bars show the scores for students who had not taken Cell Structure and Function prior to enrolling in Genetics. The filled bars show the scores for students who had taken Cell Structure and Function prior to enrolling in Genetics. An ANOVA analysis of the data was done with an online program (Vasavada, 2016), and post-test analysis indicated that the only significant differences were between the scores on Exam 4 and Exam 5 for each group.

and involved several subparts. In addition to the exams, there were eight to ten homework assignments each year that could be completed individually or in small groups. Again, depending on the material, the homework assignments consisted of genetic problems of different types or short-answer questions. I also had the students in the Fall 2014 class write a term paper on a topic of their own choosing about genetics, but I dropped this assignment for the Fall 2015 class.

There were several reasons for changing the approach from "Mendel first" to "molecules first" in the second year. First, I found that students who had Cell Structure and Function before taking General Genetics appeared to do better on each of the five exams in 2014 (Figure 1). Exams 1 and 2 focused on Mendelian genetics, Exams 3 and 4 focused on molecular genetics, and Exam 5 covered a mixture of topics. Although the standard deviations were relatively large, an ANOVA analysis of the data (Vasavada, 2016) indicated significant differences among all 10 of the exam

scores (F -statistic = 6.23, p -value = 8.34×10^{-8}). A Tukey HSD (honestly significant difference) post-test analysis as well as Scheffé and Bonferroni and Holm comparisons indicated the most consistent significant differences were between the scores on Exam 4 and the scores on Exam 5. However, on any individual test, there were no significant differences between those students who had taken Cell Structure and Function and those who had not.

Second, I thought that some Mendelian concepts like dominance, epistasis, and variable penetrance would make more sense if students understood how genes worked and contributed to a phenotype. If a student does not know that genes direct the synthesis of proteins, and that gene products interact to produce observable phenotypes, these concepts are often obscure. In a similar way, genetic exchange and gene mapping in bacteria are better understood if a student knows how DNA replication and molecular recombination work. Third, I felt that because the material in molecular genetics is somewhat more descriptive, students might do better on the exams at the beginning of the course. They would thus develop enough confidence to cope better with the more difficult analytical material that is involved in the interpretation of crosses in Mendelian genetics.

○ Student Performance

Figure 2 summarizes the percentage scores on the exams for the two years I taught General Genetics at Creighton University. The data shown in Figure 1 were pooled to give the results for 2014, and as before, the standard deviations were relatively large. The exams were similar in difficulty, but because of the differences in the sequence of topics, they varied in the specific questions on each test. The average percentage score for all of the exams in the Fall 2014 class was 65.77 with a standard deviation of 12.79. The average percentage score for all of the exams in the Fall 2015 class was 65.02 with a standard deviation of 13.12. Qualitatively, the scores appeared to increase in 2014 (except for Exam 5) as we moved from Mendelian genetics to molecular genetics, and to decrease in Fall 2015 as we moved from molecular genetics to Mendelian genetics.

An ANOVA analysis of the data (Vasavada, 2016) indicated statistically significant differences among the exams. Across all nine exams, the F -statistic was 5.48 for a p -value of 3.87×10^{-7} . As a test of the hypothesis that presenting molecular genetics first might lead to better performance on the Mendelian analysis questions, I compared the scores on Exams 3 and 4 from 2015, which focused on

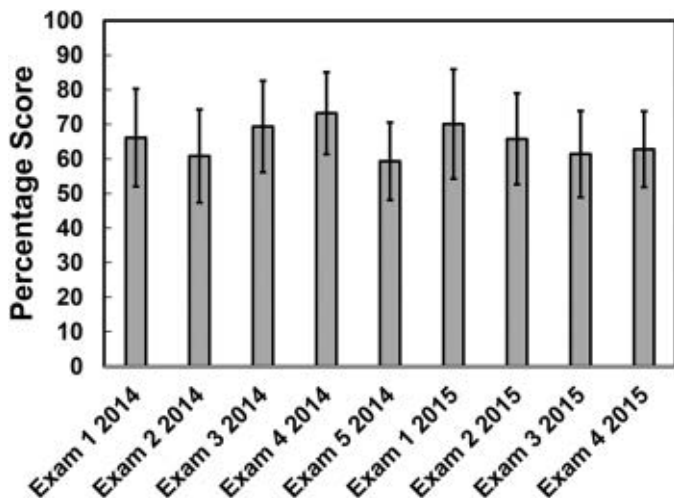


Figure 2. Mean percentage scores (± 1 SD) for the five exams in the Fall semester of 2014 and for the four exams in Fall semester of 2015. An ANOVA analysis of the data was done with an online program (Vasavada, 2016), and post-test analysis indicated no significant differences based on the order of the topics.

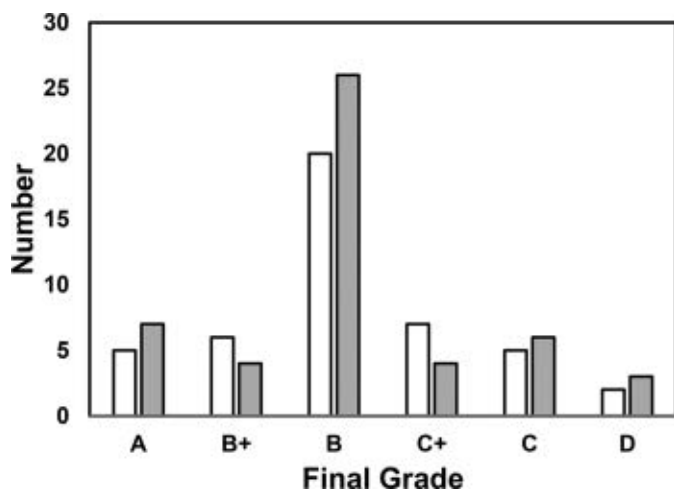


Figure 3. Distribution of final grades in General Genetics for the Fall semester of 2014 (open bars) and the Fall semester of 2015 (filled bars).

Mendelian genetics, with those of Exams 1 and 2 of 2014. The averages for 2015 were 61.41 ± 12.5 and 62.81 ± 10.96 , respectively. The averages for 2014 were 66.14 ± 14.15 and 60.83 ± 13.47 , so there was no apparent improvement. The post-test Tukey HSD analysis indicated no significant differences as a result of changing the sequence of topics. In a same way, the averages for Exams 3 and 4 in 2014, which covered molecular genetics (69.37 ± 14.15 and 73.21 ± 11.88), were similar to those for Exams 1 and 2 in 2015 (70.08 ± 15.83 and 65.8 ± 13.2). Again, the post-test Tukey HSD analysis indicated the differences were insignificant.

Figure 3 shows the final grade distributions for the two years. Creighton does not use minus grades, so only grades of A, B+, B, C+, C, and D are shown. About 10 percent of the students withdrew from

the course each year before the end of the semester. Although the averages on the exams were consistently less than 70 percent, the students were able to raise their total points for the semester through the open-book, group homework assignments, a supplementary problem set, and a term paper in 2014, and a 5 percent grade inflator in 2015. I also adjusted the final percentage cut-offs slightly from the standard Creighton grading scale (A = 90–100%, B+ = 87–89.9%, B = 80–86.9%, etc.). I confirmed with the other instructors and the Department Chair that these grades were typical of past performance in the class.

○ Perspectives and Conclusions

This natural experiment indicates that in the General Genetics course at Creighton University, it makes little difference whether Mendelian genetics is presented before molecular genetics or vice versa. I found that in both 2014 and 2015, some students did better in the section on molecular genetics than they did in the section on Mendelian genetics. Other students did better in the section on Mendelian genetics than they did in the section on molecular genetics. With the addition of points from the homework assignments and other supplementary points, the scores balanced out so the final percentage scores and grade distributions were similar. In general, however, students had more difficulty with the Mendelian genetics section than they did with the molecular genetics section.

The student course evaluations did not differ with the approach and were decidedly mixed each year (average of about 3.5 out of 5 on the most general questions). For some students, I was one of the best instructors they had ever had, but for others I was one of the worst. For some, the workload and exams were reasonable, but for others the material was too difficult and the exams were too long and too hard. For some, there was a good correlation between the class presentations and the exams, but for others these were disconnected. Interestingly, none of the students commented on the sequence of topics in the semester that they took the class. The fact that the means on the exams were around 60–65 percent was a source of much anxiety. Many biology majors at Creighton have high aspirations and are focused on health-related careers. The possibility of not getting an A or B+ was a major concern. Other faculty in the department told me that is not unusual for this class and that my experience there was normal.

My general conclusion from this experiment is that “Mendel first” or “molecules first” is not the right question to ask about a General Genetics course. Better questions to ask are, what do the students find difficult about each of these aspects of genetics, and how can their performance on exams in each area be improved. In the case of Mendelian genetics, the major difficulties were related to data interpretation and analysis. For many students, this was an entirely new experience that required the ability to think through various possibilities and a level of numerical fluency they had not yet attained. Although I assigned many problems from the textbook and gave them extensive homework assignments, many students struggled with these types of questions. I gave them handouts with suggestions for how to do this, including references to specific books (Kowles, 2001; Elrod & Stansfield, 2010; Nickla, 2010), links to various web sites, and examples from other textbooks. I also spent many hours each week helping students with the homework problems. However, many of them never figured out

how to solve genetic problems effectively. They often looked for quick short-cuts or formulas, and would not take the time needed to work through the possibilities. As the course moved along, they sometimes forgot about topics we had discussed before. The low scores on Exam 5 in 2014 were a clear example of this.

In the case of the molecular genetics section, the major difficulties were related to the complexity of some of the material that was not in the textbook, to the need to recall concepts at different times during the class, and to the students' abilities to deal with the short-answer questions on the exams. Although the textbook by Griffiths et al. is very good at presenting genetic analysis and contains extensive end-of-the-chapter problem sets, its discussion of molecular and microbial genetics is largely historical. I had to supplement the material on DNA replication, transcription, translation, and DNA repair with additional slides in the PowerPoint presentations. Likewise, I had to present many modern techniques like high-throughput DNA sequencing, genome analysis, and transcriptome and proteome analysis, which were not covered in the book at all. Although I had thought that the students might do better on the exams dealing with this material, that was not consistently the case. They often did not remember the basics of DNA structure and metabolism when the issues of DNA repair and recombination or transposition came up later in the course. Many students came into the class from large courses like General Biology where the exams were composed entirely of multiple-choice questions. They had difficulty in understanding what was expected to get full-credit on a short-answer question and in writing good answers during the exam itself. Although I included similar questions as part of the homework assignments, they often complained even when they were given partial credit on an exam question. To them, getting 7 out of 10 possible points looked like 70 percent and a C. Getting 5 or 6 points was even worse.

From this experiment, I believe each instructor needs to determine the best sequence for their students, based on the organization of their department's curriculum, his or her personal background and experience, and the choice of textbook. Most genetics textbooks are now very large (700–800 pages) and contain more material than can be reasonably covered in one semester. For a survey course that attempts to cover both Mendelian genetics and molecular genetics, I still think the “molecular first” approach may be the best, particularly if the students do not have to take a course like Cell Structure and Function first. My own background is in microbial genetics and molecular biology, so these are topics in which I have a particularly strong interest. Other instructors whose interests are in genomics, evolution, or human genetics may have other preferences. Because genetic analysis is so hard for many students today, it might be preferable to move most of the molecular material into a separate course in Molecular Genetics (Molecular Biology) or to include it in a course in Cell Structure and Function. By doing this, the General Genetics course could move at a slower pace and give students sufficient time to learn how to deal with the analytical problems. The ability of the students to deal with both genetic problems and short-answer questions seems to be related primarily to the amount of practice they get.

As noted in the Introduction, the AAAS proposal entitled *Vision and Change*, which suggests major revisions in undergraduate biology, refers to several topics that are included in a General Genetics course. Of the five core concept areas, General Genetics is most important for Evolution and for Information Flow, Exchange, and

Storage. Of the six core competencies, General Genetics is critical to the Ability to Apply the Process of Science, the Ability to Use Quantitative Reasoning, the Ability to Use Modeling and Simulation, and the Ability to Understand the Relationship between Science and Society. Although the *Vision and Change* document is very useful in outlining these core concepts and competencies, it is less clear about the order in which specific courses might be taken and about how students might gradually develop their scientific and intellectual skill. Mead et al. (2017) found that teaching Genetics before Evolution to high school students in the United Kingdom helped student understanding but did not change their acceptance of the evolutionary process.

Some genetics instructors have suggested discarding the “canon” found in most textbooks and restructuring the General Genetics course completely (Redfield, 2012). However, to the extent that the course needs to prepare students to take standardized tests like the Graduate Record Exam (GRE) or Medical College Admissions Test (MCAT), I do not think the basic content of Mendelian genetics and molecular genetics can be eliminated completely. Nevertheless, there is a need to discuss how best to present this material to contemporary students. Several authors have looked recently at these issues (Pavlova & Kreher, 2013; McElhinny et al., 2014; Smith & Wood, 2016), and it is important for this conversation to continue. To assess how different curricula or teaching approaches affect student learning, some instructors may want to use a standardized concept assessment test for either Mendelian genetics (Smith et al., 2008) or molecular genetics (Couch et al., 2015). These tests might be used as either pre-tests or post-tests. Further explorations of the “Mendel first” or “molecules first” question in different institutions with varying student populations would be very helpful.

○ Acknowledgments

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References

- American Association for the Advancement of Science (AAAS). (2011). *Vision and change in undergraduate biology education: A call to action*. Washington, DC.
- Andrews, T. M., Leonard, M. J., Colgrove, C. A. & Kalinowski, S. T. (2011). Active learning *not* associated with student learning in a random sample of college biology courses. *CBE-Life Sciences Education*, 10, 394–405.
- Brooker, R. J. (2017). *Genetics: Analysis and principles* (6th ed.). New York: McGraw-Hill Education.
- Brownell, S. E., Freeman, S., Wenderoth, M. P., & Crowe, A. J. (2014). BioCore Guide: A tool for interpreting the core concepts of vision and change for biology majors. *CBE-Life Sciences Education*, 13, 200–211.
- Cheesman, K., French, D., Cheesman, I., Swails, N., & Thomas, J. (2007). Is there any common curriculum for undergraduate biology majors in the 21st century? *BioScience*, 57, 516–522.
- Couch, B. A., Wood, W. B., & Knight, J. K. (2015). The molecular biology capstone assessment: A concept assessment for upper-division molecular biology students. *CBE-Life Sciences*, 14, 1–11.

- Duncan, R. G., Castro-Faix, M., & Choi, J. (2016). Informing a learning progression in genetics: Which should be taught first, Mendelian inheritance or the central dogma of molecular biology? *International Journal of Science and Mathematics Education*, 14, 445–472.
- Elrod, S. L., & Stansfield, W. D. (2010). *Schaum's Outline of Genetics* (5th ed.). New York: McGraw-Hill Education.
- Genetics Society of America (GSA). (2015). Core concepts and competencies in genetics. Retrieved from http://www.genetics-gsa.org/education/GSAPREP_CoreConcepts_CoreCompetencies.shtml
- Griffiths, A. J. F., Wessler, S. R., Carroll, S. B., & Doebley, J. (2015). *Introduction to Genetic Analysis* (11th ed.). New York: W. H. Freeman and Company (10th edition, 2012).
- Hartl, D. L., & Ruvolo, M. (2012). *Genetics: Analysis of genes and genomes* (8th ed.). Burlington, MA: Jones and Bartlett Learning.
- Hartwell, L. H., Goldberg, M. L., Fischer, J. A., Hood, L., & Aquadro, C. F. (2015). *Genetics: From genes to genomes* (5th ed.). New York: McGraw-Hill Education.
- Klug, W. S., Cummings, M. R., Spencer, C. A., Palladino, M. A., & Killian, D. (2015). *Concepts of Genetics* (11th ed.). Boston: Pearson Education.
- Kowles, R. (2001). *Solving Problems in Genetics*. New York: Springer-Verlag.
- Lee, W. T., & Jabot, M. E. (2011). Incorporating active learning techniques into a genetics class. *Journal of College Science Teaching*, 40, 94–100.
- McElhinny, T. L., Dougherty, M. J., Bowling, B. V., & Libarkin, J. C. (2014). The status of genetics curriculum in higher education in the United States: Goals and assessment. *Science & Education*, 23, 445–464.
- Mead, R., Hejmadi, M., & Hurst, L. D. (2017). Teaching genetics prior to teaching evolution improves evolution understanding but not acceptance. *PLOS Biology*, 15, e2002255.
- Nickla, H. (2010). *How to Solve Genetics Problems*. San Francisco: Benjamin/Cummings Pearson Education.
- Pavlova, I. V., & Kreher, S. A. (2013). Missing links in genes to traits: Towards teaching for an integrated framework of genetics. *American Biology Teacher*, 75, 641–649.
- Pierce, B. A. (2016). *Genetics: A conceptual approach* (6th ed.). New York: W. H. Freeman and Company.
- Redfield, R. J. (2012). “Why do we have to learn this stuff?”—A new genetics for 21st century students. *PLoS Biology*, 10(7), e1001356.
- Russell, P. J. (2005). *iGenetics: A Mendelian approach* (1st ed.). Boston: Benjamin/Cummings Pearson Education.
- Russell, P. J. (2009). *iGenetics: A molecular approach* (3rd ed.). Boston: Pearson Education.
- Sanders, M. F., & Bowman, J. L. (2015). *Genetic analysis: An integrated approach* (2nd ed.). Boston: Pearson Education.
- Smith, M. K., & Wood, W. B. (2016). Teaching genetics: Past, present, and future. *Genetics*, 204, 5–10.
- Smith, M. K., Wood, W. B., & Knight, J. K. (2008). The genetics concept assessment: A new concept inventory for gauging student understanding of genetics. *CBE-Life Sciences*, 7, 422–430.
- Snustad, D. P., & Simmons, M. J. (2016). *Principles of Genetics* (7th ed.). Hoboken, NJ: John Wiley & Sons.
- Vasavada, N. (2016). One-way ANOVA (ANalysis Of VAriance) with post-hoc Tukey HSD (Honestly Significant Difference) Test Calculator for comparing multiple treatments. Available at http://astatsa.com/OneWay_Anova_with_TukeyHSD/
- Waldrop, M. M. (2015). The science of teaching science. *Nature*, 523, 272–274.

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ABT AUTHORS & PHOTOGRAPHERS Guidelines

We encourage our readers, biologists with teaching interests, and biology educators in general, to write for *The American Biology Teacher*. This peer-reviewed journal includes articles for teachers at every level with a focus on high school and post-secondary biology instruction.

Revised January 2016

General Categories

The general categories of articles are:

Feature Article (up to 4000 words) are those of general interest to readers of *ABT*. Consider the following examples of content that falls into the feature article category:

- Research on teaching alternatives, including evaluation of a new method, cooperative learning, concept maps, learning contracts, investigative experiences, educational technology, simulations and games and biology standards
- Social and ethical implications of biology and how to teach such issues, genetic engineering, energy, pollution, agriculture, population, health care, nutrition, sexuality, and gender, and drugs
- Reviews and updates of recent advances in the life sciences in the form of an "Instant Update" that bring readers up-to-date in a specific area
- Imaginative views of the future of biology education and suggestions for coping with changes in schools, classrooms and students
- Other timely and relevant and interesting content like discussions of the role of the Next Generation Science Standards in biology teaching, considerations of the history of biology with implications for the classroom, considerations of the continuum of biology instruction from K-12 to post-secondary teaching environments, contributions that consider the likely/ideal future of science and biology instruction.

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continued

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Thank you for your interest in *The American Biology Teacher*. We look forward to seeing your manuscripts soon.

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Digital files must meet the following guidelines:

- Minimum resolution of 300 DPI, though 600 DPI is preferred.
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Drowsy *Drosophila*: Rapid Evolution in the Face of Climate Change

RECOMMENDED
FOR AP Biology

JENNIFER BROO, JESSICA MAHONEY, JULIE BOKOR, DANIEL HAHN



ABSTRACT

Climate change can drive evolution. This connection is clear both historically and in modern times. The three-lesson curriculum described below provides opportunities for students to make connections between climate change and evolution through various modes of inquiry and self-investigation. Students examine how genetic variation may either facilitate or limit the ability for species to survive changing climates through work with the model organism *Drosophila melanogaster*. Students are asked to layer new understanding of the mechanisms of evolution onto their observations of genetic variation in fruit fly thermotolerance, and then synthesize this information to make predictions regarding the survival of species threatened by climate change.

Key Words: climate change; evolution; *Drosophila melanogaster*; thermotolerance.

○ Introduction

Climate change is an important and timely topic that unites many concepts in the biology curriculum. A recent study of 1500 public middle- and high-school science teachers from all 50 U.S. states found that although most students will be exposed to climate change in a science class, the average teacher devotes only one to two hours of instructional time to the topic (Plutzer et al., 2016). With so little time devoted to climate change education, teachers are missing a valuable opportunity to use climate change as a unifying concept to educate students about the nature of science and evolution. Despite the fact that climate change is driving both ecological and evolutionary change in contemporary populations, many teachers cover these topics as separate, discrete units. As classroom teachers, we felt this unconnected, multiunit approach could result in student misconceptions and a general lack of understanding about the importance of climate change on evolution. We emphasize that climate change can

Climate change is an important and timely topic that unites many concepts in the biology curriculum.

result in rapid, measurable, real-time evolutionary events. Many scientific studies show a range of species are experiencing genetic changes in response to recent, rapid climate change (Bradshaw & Holzapfel, 2006; Hoffmann & Sgro, 2011; Penuelas et al., 2013; Scheffers et al., 2016). Unfortunately, this perspective of rapid modern evolution is not covered in most classrooms.

In this three-lesson curriculum unit (Table 1), we strive to develop student understanding of the impact climate change can have on microevolution in contemporary populations using the model organism *Drosophila melanogaster*. The objective of this curriculum is to unify teaching of climate change and evolution by providing students opportunities to assess natural genetic variation for cold tolerance in *D. melanogaster* and discuss the implications for genetic variation to allow for adaptation by natural selection and species persistence despite a changing climate.

We open with a data-collecting activity using species “information trading cards” to give students a broader understanding of climate change impacts on various species. Students discover that not all species have the capacity to survive rapid climate change. These cards illustrate climate change “winners” and “losers,” and emphasize the critical concept that some species may benefit from climate change by expanding their ranges, population sizes, or number of generations per year, whereas other species may suffer more with reduced ranges, decreased population sizes, and even extinction (Foden et al., 2013). In the second lesson, students perform a laboratory investigation using genetically defined lines of the fruit fly *D. melanogaster* to investigate genetic variation in climate-change-related phenotypes. Students relate their laboratory observations to the mechanisms of evolution and perform statistical analyses. In the third lesson, we extend our discussion of climate change driving natural selection using a series of examples that highlight the diversity of species’ responses to climate change and reinforce students learning the forms of natural selection: directional, stabilizing,

Table 1. A suggested pacing guide that can be used with the curriculum based on 45-minute class periods. Since the classroom teacher knows his or her students best, the teacher should decide the sequencing of lessons. The curriculum is available at <https://www.cpet.ufl.edu/resources/curricula/>.

	Week 1	Week 2
Day 1	Homework Prior to Lesson ONE: Background article reading with guided questions. Administer pre-assessment (if using).	Lesson TWO (extension): Hardy-Weinberg extension lesson and practice set.
Day 2	Lesson ONE: Winners and Losers of Climate Change— Debrief background reading guide. Complete Winners and Losers activity with species cards; debrief.	Lesson THREE: Patterns of Natural Selection— Types of Selection lesson and practice.
Day 3	Lesson TWO: Chill Coma Assay and Evolution Investigation— Assay background presentation. Run assay; collect raw data.	Lesson THREE: Patterns of Natural Selection— Types of Selection lesson and practice.
Day 4	Lesson TWO: Chill Coma Assay and Evolution Investigation— Data analysis and lab wrap-up questions.	Administer post-assessment.
Day 5	Lesson TWO: Chill Coma Assay and Evolution Investigation— Mechanisms of Evolution student investigation pages.	

and disruptive selection. Students are asked to synthesize their understanding of evolution and climate change to make predictions about the survival of species currently threatened by climate change. Taken together, these three lessons provide students the opportunity: (1) to learn that species may differ in their responses to climate change; (2) to assess natural genetic variation for cold hardiness, a climate-related trait in *D. melanogaster*; and (3) to discuss the implications for genetic variation to allow for adaptation by natural selection and species persistence despite a changing climate.

***Drosophila melanogaster* as a Model Organism for Studying Climate Change**

One aim of the *Next Generation Science Standards* is to more closely align science teaching and scientific processes. Thus, students must be exposed to the concept that some organisms can serve as models for much broader groups of organisms or for a set of scientific topics. Models are representations of complex phenomena that help students understand content, and do so in a way consistent with what scientists actually do in both laboratory and field settings (Bryce et al., 2016). Expanding the concept of using models, we found that *D. melanogaster* as a model organism was a good way to represent the larger biological phenomena of evolution and responses to climate change. *D. melanogaster* and other *Drosophila* species have served as models for thermal biology for decades, covering concepts from genetics of high-temperature responses to seasonal ecology, where they have often been used to model effects of climate change. Fruit flies are frequently chosen as a model organism for scientific studies because they have short life cycles, are more cost-effective than many other organisms (e.g., mice), and are easy to care for with limited space and effort. These same characteristics also make them ideal for classroom use.

D. melanogaster is a world-wide invasive species spread by people in stored foods and refuse that now occurs on almost every continent (David et al., 2007; Keller, 2007). This small fly originated in equatorial Africa, an area characterized by limited seasonality and generally warm temperatures. As the fly spread into more seasonal environments in the temperate zones of both the northern and southern hemispheres, populations have undergone rapid adaptation for cold hardiness and other seasonal traits (David et al., 1998; Hoffmann et al., 2002; Schmidt & Paaby, 2008). Thermal hardiness traits show a strong heritable genetic component across populations of *D. melanogaster*, as well as other invertebrates, along latitudinal clines from tropical to temperate locales consistent with directional natural selection (David et al., 2003; Gibert & Huey, 2001; Hoffmann, 2010; Sinclair et al., 2012).

Although multiple axes of cold hardiness differ among *Drosophila* populations, one of the most commonly measured traits which varies with latitude is chill coma recovery time. At low temperatures (below 4–7°C), insects and other ectotherms lose coordinated movement, resulting in an inactive state known as a “chill coma” (Sinclair et al., 2012). Flies sampled from populations originating in seasonally cool temperate locales typically recover more quickly from cold exposure than flies from warmer tropical locales (David et al., 2003; Gibert & Huey, 2001; Hoffmann, 2010; Sinclair et al., 2012). Because flies in chill coma cannot move to disperse, find mates, court, feed, or avoid predators, it is thought that the ability to recover quickly from chill coma is adaptive in seasonal habitats where flies may experience cold temperatures overnight that warm quickly in the morning. Chill coma recovery time has a clear, heritable genetic basis with polygenic quantitative patterns of inheritance. Individual genotypes within many populations clearly differ in this trait, showing that there is substantial naturally segregating variation in chill

coma recovery time (Hoffmann et al., 2002; Morgan & Mackay, 2006; Norry et al., 2008; Mackay et al., 2012).

○ Methods

Chill Coma Assay Design

This curriculum was developed in collaboration with a university researcher to bring an existing research protocol, known as the chill coma recovery time assay, from the field of evolutionary thermal biology to the high school classroom. In the chill coma assay protocol, vials containing approximately 10 flies from six genetically distinct lines of *D. melanogaster* (for a total of 60 flies) are placed on ice for three hours to induce a chill coma. The recovery times of flies, defined as a fly's ability to walk, from each line are then measured.

Fly lines were taken from the *Drosophila* Genetic Reference Panel (DGRP, Mackay et al., 2012). Each line was produced from a separate, individually housed, wild-caught gravid female from a single population of flies in Raleigh, North Carolina. Offspring of each individual female were inbred >30 generations, producing >200 true-breeding isofemale lines (Mackay et al., 2012). True-breeding isofemale lines are useful for studying the role of genetic variation in phenotypic variation because allelic variants become fixed within each line, but differ between lines. Differences in chill coma recovery times among DGRP lines indicate substantial genetic variation for chill coma recovery in the original population (Mackay et al., 2012). Thus, there is potential for natural selection to act on this trait in response to contemporary climate change.

These lines can be ordered from the Bloomington *Drosophila* Stock Center (University of Indiana; <http://flystocks.bio.indiana.edu/Browse/RAL.php>). We chose lines that recovered quickly (7–12 minutes, 25186/RAL-360, 25198/RAL-555, and 28178/RAL-356) and lines that recovered slowly (>20 minutes, 28253/RAL-861, 28254/RAL-879, and 28260/RAL-897) to maximize phenotypic differences students would observe. We suggest these lines to others, but instructors could select from any number of lines with differences in chill coma recovery time or other phenotypes of interest (Mackay et al., 2012).

Lesson ONE: The Winners and Losers of Climate Change

In Lesson ONE, students addressed the key question, “Are all species equally affected by climate change?” Students were assigned two homework articles to prepare for a class activity involving climate change “winners” and “losers.” In the article “More Evidence that Global Warming is Intensifying Extreme Weather” (Abraham, 2015), students learned how climate change is projected to produce not only higher average temperatures, but also greater frequency of extreme weather events including both heat waves and cold snaps. This article confronts a common misconception that climate change results only in rising temperatures. In the second article, “Evolutionary Response to Rapid Climate Change” (Bradshaw & Holzapfel, 2006), students learned about phenotypic plasticity, the ability of one genotype to produce more than one phenotype when exposed to different environments, and examined several examples of genetic changes that have already occurred in species due to climate change. Following a class discussion of the pre-reading, students worked in small groups (2–4 students) to analyze eight climate-affected species

(included in the curriculum) and predicted which species populations are likely to increase (“winner”) or decrease (“loser”) in response to climate change trajectory. The cards are based on authentic data available from a study that analyzed species vulnerable to climate change (Foden et al., 2013). Students examined traits like dispersal ability, interspecific interactions, and habitat needs. Using additional information provided in a figure from Foden and colleagues (2015), students made a prediction about each species' ability to benefit or struggle due to climate change (Figures 1 and 2). This activity can correct two common misconceptions: (1) evolution only occurs over very long, geologic time periods; and (2) climate change will negatively affect all species. As an extension, students could conduct their own research and create new climate-affected species cards. Students could then trade cards with other groups and add new species to their Species Vulnerability Matrix.

Lesson TWO: Chill Coma Assay and Evolution Investigation

After students learned to identify species' vulnerability to climate change, Lesson TWO challenged students to determine the role of evolution and natural selection in the long-term survival of a



Figure 1. Students analyzed information provided on species cards to complete a matrix to assist in the prediction of species survival based on current climate change trajectories (see Figure 2).

SPECIES VULNERABILITY MATRIX: Check any of the following boxes for each species if the factor is contributing negatively towards the species continued success given the current impact of climate change. Column “e, rarity” has been completed for you, for all 8 species.

	a. specialized habit	b,c. environmental tolerances/dependence on environmental triggers	d. interspecific interaction dependence	e. rarity	f. poor dispersal ability
African Reed Frog					
Asian Tiger Mosquito					
Four Toed Lizard				x	
Coral				x	
Fantail Warbler					
Common Coqui				x	
Fingered Poison Frogs				x	
Hornbill					

Figure 2. Students determined vulnerability to extinction due to climate change using this matrix (adapted from Foden et al., 2013).

species. Lesson TWO is a multiple-day activity in which students conducted the chill coma recovery time assay and performed basic statistical analyses of results to answer the driving question, “Is there potential for natural selection to act upon chill coma recovery in *D. melanogaster*?” This lesson is engaging and, importantly, models real scientific research because students performed the authentic chill coma recovery time assay with live *D. melanogaster* specimens.

Students again worked in small groups to measure chill coma recovery time on one line of flies. As a class, a standardized procedure was developed based on the authentic protocol so group results could be accurately compared. At the conclusion of the experiment, class data were compiled to include replicates. Students created graphs of the mean recovery time for each of the six genetically distinct lines, and included standard errors of the means. A class discussion of the data was held in which students identified that the six genetically distinct fly lines vary in chill coma recovery time, indicating that chill coma recovery time has a genetic basis and is a trait upon which natural selection could occur.

Next, students explored the mechanisms of evolution via self-investigation. Students read passages discussing five classic mechanisms of evolution: mutation, gene flow, non-random mating, genetic drift, and natural selection. Passages can be modified by the instructor to tailor the lesson to students’ ability or prior knowledge. Students applied their new knowledge of evolution to further interpret the laboratory data via a post-lab question set that begins with basic knowledge questions and proceeds to more open-ended questions in which students made their own connections between the content and laboratory activity.

As an extension of the lab investigation and mechanisms of evolution lesson, AP-level students explored the Hardy-Weinberg principle to quantify evolutionary change in a population of hypothetical fruit flies. This activity further expanded the students’ understanding of biostatistics using the Hardy-Weinberg equations and the chi-squared statistical test. At the conclusion of Lesson TWO, AP-level students could be required to write a formal lab report that includes a discussion in which students expound upon the statistical results of their experiment and make predictions about the ability of the fruit fly population to respond to climate change based on their newly acquired knowledge of evolutionary mechanisms.

Lesson THREE: Patterns of Natural Selection

The third lesson asked students to synthesize their understanding of microevolution and climate change to answer the essential question, “What patterns of natural selection might occur as a result of climate change?” using teacher-selected species from the Foden study. In this one-day lesson, students learned about three forms of natural selection: directional, stabilizing, and disruptive selection. Students completed a practice set with different population scenarios and predicted what form of natural selection the population could undergo. As a formative assessment, students worked in groups of three to four to complete the Natural Selection in the Face of Climate Change activity, where students identified how a real population of organisms might respond to climate change. Students received a fact sheet with a species description and a problem the species faces as a direct result of climate change. Groups drew graphs to represent the selection event, before and after the effects of climate change, and wrote an explanation of their predictions to support how species could possibly avoid extinction (c.f. Figure 3). Student groups presented

SPECIES 1 - SNOW SHOE HARE

Climate Change Issue

Snow doesn't come until later in the winter. Snowshoe hares that turn white later in the winter and/or turn brown earlier in the spring are camouflaged more effectively because they don't stand out to predators.



Figure 3. Sample answer key for a problem in the natural selection group modeling activity.

to the whole class, and students filled in a graphic organizer listing problems created by climate change and possible adaptations in response to those problems (Figure 4).

This lesson concluded with a class discussion on the limits of evolution: specifically, that species do not evolve because they need and/or want to, and that species are limited by existing genetic variation. We emphasized that evolution occurs at the population level, not the individual level. Finally, we discussed that the pace of evolution in a species may or may not keep up with environmental changes due to climate change.

Results

Student Outcomes

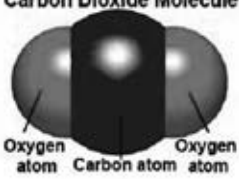

We implemented the curriculum in two different schools in AP Biology and honors-level biology classes. In the AP setting, this curriculum was used to begin the first unit on evolution. Students were asked to draw on their previous knowledge of evidence of evolution and basic genetic inheritance patterns from prior coursework. Students were previously introduced to statistical analysis in prior activities. In the honors-level class, the curriculum was used as a culminating piece, after the completion of a genetics unit, to tie microevolution to macroevolution, which had been introduced earlier in the course.

As teachers we observed higher levels of engagement using live model organisms compared to lessons from previous years using artificial representations of natural selection events (e.g., cut-out peppered moth activity). In addition, students made many positive comments about the curriculum on surveys. One stated: “I learned the most from this lab because it gave me a chance to find answers on my own, instead of just being told the answers.” Another shared that “it was really cool to use live organisms and now I see how species might change because of climate change.”

Modifications

For those who do not wish to or cannot use live fruit flies in the classroom, Lesson TWO could be approached as a case study, and students could be given data to analyze rather than collect their own. A data set is available from the authors. To better understand the impact of this curriculum on student understanding, a content knowledge assessment (available in the curriculum) and measure of evolution acceptance (GAENE, Smith et al., 2016) will be used as a repeated measure and allow comparison across classrooms in future studies.

CLIMATE CHANGE: a change in global or regional climate patterns; attributed largely to the increased levels of atmospheric carbon dioxide produced by the use of fossil fuels.

Problems Created By Climate Change	As a Result of Climate Change Natural Selection Would Favor*
<p style="text-align: center;">Carbon Dioxide Molecule</p>  <p style="text-align: center;">Oxygen atom Carbon atom Oxygen atom</p> <ol style="list-style-type: none"> 1. Acclimation strategies become out of sync with changing seasons 2. Breeding ranges may shrink 3. More rainfall and severe storms due to increased evaporation from warmer temperatures 4. Ocean acidification occurs when CO₂ is absorbed by the ocean and creates carbonic acid 5. Loss of snowpack in mountainous regions 6. Food sources may be out of sync with migration patterns 	 <ol style="list-style-type: none"> 1. Snowshoe hares that turn white later in the winter and/or turn brown earlier in the spring 2. Baltimore Orioles that expand their range to higher latitude during the breeding season 3. Juvenile Magellanic Penguins with more fat (to avoid hypothermia) and/or that develop adult feathers earlier in life 4. Shellfish with thicker shells 5. Cascades tadpoles with a shorter larval period and/or are more resistant to desiccation 6. Red knots that can find other food sources and/or arrive earlier in Delaware so arrival is synced with horseshoe crab egg laying

Limitations to Evolutionary Adaptability:

- 1) There may not be enough (or any) genetic variation in the population for a given trait
- 2) A population may not be able to evolve fast enough (especially organisms with longer generation times) to keep up with the pace of climate change

Figure 4. Teacher key of completed graphic organizer from Lesson Three.

Conclusion

This curriculum facilitates diverse teaching and learning opportunities by combining science and mathematics and asking students to engage in critical thinking the concepts presented. Our goal was to have students engage in inquiry-based learning and naturally ask their own questions about the effects of climate change and the future of life on Earth. These questions will naturally touch on diverse topics including genetics, evolutionary biology, emerging pathogens, ecology, and the nature of science. Beyond science, it was our hope that students would also engage in cross-curriculum-based inquiry, especially involving the ongoing intersection of climate change science and the politics of climate change policy worldwide. By asking students to identify questions and concepts that guide scientific inquiry, and having them learn how to conduct a scientific investigation on their own, we believe students will

develop a deeper understanding of concepts presented and be more prepared to educate themselves, their peers, and even their parents on topics of climate change and evolution.

For teachers interested in implementing this curriculum unit, all materials and detailed instructions can be found at: <https://www.cpet.ufl.edu/resources/curricula/>

○ Acknowledgments

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References

- Abraham, J. (2015, July 1). More Evidence That Global Warming Is Intensifying Extreme Weather John Abraham. *The Guardian*. Retrieved from <https://www.theguardian.com/environment/climate-consensus-97-per-cent/2015/jul/01/more-evidence-that-global-warming-is-intensifying-extreme-weather>
- Bradshaw, W. E., & Holzapfel, C. M. (2006). Evolutionary response to rapid climate change. *Science*, *312*, 1477–1478.
- Bryce, C. M., Baliga, V. B., De Nesnera, K. L., Fiack, D., Goetz, K., Tarjan, L. M., . . . Gilbert, G. S. (2016). Exploring models in the biology classroom. *American Biology Teacher*, *78*, 35–42.
- David, J. R., Gibert, P., Pla, E., Petavy, G., Karan, D., & Moreteau, B. (1998). Cold stress tolerance in *Drosophila*: analysis of chill coma recovery in *D. melanogaster*. *Journal of Thermal Biology*, *23*, 291–299.
- David, J. R., Gibert, P., Moreteau, B., Gilchrist, G.W., & Huey, R. B. (2003). The fly that came in from the cold: Geographic variation of recovery time from low-temperature exposure in *Drosophila subobscura*. *Functional Ecology*, *17*, 425–430.
- David, J. R., Lemeunier, F., Tsacas, L., & Yassin, A. (2007). The historical discovery of the nine species in the *Drosophila melanogaster* species subgroup. *Genetics*, *177*, 1969–1973.
- Foden, W. B., Butchart, S. H. M., Stuart, S. N., Vié, J.-C., Akçakaya, H. R., Angulo, A., . . . Mace, G. M. (2013). Identifying the world's most climate change vulnerable species: A systematic trait-based assessment of all birds, amphibians and corals. *PLOS ONE*, *8*(6). doi:10.1371/journal.pone.0065427
- Gibert, P., & Huey, R. B. (2001). Chill coma temperature in *Drosophila*: Effects of developmental temperature, latitude, and phylogeny. *Physiological and Biochemical Zoology*, *74*(3), 429–434. doi:10.1086/320429
- Hoffmann, A. A. (2010). Physiological climatic limits in *Drosophila*: Patterns and implications. *Journal of Experimental Biology*, *213*, 870–880.
- Hoffmann, A. A., & Sgro, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, *470*, 479–485.
- Hoffmann, A. A., Anderson, A., & Hallas, R. (2002). Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. *Ecology Letters*, *5*, 614–618.
- Keller, A. (2007). *Drosophila melanogaster*'s history as a human commensal. *Current Biology*, *17*, R77–R81.
- Mackay, T. F., Richards, S., Stone, E. A., Barbadilla, A., Ayroles, J. F., Zhu, D., . . . Richardson, M. F. (2012). The *Drosophila melanogaster* genetic reference panel. *Nature*, *482*(7384), 173–178.
- MacMillian, H. A., & Sinclair, B. J. (2001). Mechanisms underlying insect chill coma. *Journal of Insect Physiology*, *57*, 12–20.
- Morgan, T. J., & Mackay, T. F. C. (2006). Quantitative trait loci for thermotolerance phenotypes in *Drosophila melanogaster*. *Heredity*, *96*, 232–242.
- Norry, F. M., Scannapieco, A. C., Sambucetti, P., Bertoli, C. I., & Loeschcke, V. (2008). QTL of the thermotolerance effect of heat hardening, knockdown resistance to heat and chill coma recovery in an intercontinental set of recombinant inbred lines of *Drosophila melanogaster*. *Molecular Ecology*, *17*, 4570–4581.
- Penuelas, J. P., Sardans, J., Estiarte, M., Ogaya, R., Carnicer, J., Coll, M., . . . Jump, A. S. (2013). Evidence of current impact of climate change on life: A walk from genes to the biosphere. *Global Change Biology*, *19*, 2303–2338.
- Plutzer, E., Mccaffrey, M., Hannah, A. L., Rosenau, J., Berbeco, M., & Reid, A. H. (2016). Climate confusion among U.S. teachers. *Science*, *351*(6274), 664–665. doi:10.1126/science.aab3907
- Scheffers, A. R., De Meester, L., Bridge, T. C. L., Hoffmann, A. A., Pandolfi, J. M., Corlett, R. T., . . . Watson, J. E. M. (2016). The broad footprint of climate change from genes to biomes to people. *Science*, *354*, 719–731.
- Schmidt, P. S., & Paaby, A. B. (2008). Reproductive diapause and life-history clines in North American populations of *Drosophila melanogaster*. *Evolution*, *62*, 1204–1215.
- Schou, M. F., Loeschcke, V., & Kristensen, T. N. (2015). Strong costs and benefits of winter acclimatization in *Drosophila melanogaster*. *PLOS ONE*, *10*(6). doi:10.1371/journal.pone.0130307
- Sinclair, B. J., Williams, C. M., & Terblanche, J. S. (2012). Variation in thermal performance among insect populations. *Physiological and Biochemical Zoology*, *85*, 594–606.
- Smith, M. U., Snyder, S. W., & Devereaux, R. S. (2016). The GAENE—Generalized Acceptance of Evolution evaluation: development of a new measure of evolution acceptance. *Journal of Research in Science Teaching*, *53*(9), 1289–1315.

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Using Project-Based Learning to Teach Phylogenetic Reconstruction for Advanced Undergraduate Biology Students: Molluscan Evolution as a Case Study

• ANDREW A. DAVID

ABSTRACT

Phylogenetics plays a central role in understanding the evolution of life on Earth, and as a consequence, several active teaching strategies have been employed to aid students in grasping basic phylogenetic principles. Although many of these strategies have been designed to actively engage undergraduate biology students at the freshman level, less attention is given to designing challenges for advanced students. Here, I present a project-based learning (PBL) activity that was developed to teach phylogenetics for junior and senior-level biology students. This approach reinforces the theories and concepts that students have learned in their freshman courses along with incorporating Bioinformatics, which is essential for teaching zoology in the 21st century.

Key Words: systematics; morphology; discussion; pedagogy; active; learning.

○ Introduction

Phylogenetics is the study of the evolutionary relationships of individual species and groups (Nei & Kumar, 2000). The discipline plays a central role in the understanding of modern evolutionary theory but also extends to other fields such as DNA barcoding, phylogeography, and conservation biology (Avice, 1989; Hajibabaei et al., 2007). Undergraduates in most biology programs across the United States are usually introduced to phylogenetics early in their introductory coursework with technical terms such as “monophyly,” “parsimony,” and “synapomorphy/symplesiomorphy” becoming staple terms in their vocabulary by the end of their freshman year. Students are also expected to have covered the “morphological versus molecular tree” debate in which themes such as convergent evolution and homology are reinforced. In upper-level biology courses, where advanced students are expected to delve into the literature in detail, many instructors often assume that their class has at least a basic understanding of phylogenetics and is able to interpret the topology of a phylogenetic tree if one is included in an assigned research paper. However, previous studies have shown that even advanced biology

students often harbor a variety of misconceptions about building and interpreting phylogenetic trees (Lents et al., 2010).

The obvious solution would be to implement more effective pedagogical techniques in introductory biology classes for teaching phylogenetics. Multiple case studies have attempted this, and the use of tree-building exercises have been shown to improve students’ abilities to read and interpret tree topologies (Eddy et al., 2013). In this paper, I describe a project-based approach to teaching phylogenetic reconstruction. Project-based learning (PBL) consists of a broad range of pedagogical strategies that use projects as a central component. These projects extend over a period of time, vary in complexity based on the students’ aptitude, and actively engage them in design, problem-solving, investigative activities and decision making (Blumenfeld et al., 1991; Bell, 2010). This strategy facilitates autonomous work, stimulates critical thinking, and often culminates in a final realistic product such as a presentation or report. A major component of PBL is instructor feedback at crucial intervals during the course of the project. It is during these intervals that the important processes of reflection and recalibration occurs, which in turn, facilitate deep learning.

Significance of the Mollusca as the Focal Phylum for Investigation

In this project we use the phylum Mollusca as a case study. Molluscs are the second most speciose group of animals on our planet, consisting of more than 85,000 extant species with many more represented in the fossil record. Even more impressive is their ecological and physiological diversity and the fact that the diverse body plans observed across the different families is believed to have evolved from a basic ground pattern. Despite a series of systematic and phylogenomic studies in the late 1990s and 2000s, the monophyly of the group remains a contentious issue. Furthermore, some important questions on molluscan evolution remain unanswered. For example, does the shell-less condition of the Aplousobranchia represent an ancestral condition? This question is critical for molluscan evolution as it could shed light on the origin of the molluscan shell and

whether it is truly an ancestral trait for this group. Also, how can one explain the extreme divergence of many cephalopods such as squids and octopuses, whose complex sensory structures such as eyes and advanced cognitive capabilities place them in stark contrast to their gastropod (snails) and bivalve (clams, oysters, scallops) cousins? The Mollusca therefore represents an ideal phylum for zoology students to investigate through PBL as it allows them to address broad evolutionary questions through phylogenetic reconstruction of highly diverse taxa.

○ Methods

This project was developed as part of a student-centered introductory zoology course geared toward advanced biology students. The class size was 16, comprised of juniors and seniors. Prerequisite coursework included introductory-level molecular biology along with ecology and evolution. It is advisable that instructors complete their lectures on molluscs prior to assigning this project. The project was carried out over four class periods of 75 minutes each: Day 1, prep stage; Day 2, morphological analysis; Day 3, molecular analyses; Day 4, presentation and wrap up (Figure 1). On Day 1 students were organized into groups of four, briefed on the project (~20 minutes) and watched a 15-minute video on Mollusca (<https://www.youtube.com/watch?v=xKjeJlfdCBQ>). The debriefing should consist of an objective for the entire class—e.g., “The objective of this project is to elucidate the evolutionary relationships among selected molluscan taxa using morphological and molecular data”—a timeline for completion (Figure 1), and a grading rubric, along with expectations and a list of deliverables for the project. Students are then instructed to organize into groups (five to six students per group worked well in my class) and choose their group leader. Leaders designate tasks and must be willing to accept responsibility for completion of the project. After assigning this project in 2015 and again in 2017, I found that the most productive group configuration involved having two students complete the morphology tree (one student builds the character matrix, the other constructs the tree), two students on the molecular tree (one student mines and compiles sequences, the second edits, aligns, and builds the tree), and a fifth student who mines the literature and helps the group leader with the progress report and presentations. The

progress report will include a summary of each group member’s contribution for each time the group meets (either outside or during class), problems that arose, and possible solutions. This configuration controls for “free-riding,” ensuring that all students in a group contribute to the final product, however it does incur “transaction costs” as groups must also meet outside the classroom (Yamane, 1996).

On Day 1, groups are also given eight molluscan taxa representing six classes and an outgroup taxon to root their trees (Table 1). In this project, students used the annelid *Chaetopterus cautus* as an outgroup because phylogenomic analyses have placed annelids as the sister group to Mollusca (Kocot et al., 2011). It should be noted that in Table 1 some classes (e.g., Bivalvia) are represented by more than one species. The purpose of this is to reinforce the concepts of “sister taxa” and monophyly by having students observe the bifurcating branches that they will recover from their analyses.

Morphological Tree Construction

On Day 2, each group must choose at least five phylogenetically informative traits to be used in creating their character matrix for their morphology tree (Table 2). The simplest way to execute this task is to have students review lecture notes and their textbook and search for traits that distinguish the higher molluscan groups (e.g., gastropods vs. polyplacophorans, cephalopods vs. aplacophorans, etc.). As these molluscan groups are very distinct, there are a variety of traits from the basic molluscan body plan that can be used for the morphological analysis. Once the character matrix is completed, student groups are then tasked with manually creating the most parsimonious morphological tree. Based on the level of difficulty desired, instructors can also provide a “trait bank” to help students or give a list of mandatory traits for use to ensure that all groups are using the same characters.

There are two options for constructing morphology trees: the first involves using software such as PAUP* (Swofford, 1993) or Mesquite (Maddison & Maddison, 2003), and the second is by hand. The problem with using PAUP* and Mesquite is that traits will need to be coded, which is not as straightforward as working with molecular data (Pleijel, 1995). However, if the instructor is familiar with coding morphological traits, then they may opt for this option, keeping in mind that the project duration will be extended to accommodate the time needed for students to familiarize themselves with additional software. For the sake of simplicity, groups in my course constructed their morphology trees by hand using the following steps:

Step 1: Choose at least five phylogenetically informative traits. Instructors should provide either a trait bank or have student choose their own traits, which they can discuss with the instructor prior to attempting tree construction. Students should be aware that increasing the number of traits increases the number of possible trees that can be produced.

Step 2: Create a character matrix using both the ingroup and outgroup taxa. The outgroup taxon will be least similar to all of the ingroup taxa and as such will be used to “root” the tree. Table 2 shows an example of a character matrix developed by one of the groups in the class (hereafter referred to as Group A).

Step 3: Construct the tree in such a way that you can see when each of the traits either develops or is lost, and ensure that organisms are grouped by shared traits. At this point instructors should

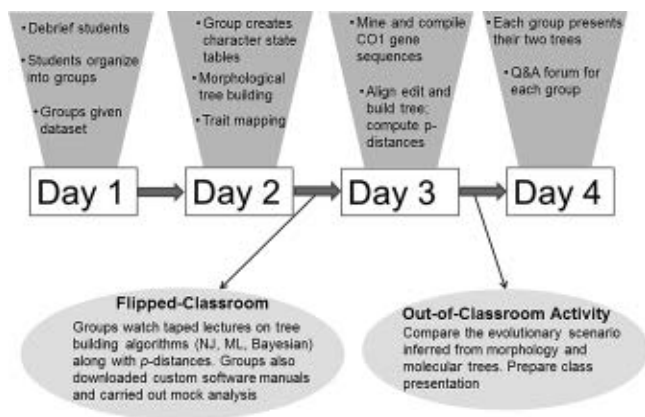


Figure 1. Summary of upper-level phylogenetics project for zoology course.

Table 1. List of molluscan taxa to be used in the phylogenetics project along with GenBank accession numbers for access to cytochrome c oxidase I (CO1) sequence data corresponding to each taxon.*

Taxa	Molluscan Groups	Accession Number**
<i>Parachiton politus</i>	Polyplacophora	HQ907881
<i>Falcidens halanychi</i>	Aplacophora	DQ371484
<i>Crepidula fornicata</i>	Gastropoda	KU566746
<i>Haliotis midae</i>	Gastropoda	AB236717
<i>Crassostrea gigas</i>	Bivalvia	AF280608
<i>Mytilus edulis</i>	Bivalvia	EU915580
<i>Pulsellum salishorum</i>	Scaphopoda	AY260832
<i>Loligo reynaudi</i>	Cephalopoda	AF075406

*Outgroup taxon: *Chaetopterus cautus* (Annelida), GenBank Accession number KX896507.

**Students are given accession numbers on Day 3.

Table 2. Example of Group A's character matrix for in-group and outgroup taxa.

	Trochophore larvae	CaCO ₃ shell	Plated Shell	Radula	Torsion	Byssus	Gladius (internal shell)	Captacula
<i>C. cautus</i> (outgroup)	1	0	0	0	0	0	0	0
<i>P. politus</i>	1	1	1	1	0	0	0	0
<i>F. halanychi</i>	1	0	0	1	0	0	0	0
<i>C. fornicata</i>	1	1	0	1	1	0	0	0
<i>H. midae</i>	1	1	0	1	1	0	0	0
<i>C. gigas</i>	1	1	0	0	0	1	0	0
<i>M. edulis</i>	1	1	0	0	0	1	0	0
<i>P. salishorum</i>	1	1	0	1	0	0	0	1
<i>L. reynaudi</i>	1	1	0	1	0	0	1	0

reiterate the philosophy of parsimony in constructing phylogenetic trees, i.e., the most preferred tree is the one with the least number of mutational steps/character state changes.

Step 4: Groups should be prepared to give a five-minute presentation of their morphology tree to which instructors can provide feedback. These presentations are not graded (although that can be left to the preference of the instructor) but are used to help students produce an improved phylogeny in the final presentation.

Molecular Tree Construction

On Day 3, each group is given GenBank accession numbers corresponding to each taxon (Table 1). For this project, I use archived sequences from the cytochrome c oxidase I (CO1) gene due to its ubiquity in phylogenetic analyses and the short sequence lengths (400–700 bp), which is convenient for teaching purposes. Two Bioinformatics software were used, BioEdit (Hall, 1999) and MEGA (Tamura et al., 2007), both of which are available for free online and offer a graphical user interface (GUI) that allows for a user-friendly working environment. BioEdit is a very simple Windows-based program that possesses extensive and

easy-to-use sequence editing capabilities, and MEGA is the preferred program for tree building in many undergraduate classrooms and compatible with both Windows and Macintosh operating systems (Newman et al., 2016). I created a simple one-page manual for using both programs but explicitly omitted troubleshooting, as having advanced students struggle with the computational glitches and coding issues that may occur is a crucial part of the learning process (especially considering that these struggles are also common among professional biologists!). Each group leader delegates the following tasks to specific group members:

1. Mine and compile the sequence data from GenBank using the accession numbers provided. Choose the FASTA format in GenBank, and copy and paste all the sequences into a text-editing file (Figure 2).
2. Use the ExPASy online translational tool to ensure gene functionality, i.e., ensure that each sequence can be translated into a functional protein (<http://web.expasy.org/translate/>). This is important as it ensures that the archived sequences being used are not pseudogenes and do not contain sequencing errors. In


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>HQ907881.1 Parachiton politus cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
ACTTTGTATATTGTTTTGGGATTGATCTGGTTTTGGTGGGTACAGCACTAAGGTTGTTGATTGCGGCTG
AGTTAGGTCAGCCTGGGGCATTATTAGGGGATGATCAGCTTTATAATGTGATTGTGACGGCTCATGCATT
TGTTATGATTTTTTTTTGGTTATGCCAATAATGATTGGGGTTTTGGAAATTGACTTGTTCCTTTGATG
TTGGGTGCGCCGATATGGCATTTCCTCGATTAATAATATGAGATTTGGCTTCTCCTCTCTTTAA
CTTTACTTTTGGCTTCCTGCTGGGTAGAGAGGGGGTGGGTACTGGTTGAAGTGTATCCCTTTGGC
TGGGAATGTGGCGCATGCCGGGGGCTGTCGATTTGGCCATTTTTCTCTTCACTTGGCCGGGTTTCT
TCAATTTAGGAGCAGTGAATTTTACTACTATTATTAATATACGTTGATGTGGCATGCAGTTGGAGC
GGGTTCCTTTGTTGTTGGTCTGTTAAAATTACTGCTATTTTACTTCTCTTTCTTTCCTGTGTTAGC
TGGGGCAATTACTATGCTTTAACTGATCGAAATTTAATACTGCTTTTTTACCCTGCTGGTGGTGA
GACCCAATTTGTATCAACATTTATTT

>DQ371484.1 Falcidens halanychi cytochrome c oxidase subunit I gene, partial cds; mitochondrial
TCAGGACTAATTGGAGCAAGCCTAAGGATAATAATTCGAATTGAGTTAGGACAGCCTGGGATAAGCTTTA
TAGGAAACGACCAACTTTTTAATGTTATTGTTACTGCACATGCTTTCGTCATAATCTCTTTATGGTAAT
ACCAATAATAATAGGAGGCTTCGGAAACTGACTTCTTCTCTCATATTAGGGATCCAGATATGGCATTCC
CCCGAATAAATAATAAGATTCTGACTTTTGGCCCCCTCTCTTACTTCTACTAAGATCCGAGGGG
TAGAAAGAGGGGCAGGAACGGATGAACAGTATACCCCTCTATCAGGACTATAAGCCACGCCGGAGC
CTCAGTAGACTTAGCAATCTTCTCTCCATCTTGCAGGAGCCTCCTCATATAGGATCTATCACTTT
ATTACCACAGTTATCAATATACGATCACAAGGGCTTACGCTAGAAGCCCTACCGCTATTTGTATGATCCG
TAAAAATCACAGCCATCTTCTACTTCTAAGGCTCCAGTCTTTCAGGAGCAATCACAATACTTTAACC
AGACCGAAACTTTAATACCGCTTTTTTGGACCCAG

>KU566746.1 Crepidula fornicata voucher AVP104 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial
TACTTCATATATTTTATTGGTATATGATCTGGACTAGTAGGAACAGCTCTAAGATTATTAATTCGAGCT
GAACCTGGACAACCAGGTGCTTCTTAGGGGATGATCAACTATACAATGTAATTGTTACAGCACACGCTT
TTGTTATAATTTTTTCTAGTAATACCTATAAATCGGGGATTGGTAATTGGTTAGTTCATTAAT
ATTAGGTGCTCCTGATATAGCTTTTCTCGACTTAATAATATAAGTTTCTGACTATTACCTCCAGCATT
TTACTATTGCTATCTCGGCCGAGTAGAAAGAGGAGTTGGGACCGGTTGAAACGGTTATCCTCTTTGT
CTGAAACCTGGCTCAGCCGGTGGATCTGTTGATTTAGCAATTTTTCTTACATCTTGCTGGTGTTC
ATCTATTTAGGAGCTGTAATTTTATTACTACTGTAATTAACATACGTTGACAGGGAGTTCAATTTGAA
CGACTTCTTTATTGTATGATCAGTAAAATTACAGCCATCTATTACTTTCTTACCAGTGTAG
CCGGAGCAATTACGATGCTTTTAAACAGACCGAAATTTAATACTGCTTTTGTGATCTGCAGGAGGAG
TGATCTATCTTATATCAGCATTATTTTGGTCTTT

```

Figure 2. Partial compilation of CO1 sequences of selected taxa in FASTA format using the notepad text editor.

introductory biology courses, students learn to translate the nucleotide sequence of a protein-coding gene into an amino acid sequence using the mRNA codon chart. In ExPASy, students copy and paste their edited sequences into a translation window, which executes the same process in a much shorter time. Students can confirm that the gene is functional if an open reading frame (ORF) is available.

3. The compiled text file with each sequence in FASTA format can then be imported into BioEdit for alignment and editing (Figure 3). Once imported, the Clustal W alignment tool should be selected to align the sequences. For simplicity, editing consists of eliminating gaps in the flanking regions of the sequences so a uniform size is recovered.
4. The edited sequence file can then be exported into a separate file, and then imported into MEGA to build a neighbor-joining (NJ) tree. In this exercise, I used a step-by-step instructional video provided by the National Institute of Health (https://www.youtube.com/watch?v=d_-NTsJDvn8) for building the NJ tree in MEGA (parameters are also specified in the video). The NJ method was used as opposed to the maximum-likelihood (ML) method because it is computationally faster and more accurate than the latter when dealing with smaller datasets, such as the one being used in this exercise (Tamura et al., 2004).

that may arise during the class activity. Alternatively, the instructor may secure a computer lab on campus for this activity, as it would allow for consistent hardware and software performance across all groups and thus less frustration on the part of both instructor and students.

Out-of-Classroom Activities

To facilitate Day 3 activities, I have found it useful to incorporate a flipped classroom approach. Prior to Day 3, I uploaded taped lectures along with PowerPoints, which students were required to watch and read. These materials explained the general practice of aligning and editing sequences, and background information on the different types of tree building such as the NJ and ML methods. In addition, the instructional video for using the MEGA software can be assigned as an out-of-classroom activity, which allows students to toggle with the settings and become familiarized with the program before attempting to build the molecular tree on Day 3. Students were encouraged to start forum threads on the course webpage if they had any questions, and either I or another student who understood the material would post responses to begin discussing the material in more detail. Extra-credit points were used as incentives for these out-of-classroom activities. Finally, all groups were required to meet outside the classroom to compare and reconcile their morphology and molecular trees and to provide an explanation for their results. Once these were accomplished, each group

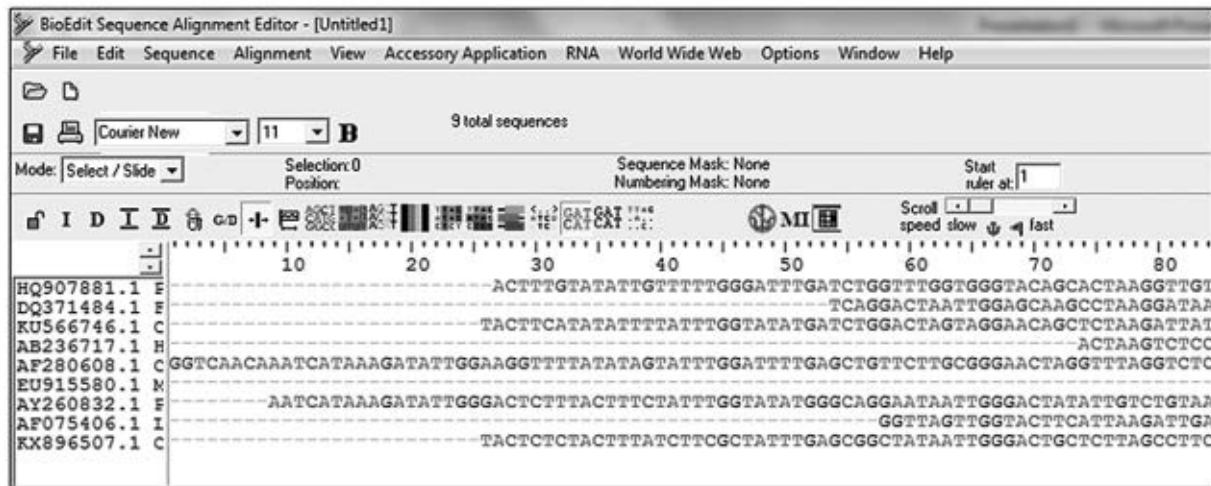
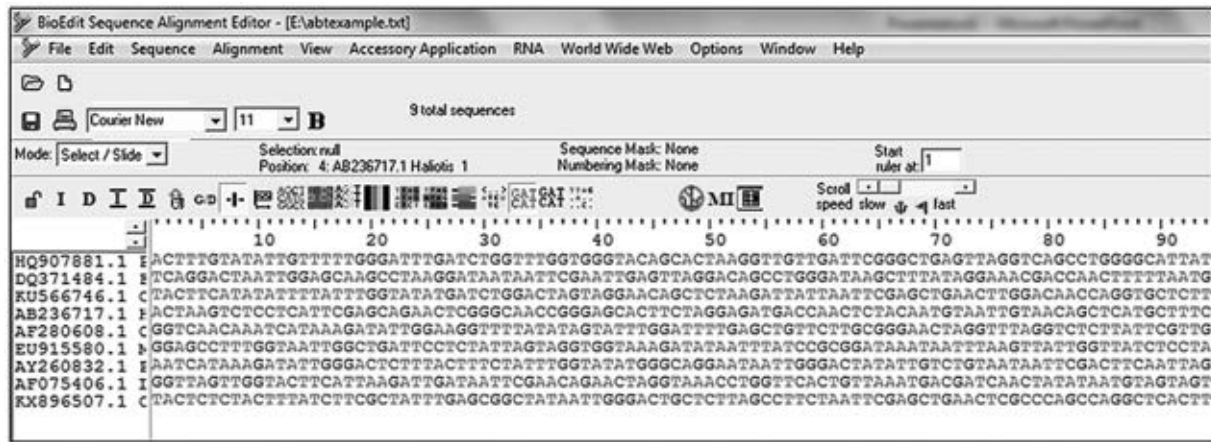


Figure 3. Compiled CO1 sequences in BioEdit before and after alignment (top and bottom panels, respectively).

was required to give a 15-minute presentation on the final day of the project (Day 4). Groups should also be prepared to meet outside class to prepare their presentations for Day 4. I have found it useful to arrange time slots to meet with groups to discuss any issues or problems they may have encountered during their final analyses.

Oral Presentation

If the instructor is not a trained zoologist or does not carry out research in phylogenetics, it may be helpful to students if a faculty member in this research area can be persuaded to sit in on the presentations. Evaluating student work at this point is completely up to the instructor, but for this zoology course, each group was required to present three deliverables in their oral presentations:

1. Morphological and molecular trees—Groups should present both trees along with the methodology used to construct them and the revisions that were made after instructor feedback and peer-review from their classmates.
2. Interpret and compare both trees—Groups should be prepared to discuss similarities and differences between their morphology and molecular trees. In addition, students should also be able to compare their own results against published studies, providing proper explanation for any differences observed.

3. Caveats identified during the study—This is perhaps the most critical aspect of the project since groups should be able to explain the limitations of their methodology that could have affected their results. For example, would altering one or two traits in their character matrix significantly alter the morphology tree? How different would the molecular tree be if a nuclear gene or multiple genes were used in the analysis instead of a single mitochondrial gene fragment? Here, the instructor may explain the difference between gene trees and species trees, and why that difference is important for interpreting molecular phylogenies.

Results & Discussion

Examples of Group A's morphology and molecular trees are shown in Figures 4 and 5. In the morphology tree, the shell-less condition of the Aplousobranchia is ancestral, and as a consequence, it is likely that the ancestor to all molluscs did not have a shell. Their tree also placed cephalopods and gastropods as sister taxa along with the bivalves and scaphopods, but interestingly, they did not identify a synapomorphic trait that would justify the two clades. The molecular tree complemented the morphology tree by recovering a distinct clade of gastropods and bivalves; however, the Cephalopoda was more closely related to the bivalves than the gastropods in the

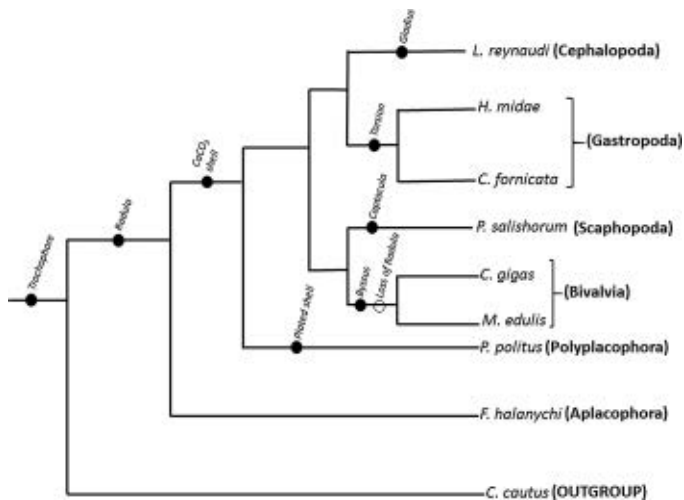


Figure 4. Example of Group A’s morphology tree constructed using the character matrix compiled in Table 1.

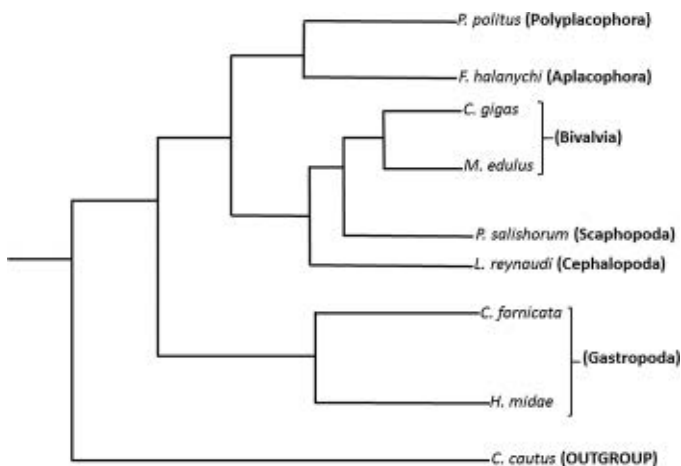


Figure 5. Example of Group A’s molecular tree constructed using the CO1 data from Table 1.

molecular tree. In addition, a third clade consisting of Polyplacophoran and Aplacophoran specimens was recovered using the molecular data. Group A concluded that their molecular tree was more accurate as it “conformed” more closely to the trees presented in the latest phylogenomic study on molluscs.

In terms of timing, the project was assigned after two days of lectures and labs on molluscan diversity. During the project, it is important to reinforce to students that each phylogenetic tree is a hypothesis because the dataset used to generate it is limited. This is obvious in the morphological part of the study, as students must manually draw different trees and choose the most parsimonious evolutionary scenario. In contrast, for the molecular part of the study, the tree-building algorithm built into MEGA automatically chooses the best possible tree. In this case, I often direct students to review the metadata generated during the heuristic search so they can observe the number of tree arrangements that have occurred before the program produces the “best possible” tree.

This project emphasizes the importance of using PBL to actively engage students in the scientific process by having them complete

the procedures that were used to generate the results and theories that they learn about during lecture (Kolb & Kolb, 2005). In addition, we incorporated certain aspects of Bioinformatics into the project, such as DNA sequence mining and editing, along with multiple alignments. This is crucial training for undergraduates at all levels, as the future of the Biosciences is strongly associated with the field of Bioinformatics (David, 2017). One caveat of this project is that it was designed for a zoology course in which phylogenetics played a central role in the course curriculum. As many professors know, zoology is arguably the broadest discipline of all the biological sciences and can be taught in a variety of styles, with some instructors opting for alternative approaches to the phylogenetic-based framework. Furthermore, for this project to be worthwhile to students, it is imperative that they have a firm understanding of phylogenetic theory, which can be accomplished by reinforcing basic concepts in the early lectures and assigning key phylogenetic papers during the semester. Papers that were assigned that I considered to be most critical to zoology include a landmark review piece by Halanych (2004), who addressed the influence of molecular data on the tree of life, along with papers by Struck et al. (2007) on Annelid evolution, Kocot et al. (2011) on molluscan evolution, and more recently, a controversial but informative commentary by Halanych (2015) and Whelan et al. (2015), both of whom argue that the basal position on the tree of life should be occupied by ctenophores, not sponges—a hypothesis that is still met with considerable backlash by some zoologists.

Aside from organismal-centered courses, this exercise is also appropriate for any upper-level biology course where phylogenetic trees play a central role, e.g., Systematics, Bioinformatics, Population Genetics, and Evolution. In addition, this project could also be given as a challenge to high-performing students in introductory biology courses, but will need to be repackaged and executed in a different manner than is outlined in this paper. I would suggest that at the freshman or sophomore level, instructors should edit and align the sequences so that students would only be required to execute the NJ analysis in MEGA. The instructor should also omit the dense background information on NJ and ML methods of building phylogenetic trees, and should provide a one-page info sheet with biological information (physiological and ecological) on the ingroup taxa along with pictures of each taxon. Although we used molluscan evolution as a case study, the instructor could in theory choose any group providing that (a) enough taxonomic information is available to clearly distinguish the in-group taxa morphologically, and (b) DNA sequences for a specific genetic marker are available for each taxon on public online repositories that students can access.

○ Acknowledgments

Fruitful discussions at the National Academy of Sciences’ (NAS) Summer Institute workshop in June 2016 were instrumental in the development of this project. The financial support of the Biology Department at Clarkson University was also extremely helpful in designing a zoology course consistent with the NSF’s *Vision and Change* manifesto. Finally, this paper is dedicated to Clarkson’s Biology undergraduates, whose critical feedback over the past two years has played the biggest role in changing my view of how zoology should be taught in the 21st century.

References

- Avise, J. C. (1989). Gene trees and organismal histories: A phylogenetic approach to population Biology. *Evolution*, 43, 1192–1208.
- Bell, S. (2010). Project-based learning for the 21st century: Skills for the future. *The Clearing House*, 83, 39–43.
- Blumenfeld, P. C., Soloway, E., Marx, R. W., Krajcik, J. S., Guzdial, M., & Palincsar, A. (1991). Motivating project-based learning: Sustaining the doing, supporting and learning. *Educational Psychologist*, 26, 369–398.
- David, A. A. (2017). A student-centered framework for teaching undergraduate Parasitology. *Trends in Parasitology*, 33, 420–423.
- Eddy, S. L., Crowe, A. J., Wenderoth, M. P., & Freeman S. (2013). How should we teach tree-thinking? An experimental test of two hypotheses. *Evolution Education and Outreach*, 6, 13.
- Hajibabaei, M., Singer, G. A., Herbert, P. D., & Hickey, D. A. (2007). DNA barcoding: How it complements taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics*, 23, 167–172.
- Halanych, K. M. (2015). The new view of animal phylogeny. *Annual Review of Ecology, Evolution and Systematics*, 35, 229–256.
- Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.
- Kocot, K. M., Cannon, J. T., Todt, C., Citarella, M. R., Kohn, A. B., Meyer, A., . . . Halanych, K. M. (2011). Phylogenomics reveals deep molluscan relationships. *Nature*, 477, 452–456.
- Kolb, A. Y., & Kolb, D. A. (2005). Learning styles and learning spaces: Enhancing experiential learning in higher education. *Academy of Management Learning and Education*, 4, 193–212.
- Lents, N. H., Cifuentes, O. E., & Carpi A. (2010). Teaching the process of molecular phylogeny and systematics: A multi-part inquiry-based exercise. *CBE—Life Sciences Education*, 9, 513–523.
- Maddison, W. P., & Maddison, D. R. (2003). Mesquite: A modular system for evolutionary analysis. Version 1.0. <http://mesquiteproject.org>
- Nei, M., & Kumar, S. (2000). *Molecular Evolution and Phylogenetics*. Oxford: Oxford University Press.
- Newman, L., Duffus, A. L., & Lee, C. (2016). Using the free program MEGA to build phylogenetic trees from molecular data. *American Biology Teacher*, 78, 608–612.
- Pleijel, F. (1995). On character coding for phylogeny reconstruction. *Cladistics*, 11: 309–315.
- Struck, T. H., Schult, N., Kusen, T., Hickman, E., Bleidorn, C., McHugh, D., & Halanych, K. M. (2007). Annelid phylogeny and the status of Sipuncula and Echiura. *BMC evolutionary Biology*, 7, 57.
- Swofford, D. L. (1993). PAUP*, phylogenetic analysis using parsimony, version 4.0b8. Illinois Natural History Survey, University of Illinois.
- Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences*, 101, 11030–11035.
- Tamura, K., Dudley, J., Nei, M., & Kumar, S. (2007). MEGA4: Molecular evolutionary genetics analysis (MEGA), software version 4.0. *Molecular Biology and Evolution*, 24, 1596–1599.
- Whelan, N. V., Kocot, K. M., & Halanych, K. M. (2015). Employing phylogenomics to resolve the relationships among cnidarians, ctenophores, sponges, placozoans, and bilaterans. *Integrative and Comparative Biology*, 55, 1084–1095.
- Yamane, D. (1996). Collaboration and its discontents: Steps toward overcoming barriers to successful group projects. *Teaching Sociology*, 24, 378–383.

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Using Environmental DNA to
Connect Lab Science with Field
Practice

RECOMMENDATION

JAMES MCNEIL, ANNEKE DELUYCKER,
SARAH PUTMAN

ABSTRACT

Experiential learning helps students make connections between different skill sets and allows them to engage in a deeper level of inquiry. To enhance the connection between field and laboratory practice for undergraduate students in our wildlife ecology curriculum, we developed an exercise using environmental DNA (eDNA) analysis. eDNA sampling involves extracting and amplifying the DNA from specific organisms from an environmental sample, rather than from the organisms themselves, and has been rapidly adopted by conservation practitioners around the world. In our activity, students collect water samples from a local pond and process them to detect the presence of American bullfrogs. Practicing this procedure not only introduces them to professional skills they may utilize in their careers, but also helps create context for how laboratory science and field work support each other and can be used to connect to larger issues of conservation, environmental studies, or ecology.

Key Words: eDNA; amphibians; biodiversity sampling; experiential learning.

○ Introduction

It is a common refrain in many publications about undergraduate biology education that hands-on and experiential class and laboratory exercises benefit student learning and lead to increased science literacy (Beck & Blummer, 2012; Brownell & Kloser, 2015). The most recent report from the AAAS *Vision and Change* program highlights the successes of incorporating case studies and realistic scenarios into undergraduate courses to provide a more authentic experience of a practicing professional scientist (AAAS, 2015). Additionally, the report calls on instructors to engage in activities that cross fields of inquiry, allowing students to experience the interdisciplinary nature of science.

*Environmental DNA
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Our programming in the Smithsonian-Mason Semester for Conservation Studies (<http://smconservation.gmu.edu>) is based on this tenet that the field of environmental conservation should be taught as it is practiced, namely, as an integration of many disciplines working together to solve urgent conservation issues. Additionally, we use extensive hands-on learning activities that give students the opportunity to practice professional skills with the tools they will be using in their careers.

We offer several programs of study, but one of the clearest examples of this mission comes from our wildlife ecology curriculum. This curriculum focuses on how ecological theory informs conservation and management practice, and as such there is a strong emphasis on methods of monitoring species or populations from different taxa using traditional field biology methods (e.g., visual encounter surveys, live trapping, camera trapping). Many of our students enter the program looking for experience with these field techniques, having come through undergraduate biology courses that focus mostly on theory or laboratory skills. Most have not made the connection between field and lab studies, and how the two aspects of scientific inquiry are not mutually exclusive and are often both necessary to fully describe a system or address an environmental issue. To explicitly illustrate the connection between the two approaches, we developed a unit on the use of molecular tools to monitor species in the wild, specifically the application of environmental DNA (eDNA) analysis for monitoring aquatic species.

Environmental DNA is a relatively new technique that has found widespread adoption among conservation professionals, even those who do not have a lot of experience with molecular techniques (Lodge et al., 2012). The technique involves extracting DNA from an environmental sample, usually water or soil, and identifying which organisms have been present in the medium by probing the isolated DNA with species-specific

primers. The technique has enjoyed rapid acceptance from practitioners, who find it more cost effective and accurate than traditional techniques for many cryptic or rare aquatic organisms.

○ Methodology

There are still questions, though, surrounding the interaction between environmental factors and detection, including the longevity of DNA in the environment and how small-scale habitat differences affect detection.

Our protocols for the activity are derived from one of the first published accounts of using eDNA for species monitoring, which used American bullfrogs (*Lithobates catesbeianus*) as a test animal (Ficetola et al., 2008). Bullfrogs are widely distributed, and their obligate association with water throughout their life cycle makes them ideal candidates for eDNA sampling since, if they are present and abundant, their DNA signature will be robust and more detectable in the water body. When we first began using this activity, our goal was merely to see if we could detect the presence or absence of bullfrogs within a local pond using eDNA procedures. In three years of investigation, though, we have observed that detection is lowest near where a stream flows into the pond and highest near an outflow from the pond (Figure 1).

The emergence of this pattern has given us an opportunity to frame the activity in the context of a long-term study to which students contribute data every semester and explore the relationship

between field and lab techniques and how they support each other. We specifically test the descriptive hypothesis that we will detect bullfrogs with eDNA sampling at locations near the pond outflow more readily than at the inflow. Our learning objectives for the activity are that students will be able to independently implement both traditional field sampling techniques for amphibians and eDNA sampling, describe the relative advantages and disadvantages of both types of sampling, analyze and troubleshoot molecular data and procedures, and justify the use of particular sampling techniques in specific circumstances based on the data the class collects and the larger, long-term data set.

The entire eDNA activity spans 2.5–3 days and begins with field exercises. We bring the students to the field site (Figure 1), and introduce and demonstrate traditional field sampling techniques for amphibians, including drift fences, minnow traps, and visual encounter surveys (Figure 2). We set up the drift fence in the field, demonstrating that the fence should be oriented parallel to the bank of the pond to intercept any animals attempting to enter the pond (Figure 2A). The day before the visit to the field site, faculty set up a minnow trap for demonstration purposes that is retrieved in the field (Figure 2B). Any captured organisms are observed in a pan with water from the pond before being returned to the water. The use of a net to sample aquatic vegetation is demonstrated as a method of visual encounter survey (Figure 2C).

Following these demonstrations by faculty, students are given the coordinates of an assigned sampling station (Figure 1), a GPS unit, a net, and a pan. They navigate to their station and conduct a visual encounter survey of the aquatic vegetation at that site by taking three samples with the net, and observing and recording the number and diversity of fauna they collect. We rarely encounter adult bullfrogs using these methods, but do capture many tadpoles and salamanders. Students do not share equipment between stations to reduce risk of spreading biological material, and we only visit one pond on the field trip. Additionally, all field equipment is rinsed with a 10 percent bleach solution before being stored to reduce the risk of spreading pathogens between sites.

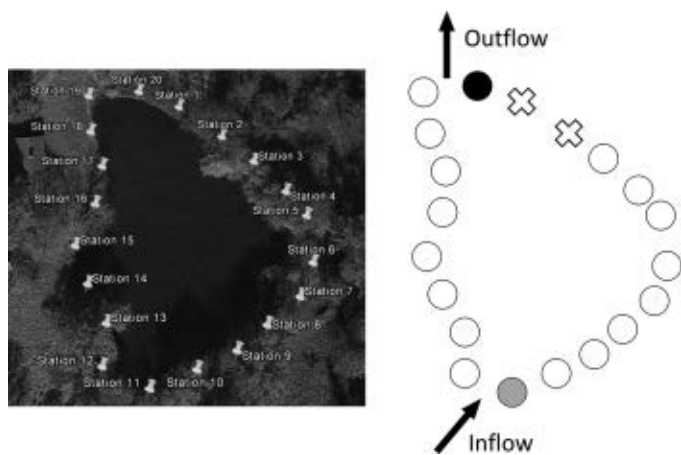


Figure 1. Sampling station locations at our local pond are depicted on the left. There are 20 stations arrayed around the border of the pond, each ~10 m from the next. The figure on the right is the output of a Hot Spot Analysis (Getis-Ord G_i^*) run in ArcMap (Environmental Systems Research Institute (ESRI), Release 10.2, Redlands, CA) on bullfrog detection data collected in four semesters from fall 2014, 2015, and 2016, and summer 2016. The black circle indicates a 90% confidence in a higher detection probability than random at that station; the gray circle indicates a 90% confidence in a lower detection probability than random at the station; and the white circles indicate no significant pattern in detection. The black arrows indicate water flow into and out of the pond. There was insufficient data available for stations 1 and 2 in the analysis (white Xs).



Figure 2. Examples of traditional field sampling techniques for amphibians. Drift fence (A) and minnow trap (B) deployment are demonstrated by faculty. Students conduct visual encounter surveys using samples collected via nets from aquatic vegetation (C).

The day after sampling the pond using traditional field methods, we return and collect water samples to process using eDNA techniques. Each student is given a GPS, a plastic graduated cylinder, a 50 mL centrifuge tube containing a solution of 1.5 mL 3M sodium acetate (ThermoFisher Scientific, #S210) and 20 mL of 100 percent ethanol (Pharmco-Aaper, Brookfield, CT; #E200), and a clipboard with a data sheet (Figure 3).

Students navigate to the same pre-determined station they used the day before and collect a 15 mL water sample that they add to the centrifuge tube. The sodium acetate helps protect the DNA from degrading; these samples are stable at room temperature for several hours or even days. On the data sheet, students note habitat features in the area near where they collected the sample. Each student has their own equipment to reduce the risk of mixing DNA between stations.

After collecting the samples, we return to the lab and extract the DNA from the water using the commercially available QIAGEN DNA mini kit (#51304, QIAGEN, Valencia, CA) used in many research laboratories. After mixing the samples by inverting the 50 mL centrifuge tubes, students pull out a 200 μ L subsample, which is then processed using the extraction procedure provided in the kit. Even though a very small volume of sample is used in the lab procedure, it is important to collect a large volume in the field because the eDNA signature is very dilute. Sample processing amounts to a series of initial reagents and incubations that extract the DNA and inactivate proteins in the sample, and then a series of spins and washes using a benchtop centrifuge to clean contaminants out of the sample and concentrate the DNA, which is stored on ice.

In addition to the chemicals provided in the kit, faculty must provide 100 percent ethanol, deionized water, 2 mL microcentrifuge tubes, micropipettors and tips, a centrifuge able to spin microcentrifuge tubes at 6,000 and 25,000 g (8,000–15,000 rpm), a vortex mixer, a digital dry bath incubator able to reach 56°C, a thermocycler, gel rig system, and an imaging system. Throughout these protocols, we highlight that our methodologies are derived from the primary literature (Ficetola et al., 2008), and we are using the same tools that researchers would use. Students must complete lab notes for the procedures they conduct, though we do provide them with the reagents and written instructions provided with the QIAGEN kit, much as if they were graduate students using this procedure for their thesis research (see Online Supplementary Material).

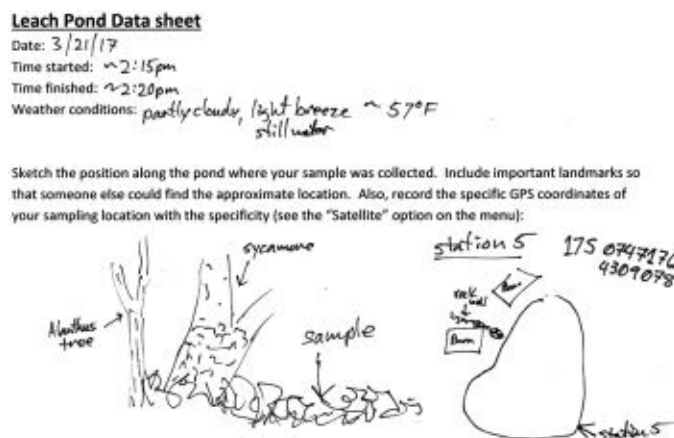


Figure 3. Example of field data sheet.

Once the students isolate the DNA, they amplify sections of DNA specific to our target organism using the polymerase chain reaction (PCR) with primers and protocols outlined in Ficetola et al. (2008). The PCR reaction is standardized to 50 μ L. We obtain Express Oligos primers (5'-GCCAACGGAGCATCATTC-3' and 5'-ATAAAGGTAG-GAGCCGTAGT-3') from Eurofins Genomics (Louisville, KY), without modifications, using salt-free purification and a 10 nmol scale. Each reaction contains 3 μ L of each primer, both with a final concentration of 1 μ M. We use 25 μ L of AmpliTaq Gold Master Mix (#4398876, ThermoFisher Scientific, Waltham, MA) in each reaction, as well as 2 μ L of 360 GC Enhancer (#4398876, ThermoFisher Scientific, Waltham, MA, USA) provided with the Master Mix. Students add 17 μ L of either DNA sample or deionized water to each reaction, but the Master Mix is the last reagent added to each reaction. Each student prepares four PCR reactions: one negative control without DNA (deionized water instead of sample), one positive control using a sample we know contains bullfrog DNA, and two replicates of their own sample. Immediately after adding the Master Mix, the samples are amplified using a BioRad C1000 Touch Thermal Cycler (Hercules, CA) thermocycler, using the temperature and time parameters described in Ficetola et al. (2008).

We emphasize at this point that we cannot predict the outcome of the PCR. Acknowledging the uncertainty here illustrates the real experience of doing research: these are real samples, so you can prepare and follow plans and protocols, but ultimately cannot control the data. After completing the PCR, we process the samples using gel electrophoresis (#3487–5000, DNA Plus gel system, USA Scientific, Ocala, FL; and #1645050, PowerPac Basic, BioRad) and determine which samples detected bullfrog DNA. We use a 2 percent agarose (#50090, NuSieve 3:1 Agarose, Lonza, Alpharetta, GA) gel with incorporated Gel Red fluorescent DNA stain (#41003, Biotium, Fremont, CA). The samples run on the gel for 1 hr at 95 V, and we visualize the DNA using a Enduro GDS gel documentation system (Labnet, Edison, NJ) UV light source with an integrated camera.

The activity concludes with a discussion of the results and how the class's data compares with that from previous semesters. We address our original hypothesis—eDNA is more frequently detected near the outflow vs. the inflow of the pond—and explore how the current class's data fits into this framework. Students are asked to pose fundamental hypotheses to explain why this pattern exists, such as the influence of flowing water on shaping the habitat in those areas, which could affect the presence of bullfrogs at particular stations. Bullfrogs are algal feeders as tadpoles, so flowing water may remove resources from the inlet and deposit them near the outflow, which could account for the differential detection. By examining whether bullfrog individuals were found at these sites using the traditional techniques, we can assess the likelihood of this scenario. Another alternative explanation is that frogs are present in the inflow, but the movement of water in the area removes the DNA signal, which could also account for the increased detection near the outflow as it accumulates there. On exams following this activity, we ask students to describe how they would test these hypotheses, using both field and lab techniques, to evaluate their understanding of the procedures and how they can be applied.

We also discuss any inconsistencies in the data, and as a class attempt to troubleshoot any unusual findings. Each class contributes to refining the protocol for the next group, just as professionals

would do in optimizing any protocol. For example, when we first began this activity, we unintentionally delayed putting the samples into the thermocycler after adding the Master Mix, and the samples incubated at room temperature for almost 20 minutes. As a result, our amplifications were a random assortment of different-sized pieces of DNA because of the unregulated amplification occurring outside of the conditions in the thermocycler. Although these results were not what we expected, this circumstance did provide an opportunity to walk through the logic of troubleshooting our protocol and results, and led to a refinement of our procedure, where the Master Mix is always added immediately before the samples are placed in the thermocycler.

Finally, we discuss in class the broader implications and applications of eDNA sampling. As mentioned previously, we rarely capture adult bullfrogs when doing our traditional sampling in the field, even in the summer and fall when they are prevalent, but always find tadpoles. However, green frog (*Lithobates clamitans*) and bullfrog tadpoles can be difficult, if not impossible, to differentiate in the field, and both species are present at our sample site. If green frog primers were to be included in this activity, this could show how eDNA is a simple way to assess whether one or both species are present at these sites, in the absence of a morphological marker for differentiating the tadpoles. We also discuss examples from the scientific literature where eDNA has been applied. For instance, Goldberg et al. (2014) used eDNA sampling to determine the distribution of American bullfrogs at Fort Huachuca, Arizona, where bullfrogs are considered an invasive species. eDNA sampling allowed those scientists to cover a wider area using fewer staff and less time than traditional methods of sampling. As a final evaluation, students must submit a short paper describing how they would apply eDNA to investigate a system or question of their own choosing. They must clearly state the question and hypothesis they would test as well as provide a rough outline of the methodology. They must also justify why a molecular technique like eDNA is more efficient than traditional sampling techniques in their chosen system. This assignment requires outside research on the part of the student to sufficiently describe how they would successfully apply the technique, and students are evaluated based on the thoroughness and specificity of their response.

○ Implications

The actual procedures for processing the samples use robust molecular techniques that are familiar to most biology majors, but the activity applies them in a new context to determine important information about the presence or distribution of species. For students who have a grounding in laboratory science, utilizing this technique helps them build on their experience and connect their findings to field practice as well. In post-course evaluations, students rate this activity very highly in terms of usefulness to their professional development: 4.5 out of 5 on a scale of helpfulness, where 4 is “much help” and 5 is “great help” ($n = 44$ students over four semesters). Many students express their excitement that the results of this activity are unknown, unlike other labs where the results are predetermined based on the sample you are given. Most importantly, students comment that before doing this activity, they did not realize how techniques learned in a laboratory setting (e.g., DNA extraction and PCR) could be applied to a field question.

Environmental DNA as a sampling tool is still being refined, so there are opportunities for students to be involved in the development of the technique in very real ways. Issues of efficiency of detection, the relationship between population size and eDNA signal detection, and how eDNA spreads in bodies of water are still being actively researched and could be incorporated into curricula at many levels. For example, students could also collect water quality and chemistry measurements when they collect their water samples, and determine whether there are correlations in detection with factors such as temperature, pH, or alkalinity. They could even experimentally manipulate these factors on collected samples back in the lab. They could also work on ways to optimize the sample collection and processing procedure for different species. Bullfrogs are well-studied in this regard, but there are many other aquatic and semi-aquatic amphibians that have not been thoroughly studied, but could benefit from using this type of tool, such as mole salamanders that are only present in water bodies for short periods of time in the spring when they are mating. If eDNA could be successfully applied to detect the presence of these species, it could help us understand their distributions better and possibly detect population declines sooner, in time to take preventative action.

Another benefit to students from this activity is the development of professional skills. Because the activity is structured using the real tools of a practicing molecular biologist, students are able to state on their resumes that they have experience with these particular kits and techniques. For students who have never worked in a laboratory setting, it helps them develop confidence that they need not be expert to be proficient at these skills, and molecular techniques can be integrated into studies in many different areas, such as species monitoring, landscape ecology, wildlife management, or even law enforcement.

Beyond the collection of the data, though, this activity also serves to demonstrate how scientific practice can move forward. By using published protocols and adapting them to our specific needs, students get experience with trouble-shooting standardized procedures in a new circumstance. Also, because our data are collected periodically in the same location over time, we are able to see long-term patterns developing. This emphasizes to students how biodiversity monitoring is an ongoing endeavor that requires persistence and time to detect patterns—a realization that will become important to them as they go into their careers. This activity provides a clear example of how applying different skill sets to problems in conservation can lead to more efficient practice, which ultimately could lead to better solutions for pressing conservation issues.

References

- American Association for the Advancement of Science (AAAS). (2015). *Vision and change in undergraduate biology education: Chronicling change, inspiring the future.*
- Beck, C. W., & Blumer, L. S. (2012). Inquiry-based ecology laboratory courses improve student confidence and scientific reasoning skills. *Ecosphere*, 3(12), 112.
- Brownell, S. E., & Kloser, M. J. (2015). Toward a conceptual framework for measuring the effectiveness of course-based undergraduate research experiences in undergraduate biology. *Studies in Higher Education*, 40(3), 525–544.

Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using environmental DNA from water samples. *Biology Letters*, 4(4), 423–425.

Goldberg, C. S., Strickler, K. M., & Fremier, A. K. (2014, February). Monitoring reptile and amphibian populations using environmental DNA, Year 1 Report. Technical Report for Legacy Project 12-616. U.S. Department of Defense. Retrieved from digitalibrary.utah.gov/awweb/awarchive?type=download&item=77403

Lodge, D. M., Turner, C. R., Jerde, C. L., Barnes, M. A., Chadderton, L., Egan, S. P., & Pfrender, M. E. (2012). Conservation in a cup of water:

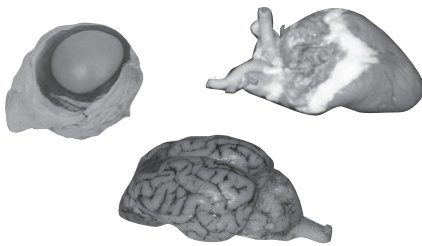
Estimating biodiversity and population abundance from environmental DNA. *Molecular Ecology*, 21(11), 2555–2558.

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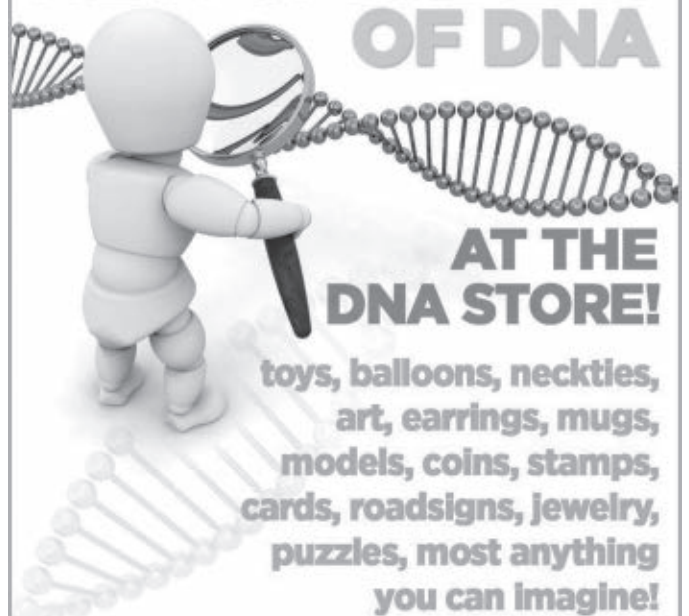
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Following Phenotypes: An Exploration of Mendelian Genetics Using *Arabidopsis* Plants

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ABSTRACT

Arabidopsis thaliana, a model system for plant research, serves as the ideal organism for teaching a variety of basic genetic concepts including inheritance, genetic variation, segregation, and dominant and recessive traits. Rapid advances in the field of genetics make understanding foundational concepts, such as Mendel's laws, ever more important to today's biology student. Coupling these concepts with hands-on learning experiences better engages students and deepens their understanding of the topic. In our article, we present a teaching module from the *Arabidopsis* Biological Resource Center as a tool to engage students in lab inquiry exploring Mendelian genetics. This includes a series of protocols and assignments that guide students through growing two generations of *Arabidopsis*, making detailed observations of mutant phenotypes, and determining the inheritance of specific traits, thus providing a hands-on component to help teach genetics at the middle and high school level.

Key Words: Mendel's laws; genetics; *Arabidopsis*; inheritance; phenotype; segregation.

○ Introduction

Topics related to genetics and genetic modification have been making regular appearances in the news for some time now. For today's students to develop the scientific literacy necessary to understand advances in this field, it is important to establish a solid understanding of basic genetic concepts. Presenting challenging content solely through lectures leaves many students with only a superficial understanding of the material (Kontra et al., 2015). By performing hands-on experiments designed to demonstrate foundational concepts such as Mendel's laws, student learning is elevated beyond what textbooks and lectures alone can accomplish (ACS, 2016; Wyatt & Ballard, 2007; Zheng, 2006).

Arabidopsis thaliana (*Arabidopsis*), the first plant to have its genome completely sequenced, has been transformed from being just a common weed to serving as a major model system for plant research worldwide (Somerville & Koornneef, 2002; Koornneef & Meinke, 2010). Its role expands beyond the laboratory to have

considerable utility in science education. *Arabidopsis* is a member of the mustard family (Brassicaceae) and a relative of Wisconsin Fast Plants, which may be familiar to some science educators. *Arabidopsis*, whose common name is mouse-ear cress, provides a launching point from which students can investigate a wide variety of scientific concepts, including adaptation to environmental conditions, how plants sense light, and the role of environment and genetics in growth and development (ABRC, 2016; Provart et al., 2016). With a short life cycle (6–8 weeks from seed to seed), self-fertility, and relatively low-maintenance growing requirements, this plant can be easily incorporated into even the most modestly equipped science classrooms (Ausubel, 2000; Pang & Meyerowitz, 1987; Zheng, 2006).

The *Arabidopsis* Biological Resource Center (ABRC) at The Ohio State University (OSU) is one of two global stock centers providing seeds, DNA, and other resources to scientists and educators worldwide. ABRC, which is home to more than 1,000,000 *Arabidopsis* stocks, provides samples to approximately 30,000 researchers in more than 50 countries annually. The Center launched its education and outreach program in 2011 by releasing 20 teaching modules, consisting of *Arabidopsis* seeds and/or DNA resources combined with lab instructions. The instructional materials have been made available through ABRC's education and outreach website (ABRC Outreach, <https://abrcoutreach.osu.edu/>), and the seeds and DNA can be ordered through The *Arabidopsis* Information Resource (TAIR, <https://www.arabidopsis.org/>). The program provides centralized access to *Arabidopsis* resources and teaching tools for K-12 and undergraduate education. Seeds are provided free of charge to K-12 teachers, along with in-depth lesson plans and supplemental materials that guide educators through the process of incorporating *Arabidopsis* into their science curriculum. The activities presented in this article are based on ABRC's "Play Mendel" module, with additional support for the procedures presented in this article available on the ABRC Outreach website. Seeds for this lesson can be ordered from ABRC through TAIR using the module's catalog number, CS19985.

○ Lesson Details

This article presents three activities that can be combined or used as stand-alone units to engage students in the process of growing, breeding, and caring for two generations of *Arabidopsis*, as well as making observations, maintaining a detailed lab notebook, collecting data, and analyzing results. By performing these activities, students gain insight into concepts such as genotype, phenotype, inheritance, and segregation of traits, which lie at the core of understanding Mendel's laws. The activities are designed for use with middle and high school classes and are aligned with the Next Generation Science Standards (NGSS), which are listed within each activity section. This module provides a structured laboratory experience, and the skills developed

through the procedures and assignments lay the foundation for future open-inquiry and student-driven experiments using *Arabidopsis*.

The full lesson (all three activities) spans four months. Together, these activities guide students through the process of growing *Arabidopsis* from the parent (P) generation (Activity 1), conducting phenotypic analysis of the segregation of a reproductive trait (Activity 2), and performing genetic crosses to obtain and analyze the phenotype of the first filial (F1) generation (Activity 3). Procedures and assignments are listed for each of the activities (Tables 1, 2, and 4). Students can be engaged in the entire process for a rich hands-on experience, or portions of the procedures can be performed by the teachers ahead of time to adapt to tighter schedules.

Table 1. Schedule of procedures and assignments for Activity 1.

Week	Activity	Estimated Time	Lab Learning Objective
1	Procedure 1: Plant P seeds Assignment 1: Observe growth	1 hour (prep) + 45 min (plant) 20 min	1, 2, 3
2–4	Water plants Assignment 1 continued	20 min twice a week 20 min per week	1, 2, 3
5	Water plants Assignment 1 continued Assignment 2: ID unique traits	20 min twice a week 20 min 1 hour	1, 2, 3 3, 4

Table 2. Schedule of assignments for Activity 2.

Week	Activity	Estimated Time	Lab Learning Objective
6	Assignment 1: Analyze inheritance	45 min	1, 2

Table 3. Data table for analysis of the inheritance of select mutations.

	Reference phenotype	Mutant phenotype	Ratio
Group 1			
Group 2			
Groups 1 & 2			

Table 4. Schedule of procedures and assignments for Activity 3.

Week	Activity	Estimated Time	Lab Learning Objective
6	Procedure 1: Perform genetic crosses	2 x 45 min	1, 2
7	Water plants Assignment 1: Observe cross outcome	20 min 20 min	1
8–9	Do not water plants Assignment 1 continued	20 min per week	1
10	Procedure 2: Collect F1 seeds	45 min	
11–12	No activity		
13	Procedure 3: Plant F1 seeds	45 min (prep & plant)	
14–15	Water plants	20 min twice a week	
16	Water plants Assignment 2: Compare phenotypes	20 min 45 min	3

○ Seed Strain Background

- Columbia (Col-1, CS28169)—This is the reference strain of *Arabidopsis*. The genome of the closely related Col-0 strain has been completely sequenced and is used as a basis for comparison with other natural strains. Col-1 is a laboratory strain that has been used to generate many mutants, including the *gl1-1* mutant used in this lesson. Col-1 is used as a reference strain for the *gl1-1* mutant in this lesson.
- *gl1-1* (CS28175)—This strain has a mutation in the *GLABROUS1* gene, which encodes a protein involved in trichome (leaf hair) formation. The Col-1 reference strain has trichomes on its stems and leaves. The homozygous *gl1-1* mutant is glabrous (hairless), with very few trichomes present.
- Landsberg erecta (Ler-0, CS20)—This laboratory strain, which is widely used to generate mutants, carries an X-ray induced mutation in the *ERECTA* gene, causing the plant to have a more upright growth habit. This is the parent strain for the *ag-1* mutant used in this lesson. Ler-0 is used as a reference strain for the *ag-1* mutant in this lesson.
- *ag-1* (CS25)—This plant has a mutation in the *AGAMOUS* gene, which encodes for a protein that controls the production of floral organs (sepals, petals, stamens, and carpels) during flower development. The term “agamous” means asexual, referring to the phenotype of the mutant plant, which is sterile. The stamens and carpels in *ag-1* mutants have been replaced by petals and sepals, producing a double flower (see Figure 2).

○ Lesson Preparation

Seeds can be ordered from ABRC through TAIR (www.arabidopsis.org), which requires individuals to register before placing an order. To streamline the registration process, it is recommended that first-time users contact ABRC directly to set up an ordering account with TAIR. To contact ABRC about setting up an account, e-mail abrccedu@osu.edu with the following information: full name, email address, school/institution name, job title (elementary/middle/high school teacher/other), phone number (for shipping), and shipping address. Seeds should be ordered at least two weeks before the planned start date of the first activity.

Arabidopsis grows best at 120–150 $\mu\text{mol}/\text{m}^2\text{s}$ continuous light and a temperature of 22–23° C (Rivero et al., 2014). Detailed protocols for growing and maintaining *Arabidopsis* plants can be found on the ABRC website (<https://abrc.osu.edu/seed-handling>). Please note that growth rates will vary when seeds are grown in different conditions. Plants may grow faster or slower than what is indicated in these protocols if the light intensity and duration, as well as the temperature, vary from what is recommended.

Supplemental materials for teachers are provided to facilitate easier implementation of this unit. Appendix 1 provides definitions of terms related to plant anatomy and Mendelian genetics. Appendix 2, designed to serve as a student hand-out, contains simplified laboratory protocols for all of the procedures.

○ Activity 1

Observation of Growth and Development of *Arabidopsis* Plants

The first activity in this lesson allows students to observe both vegetative and reproductive growth and development of *Arabidopsis* plants over a five-week period. Students will learn about plant anatomy while making detailed observations throughout this dynamic period of growth. Instructors can use this activity to discuss how the development of plant organs, tissues, and cells contribute to overall anatomy and to identify the different functions each perform in a plant organism. The schedule of procedures and assignments for this activity is listed in Table 1. This activity is aligned with the following Next Generation Science Standards (NGSS):

- MS-LS1-4, From molecules to organisms: Structures and processes
 - Disciplinary Core Idea (DCI) LS1.B, Growth and development of organisms
- MS-LS4-4, Biological evolution: Unity & diversity
 - DCI LS4.B, Natural selection

Lab Learning Objectives

1. Make detailed observations of the various growth stages in mutant and reference plants.
2. Define terms associated with the growing process as well as with the phenotypes of different *Arabidopsis* mutants.
3. Compare the phenotypes of mutant and reference strains of *Arabidopsis*.
4. Label the anatomy of a plant and identify the role of specific features in reproduction.

Materials

- 4 strains of *Arabidopsis* seeds (Catalog #: CS28169, CS28175, CS20, CS25)
- Potting soil
- 14-14-14 fertilizer (e.g., Osmocote)
- Teaspoon
- 64 plastic pots (Recommended: 1-quart round pots, 4.7”d × 4.75”h)
- 8 solid trays (Hummert, Item #11-3050-1)
- 8 trays with holes for sub-irrigation (Hummert, Item #11-3000-1)
- Cheesecloth (Fisher Scientific, Item #06-665-2513) or paper towels
- Weighing boats
- Disposable Pasteur pipettes
- Labeling tape and marker
- Plastic wrap
- Watering can
- Lab notebook
- Growth space with fluorescent lights (<http://abrcoutreach.osu.edu/growing-arabidopsis-classroom>)

Procedure 1: Plant the P Generation Seeds (Week 1)

In this procedure, students will plant two reference strains of *Arabidopsis*, a homozygous *gl1-1* mutant, and a segregating population (heterozygous for gamete formation) of the *ag-1* mutant. Divide the class into two groups, with each group completing the following planting procedure:

1. Place 1.0 cubic feet of potting soil in a bucket or other large container and wet thoroughly with water. The moisture content of the soil should resemble that of a wet sponge. Add 14-14-14 fertilizer to the soil according to the ratio provided on the product label and blend thoroughly.
2. Line 32 pots with a piece of cheesecloth or paper towel cut to fit the bottom of the pot such that soil will not escape through the drainage holes.
3. Place a tray with irrigation holes inside a solid tray. This set of two trays (one with holes, one without) will be referred to as a tray throughout the remaining procedures. Label four trays per group with the group number or name, and the name of the experiment (glabrous or agamous). Next, label eight pots with each of the seed stock names (Col-1, *gl1-1*, Ler-0, or *ag-1*).
4. Fill the pots loosely with the prepared soil, taking care not to limit aeration by compressing the soil.
5. Put a small amount of water in a weighing dish. Select the first seed stock to be planted and sprinkle a portion of the seeds into the water. Use a disposable pipette to mix the seeds by pipetting the water up and down slowly until the seeds are dispersed throughout the water. Use the pipette to draw in individual seeds and water. Dispense the seeds onto the surface of the soil, placing nine seeds evenly spaced in each pot. Continue until you have completed eight pots per seed stock. Do not cover the seeds with soil.
6. Repeat Step 5 until all four stocks have been planted (Figure 1). Use a new weighing dish and pipette for each seed stock to avoid cross-contamination.
7. Place four mutant pots and four of the corresponding reference strain in each tray. When complete, each group will have two trays with four pots each of Col-1, and *gl1-1*, and two trays with four pots each of Ler-0 and *ag-1* (Figure 1).
8. Cover pots tightly with plastic wrap. This will help maintain humidity until the seeds germinate. If possible, immediately after covering, place the trays in a dark refrigerator for 2–3 days to promote uniform germination. This process is known as stratification.
9. After planting (or after stratification), place the trays under the light source. Once the seeds have germinated and growth is seen (3–7 days after planting), remove the plastic wrap. Fill the bottom of the tray with ½ inch of water once or twice a week. It is important not to overwater the plants or let the soil dry out completely.

Observation of Genetic Traits

The short life cycle of *Arabidopsis* provides students with an opportunity to make detailed observations of the morphological changes

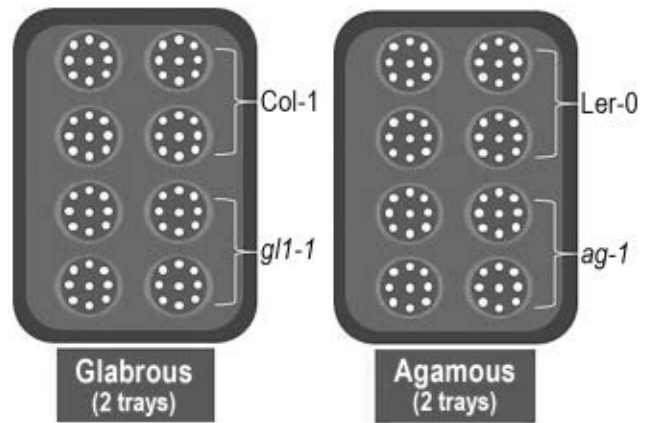


Figure 1. Illustration of seed spacing and how groups should organize pots in trays for the experiment. In the Glabrous experiment, the glabrous (hairless) *gl1-1* mutant (containing a loss-of-function mutation in the *GLABROUS* or *GL1* gene) will be compared to the Col-1 reference strain (containing a functional *GL1* gene). In the Agamous experiment, the agamous (asexual) *ag-1* mutant (containing a loss-of-function mutation in the *AGAMOUS* or *AG* gene) will be compared to the Ler-0 reference strain (containing a functional *AG* gene).

that occur in the plants throughout all stages of growth. To prepare for the following assignments, have students research the life cycle of *Arabidopsis* and become familiar with terms for basic plant structures and processes (see Appendix 1). There are a number of online resources where this information can be gathered including the ABRC Outreach website (<http://abrcoutreach.osu.edu/>) and the American Society of Plant Biologists K-12 education page (<https://aspb.org/education-outreach/k12-roots-and-shoots/>).

Assignment 1: Observe Growth (Weeks 1–5)

After planting is complete, have students record observations of the plants in their lab notebooks several times a week for the first five weeks.

Through this assignment students should:

- Make detailed drawings and notes about each of the four strains of *Arabidopsis*.
- Define growth stages of the plants based on the number of leaves, onset of flowering, silique maturation, and senescence.
- Define terms related to the growing process including stratification, germination, bolting, flowering, senescence (see Appendix 1).

Assignment 2: Identify Unique Traits (Week 5)

Have students compare all four strains of plants to identify the traits that differentiate each mutant from its corresponding reference strain. Images in Figure 2 show the distinguishing traits for each of the four seed strains.

Through this assignment students should:

- Understand the anatomy and function of plant organs.
- Describe the traits using illustrations and notes.
- Record the growth stage when the differences were first noticed.

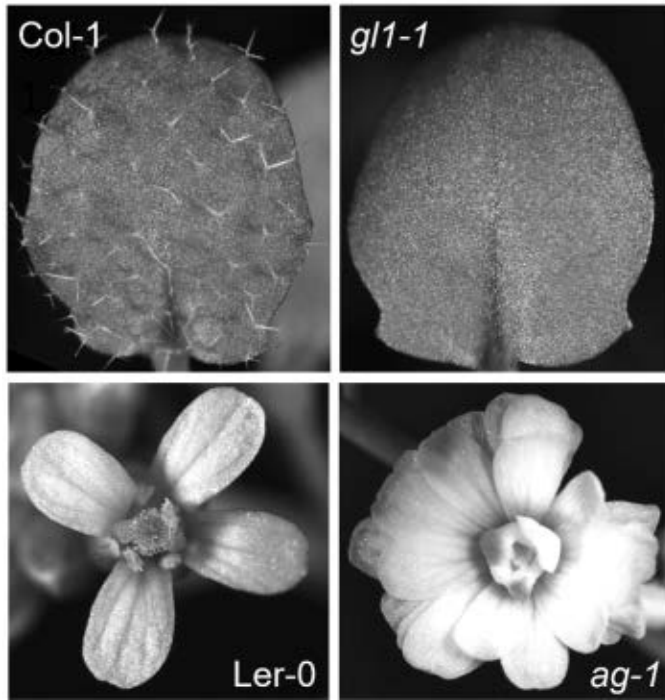


Figure 2. Different phenotypes associated with the reference (Col-1 and Ler-0) and mutant (*gl1-1* and *ag-1*) plants used in this unit.

- Compare the two different mutants with respect to growth stage when each plant's unique trait was first visible.

○ Activity 2

Phenotypic Analysis of the Segregation of the Agamous Trait

An investigation of the inheritance of a mutation in the *AGAMOUS* gene can be completed as a stand-alone activity within a single class session. This activity, which requires only six weeks of grow time, serves as a demonstration of Mendelian genetics. The schedule of assignments for Activity 2 is listed in Table 2. This activity is aligned with the following NGSS:

- MS-LS3-1 & MS-LS3-2, Heredity: Inheritance & variation of traits
 - DCI LS3.A, Inheritance of traits
 - DCI LS3.B, Variation of traits
- HS-LS3-3, Heredity: Inheritance & variation of traits
 - DCI LS3.B, Variation of traits

Lab Learning Objectives

1. Collect and analyze data to determine the inheritance of a specific trait in *Arabidopsis* mutants.
2. Define concepts central to Mendelian genetics (see Appendix 1).

Materials

- 2 trays containing 16 pots of 6-weeks old plants (*ag-1* and Ler-0, Figure 1) per group (plants obtained as described in Activity 1)
- Lab notebook

Assignment 1: Analyze Inheritance (Week 6)

In this assignment, students will analyze the inheritance of the *ag-1* mutation. Have students determine the number of plants displaying the reference strain flower phenotype and the mutant flower phenotype in the *ag-1* pots. Students should arrange their results in a table (Table 3) and conclude whether this mutation is dominant or recessive. A dominant mutation requires that only one copy of the mutant gene be present in order for the mutant phenotype to be apparent. However, a recessive mutation requires that both copies of the gene contain the mutation in order for the mutant phenotype to be displayed. This activity will demonstrate the importance of having a statistically significant sample size when analyzing data. Students will appreciate that the Mendelian ratio of 3:1 may not be observed with a random sample of small size, but that the ratio approaches 3:1 with a larger sample. The number of plants of the segregating *ag-1* mutant used in this experiment may not be sufficient for the collected data to accurately reflect the Mendelian ratio. In this case, teachers are advised either to use the data generated by previous classes or to reach out online for additional data from other teachers to aggregate with the data collected by their class. Teachers can use this experiment as an example to convey the importance of large sample sizes. Demonstrating the effect of a large sample size on the outcome of the experiment will also help reinforce student understanding and appreciation of Gregor Mendel's original discovery. Mendel counted and scored phenotypes for thousands of specimens to conclude the approximate 3:1 ratios.

Through this assignment students should:

- Calculate the ratio of reference to mutant flower phenotypes in each group.
- Predict what will happen with the ratio if the results from the two groups are combined.
- Calculate the ratio with the results from both groups combined, and explain how and why the ratio may have changed.
- Conclude whether the *ag-1* mutation is dominant or recessive (keeping in mind that a segregating population of the *ag-1* mutant was planted).
- Use a Punnett square to support the conclusion with evidence (Figure 3).

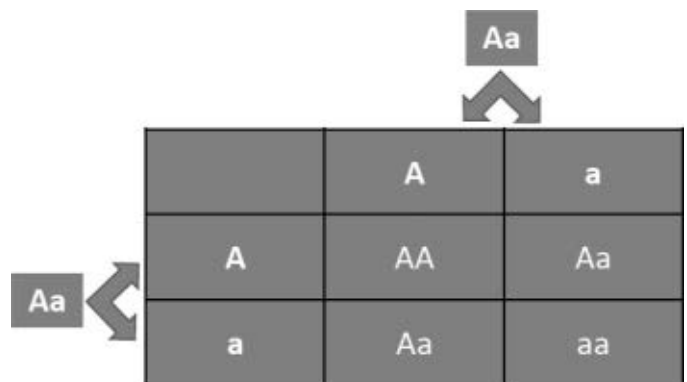


Figure 3. Punnett square demonstrating the possible outcomes of a genetic cross in which each parent possesses one each of the dominant (A) and recessive (a) gene variants (alleles).

At this point in the module, the group is done working with the Ler-0 and *ag-1* plants, and they can be discarded. Once emptied, pots and trays can be disinfected and reused for future procedures in Activity 3. To disinfect pots and trays, dilute ¼ cup of Lysol per one gallon of warm water. Allow the material to soak for 10 minutes. Use a sponge or scrub brush to remove any plant or soil material, then rinse and air dry.

○ Activity 3

Perform Genetic Crosses to Obtain F1 Heterozygotes for the Glabrous Trait

In this activity students will perform genetic crosses between a *gll-1* mutant and Col-1 reference strain, collect seeds and grow the F1 generation, and analyze the phenotype of the resulting F1 plants. The schedule of procedures and assignments for this activity is listed in Table 4. This activity is aligned with the following NGSS:

- MS-LS3-1 & MS-LS3-2, Heredity: Inheritance & variation of traits
 - DCI LS3.A, Inheritance of traits
 - DCI LS3.B, Variation of traits
- HS-LS3-3, Heredity: Inheritance & variation of traits
 - DCI LS3.B, Variation of traits.

Lab Learning Objectives

1. Generate a segregating population of plants by performing a genetic cross between mutant and reference plants.
2. Label the anatomy of a plant and identify the role of specific features in reproduction.
3. Compare the phenotypes of F1 plants to the mutant and reference strains of *Arabidopsis* to hypothesize whether the glabrous trait is dominant or recessive.

Materials

- 2 trays containing 16 pots of 6-week old plants (*gll-1* and Col-1, Figure 1) per group (plants obtained as described in Activity 1)
- Potting soil
- 14-14-14 fertilizer (e.g., Osmocote)
- Teaspoon
- 18 plastic pots (Recommended: 1-quart round pots, 4.7”d × 4.75”h)
- 3 solid trays (Hummert, Item #11-3050-1)
- 3 trays with holes for sub-irrigation (Hummert, Item #11-3000-1)
- Cheesecloth (Fisher Scientific, Item #06-665-2513) or paper towels
- Weighing boats
- Disposable Pasteur pipettes
- Labeling tape and marker
- Plastic wrap
- Scissors
- Headband magnifier (Lehle Seeds, Item #DA-10)
- Fine-tip tweezers (Lehle Seeds, Item #DV-30)

- Eppendorf tubes (Fisher Scientific, Item #05-408-138)
- Watering can
- Lab notebook
- Growth space with fluorescent lights (<http://abrcoutreach.osu.edu/growing-arabidopsis-classroom>)

Procedure 1: Perform Genetic Crosses (Week 6)

Arabidopsis is a self-pollinating plant. Once the flower has opened, pollination has already occurred. To avoid self-pollination and perform a successful cross, students will need to select buds with barely visible petals to become the female parent plant. Figure 4 demonstrates the various steps outlined in this procedure. This is a challenging procedure that requires patience. To help prepare students, have them view the Play Mendel Protocol Video available on the ABRC Outreach website (<http://abrcoutreach.osu.edu/educational-kits>). At this stage of growth, each *Arabidopsis* plant should contain multiple flower buds. This will allow students the freedom to practice preparing a flower for a cross with the understanding that mistakes will be made, while still providing enough flower buds for the completion of a successful cross. However, if students are demonstrating difficulty with this procedure or are unsure of flower anatomy, teachers may purchase cut flowers with obvious anatomy (such as lilies) from a grocer or flower shop to use as a practice specimen.

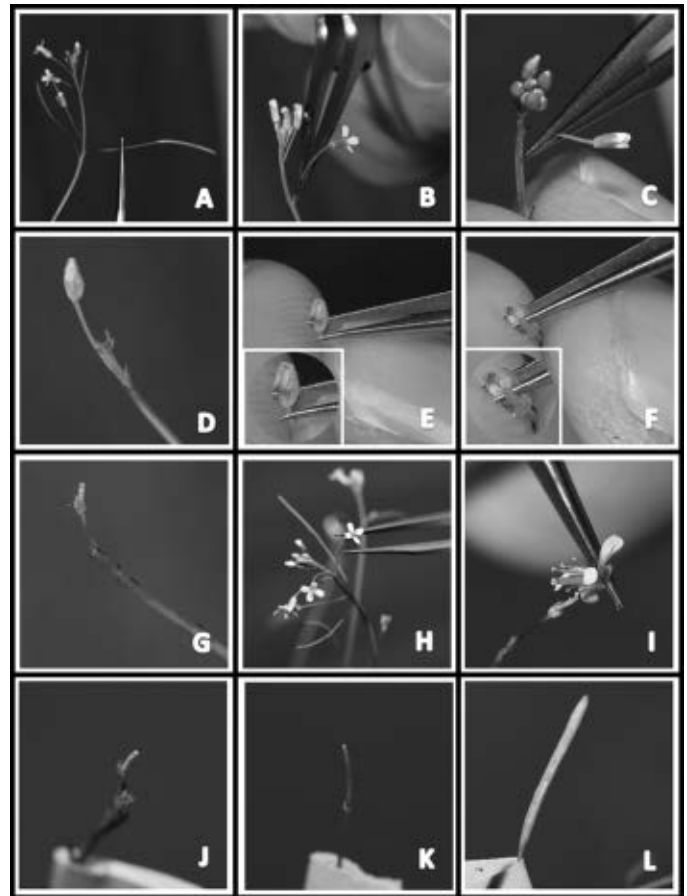


Figure 4. Steps involved in performing a cross between two *Arabidopsis* flowers. Steps A–L are explained in Procedure 1.

In this procedure, students will be performing two types of crosses. First, students will use pollen of a Col-1 reference strain plant to pollinate a *gl1-1* mutant (*gl1-1* x Col-1). Then students will use pollen from a *gl1-1* mutant to pollinate a Col-1 reference strain plant (Col-1 x *gl1-1*).

1. Have students select a *gl1-1* plant and locate an inflorescence branch containing at least two buds with barely visible petals. Students should use scissors or tweezers to remove any siliques and open flowers from the branch (Figure 4 A–D).
2. Using a headband magnifier and two pairs of fine-point tweezers, students should remove the sepals, petals, and stamens from two buds, being careful not to damage the carpel (Figure 4 E–G). If the carpel is damaged, remove the flower and proceed to the next bud.
3. Students should use tweezers to grasp a fully open flower from the Col-1 reference plant (male parent) at the peduncle level, squeezing the base to expose the anthers. Students may then pollinate the *gl1-1* female parent by brushing the anthers of the male parent over the emasculated carpel of the female flower (Figure 4 H–I). To increase the likelihood of success, each student should perform multiple crosses.
4. Students should use tape to label the cross and observe the branch for the formation of siliques (Figure 4 J–K). Let the siliques fully mature (Figure 4 L) before proceeding to Procedure 2. This process normally takes 2–3 weeks after pollination, during which time the students will have an opportunity to observe the development of siliques as part of the next assignment. A similar procedure should be performed for a reciprocal Col-1 x *gl1-1* cross, using Col-1 as a female and *gl1-1* as a male parent.

Assignment 1: Observe Cross Outcome (Weeks 7–9)

Have students observe the female parent plants and note the outcome of the crosses they performed. Approximately 1–2 days after the cross, the students should be able to tell the difference between a successful and unsuccessful cross. A successful cross will result in the formation of a silique (Figure 4 K), an unsuccessful cross will not. Have students sketch the female parent plant, noting the presence or absence of a silique in their illustration. Continue to water the plants for approximately one week after performing the crosses. Stop watering the plants and let the siliques dry out for at least two weeks, until they change from green to yellow-brown (Figure 4 L).

Through this assignment students should:

- Illustrate the results of a successful and unsuccessful cross.
- Calculate the percentage of successful crosses for the class.
- Discuss what occurs during the two weeks after pollination.
- Draw the *Arabidopsis* flower and label the structures listed below (see Appendix 1). For those structures that have a direct role in reproduction, have students define that role.
 - Stigma, stamen, carpel, inflorescence, petal, silique, sepal, peduncle, anther, pollen

Procedure 2: Collect F1 Generation Seeds (Week 10)

In this procedure, students will collect the seeds from the successful crosses and allow them to dry out to reduce the internal moisture content. This drying out process leads to improved seed germination.

1. Use scissors to carefully remove the dry siliques of successful *gl1-1* x Col-1 crosses (F1 generation) and place them in an Eppendorf tube, one silique per tube. Close tubes and label with the group number or name, plant number, generation (F1), and the type of cross.
2. Tap the tube several times to release the seeds from the siliques.
3. Repeat Steps 1–2 with the siliques of successful Col-1 x *gl1-1* crosses.
4. At this point in the module, the group is done working with the P generation plants and they can be discarded.
5. Allow the F1 seeds to dry for 2 weeks before planting.

Procedure 3: Plant the F1 Generation Seeds (Week 13)

In this procedure students will plant the F1 generation of the *gl1-1* x Col-1 and Col-1 x *gl1-1* crosses.

1. Following Steps 1–6 outlined in Procedure 1 of Activity 1, plant the seeds from the *gl1-1* x Col-1 and Col-1 x *gl1-1* crosses to produce one tray (eight pots) of each type of cross. Label the pots with your group number or name, plant number, generation (F1), and the type of cross.
2. In addition, plant one pot each of Col-1 and *gl1-1* seeds, placing 10–20 seeds in each pot. These plants will serve as controls for phenotypic observations.
3. Follow Steps 8 and 9 in Procedure 1 of Activity 1 for *Arabidopsis* growth and care.

Assignment 2: Compare Phenotypes (Week 16)

Have students observe and compare the phenotypes of the F1 and control plants. Through this assignment students should:

- Illustrate and describe the phenotypes of the F1 generation.
- Investigate if any phenotypic differences occur between the F1 plants coming from *gl1-1* x Col-1 crosses versus the Col-1 x *gl1-1* crosses. If differences are noted, have students document those in their illustrations and notes.
- Based on the comparison of the F1 plant phenotype to reference and mutant plants, have students conclude whether *gl1-1* mutation is dominant or recessive.

○ Conclusions

This unit demonstrates one of many ways that *Arabidopsis* can be utilized in a science curriculum to reinforce key concepts and engage students in hands-on learning. Although originally designed for instruction at the high school level, this module now aligns with many NGSS for other grade levels, which introduce scientific concepts such as genetic inheritance and variation of traits as early as middle school. The unit also offers teachers the ability to adjust the depth at which they cover the material, resulting in a flexible format. Therefore, this unit can easily be adapted to suit the needs of middle school classes, as well as advanced placement or other specialized high school biology classes, and college-level courses.

Additional experiments that utilize *Arabidopsis* to demonstrate a variety of other science concepts can be downloaded from the

ABRC's Outreach website (<https://abrcoutreach.osu.edu/>). As students become more comfortable with the scientific process, *Arabidopsis* represents a simple system with which they can design and conduct their own investigations (Wyatt & Ballard, 2007). Learning opportunities with *Arabidopsis* are plentiful, and educators are encouraged to fully integrate students in the inquiry process using this model system for plant research.

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Kontra, C., Lyons, D. J., Fischer, S. M., & Beilock, S. L. (2015). Physical experience enhances science learning. *Psychological Science*, 26(6), 737–749.

Koornneef, M., & Meinke, D. (2010). The development of *Arabidopsis* as a model plant. *The Plant Journal*, 61(6), 909–921.

Pang, P. P., & Meyerowitz, E. M. (1987). *Arabidopsis thaliana*: A model system for plant molecular biology. *Nature Biotechnology*, 5(11), 1177–1181.

Provart, N. J., Alonso, J., Assmann, S. M., Bergmann, D., Brady, S. M., Brkljacic, J., . . . McCourt, P. (2016). 50 years of *Arabidopsis* research: Highlights and future directions. *New Phytologist*, 209(3), 921–944.

Rivero, L., Scholl, R., Holomuzki N., Crist, D., Grotewold, E., & Brkljacic, J. (2014). Handling *Arabidopsis* plants: Growth, preservation of seeds, transformation, and genetic crosses. *Methods in Molecular Biology*, 1062, 3–25.

Somerville, C., & Koornneef, M. (2002). A fortunate choice: The history of *Arabidopsis* as a model plant. *Nature Reviews Genetics*, 3(11), 883–889.

Wyatt, S., & Ballard, H. E. (2007). *Arabidopsis* ecotypes: A model for course projects in organismal plant biology and evolution. *American Biology Teacher*, 69(8), 477–481.

Zheng, Z. L. (2006). Use of the *gl1* mutant and the *CA-rop2* transgenic plants of *Arabidopsis thaliana* in the biology lecture course. *American Biology Teacher*, 68(9), 148–153.

References

American Chemistry Society (ACS). (2016). Importance of Hands-on Laboratory Science: ACS Position Statement. Available at <https://www.acs.org/content/acs/en/policy/publicpolicies/invest/computersimulations.html>

Arabidopsis Biological Resource Center (ABRC). (2016). Education and Outreach. Available at <https://abrcoutreach.osu.edu/>

Ausubel, F. M. (2000). *Arabidopsis* genome: A milestone in plant biology. *Plant Physiology*, 124, 1451–1454.

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Appendix 1. Key terms and definitions.

General Terms	
<i>Reference strain</i>	Strain used as the phenotypic benchmark against which mutant phenotypes are compared.
<i>Mutant</i>	Strain containing a mutation (a change in the DNA sequence) that is not present in the reference strain.
<i>Genotype</i>	Genetic makeup of an organism that can refer to the specific gene. Examples of different genetic makeups: TT, Tt, tt represent different variants of that gene (alleles).
<i>Phenotype</i>	Physical appearance of an organism for a given trait. Results from the interaction of the genotype with the environment.
Terms related to Mendelian genetics	
<i>Inheritance</i>	The process in which genotypes are passed down from one generation to the next
<i>Segregation</i>	Separation of pairs of gene variants into reproductive cells
<i>Genetic variation</i>	Genetic differences found in nature among different individuals of the same species

Terms related to plant anatomy

<i>Inflorescence</i>	A cluster of flowers including the branches of the stem
<i>Peduncle</i>	A branch supporting an inflorescence
<i>Stamen</i>	Male reproductive organ that includes the anther
<i>Anther</i>	The pollen-producing, oval-shaped portion of the stamen
<i>Pollen</i>	A carrier of the male reproductive cells. Has an appearance of powder or dust.
<i>Carpel</i>	Female reproductive organ that includes the stigma
<i>Stigma</i>	The bulb-shaped portion of the carpel, where pollen lands and pollinates the plant
<i>Petal</i>	Modified leaves that often function to attract pollinators
<i>Silique</i>	Seed pod of the plant
Terms related to processes	
<i>Senescence</i>	Final developmental stage of the plant; the aging process that leads to death
<i>Stratification</i>	Cold treatment of seeds that mimics "winter." Seeds taken out of cold treatment have higher rates of uniform germination, a response to "spring."

Appendix 2. Play Mendel—Handout for students

In this exercise you will plant and observe growth of *Arabidopsis thaliana* (*Arabidopsis*), a small plant used as a research model. The whole genome sequence of this plant is known, enabling scientists to understand the relationship of a gene sequence (genotype) and the resulting appearance of this plant (phenotype). You will compare the phenotypes of two different mutants (having a change in the sequence of a mutated gene) to a reference plant with a normal (common, wild type) phenotype. The first mutant, named *gl1-1*, has a mutation in the *GL1* (*GLABROUS1*) gene, resulting in the hairless leaf trait. The respective Col-1 reference plant has leaf hairs. The second mutant, named *ag-1*, has a mutation in the *AG* (*AGAMOUS*) gene, resulting in a double flower (multiple whorls of sepals and petals) trait. The respective Ler-0 reference plant has a flower with just one whorl each of sepals and petals. You will have an opportunity to follow these specific traits as they segregate, and make conclusions about their inheritance based on the analysis of the phenotypes in the progeny resulting from crossing a mutant with a reference plant. This will help you understand the principles of Mendelian genetics, which are the same for all organisms with sexual reproduction, including humans.

Activity 1. Observation of Growth and Development of *Arabidopsis* Plants

Procedure 1: Plant the Parent (P) Generation Seeds

In this procedure, you will work as part of a group. Each group should complete the same procedure.

1. Place potting soil in a bucket and add water and fertilizer. Use gloves when handling fertilizer and fertilized soil.
2. Line the bottom of 32 pots with a piece of cheesecloth or paper towel.
3. Prepare 4 sets of two trays by placing a tray with holes into the one without. Label the four outside trays with your group number or name. Label two trays "glabrous" and two trays "agamous". Label eight pots with each of the seed stock names (Col-1, *gl1-1*, Ler-0, and *ag-1*).
4. Fill the pots with soil.
5. Four students should each select one of the seed stocks and fill a weighing dish with water. For each stock, sprinkle approximately 100 seeds into the water. Use a disposable pipette to mix the seeds by pipetting up and down. Dispense nine seeds on top of the soil of each pot (see Figure 1). Continue until you have completed eight pots per seed stock. Do not cover the seeds with soil.
6. Place four *gl1-1* mutant pots and four Col-1 pots in each of the two "glabrous" trays. Place four *ag-1* mutant pots and four Ler-0 pots in each of the two "agamous" trays (see Figure 1).
7. Cover trays with plastic wrap and place them in a dark refrigerator for 2–3 days.

8. After 2–3 days, take the trays out of refrigerator and place them under the light source. Remove the plastic wrap after seeds have germinated and the seedlings look green. Fill the bottom of the tray with ½ inch of water once or twice a week. Continue watering the plants while proceeding with the assignments, as instructed by your teacher.

Activity 3: Perform Genetic Crosses to Obtain F1 Heterozygotes for the Glabrous Trait

Procedure 1: Perform Genetic Crosses

After approximately six weeks of growth, the plants should be ready for crosses. Performing crosses on small flowers of a plant such as *Arabidopsis* requires patience and practice. Your teacher will show you a video and give you other detailed instructions to help you master this technique. Don't be discouraged if you are unsuccessful at the beginning – there are plenty of flowers to practice on and you will get better!

1. You will start by preparing the *gl1-1* flower for crossing. Select an inflorescence branch containing at least two buds with barely visible petals. Remove open flowers and any siliques from the branch.
2. To expose the carpel (female reproductive organ) of the *gl1-1* plant, remove the sepals, petals and stamens from two buds using a headband magnifier and two pairs of fine point tweezers. If you damage the carpel, remove the flower and proceed to the next bud.
3. To prepare the pollen of the Col-1 plant, select a fully open flower and use tweezers to squeeze the flower at the base to expose the anthers (male reproductive organs that carry pollen). Pollinate the prepared carpel of the *gl1-1* plant by brushing the anthers of the Col-1 plant over it.
4. Use tape to label the cross and observe the branch for the formation of siliques (seed pods). This is part of the assignment that you should complete as instructed by your teacher.
5. Perform the same procedure (Procedure 1, steps 1–4) for a reciprocal Col-1 x *gl1-1* cross, using Col-1 as a female and *gl1-1* as a male parent. Continue watering the plants for 2–3 weeks until siliques mature.

Procedure 2: Collect F1 Generation Seeds

After 2–3 weeks of silique growth and maturation, the seeds are ready to be collected.

1. Use scissors to remove and carefully place the dry siliques of successful *gl1-1* x Col-1 crosses (F1 generation) in an Eppendorf tube, one silique per tube. Close tubes and label with the group number or name, plant number, generation (F1) and the type of cross.
2. Tap the tube several times to release the seeds from the siliques.
3. Repeat steps 1–2 with the siliques of successful Col-1 x *gl1-1* crosses.
4. Keep the closed tubes in a dry place for two weeks to let the seeds dry out.

Procedure 3: Plant the F1 Generation Seeds

After two weeks of drying, the F1 seeds of both types of crosses are ready for planting.

1. Plant the seeds from the *gl1-1* x Col-1 and Col-1 x *gl1-1* crosses to produce one tray (eight pots) of each type of cross. Label the pots with your group number or name, generation (F1) and the type of cross.
2. In addition, plant one pot each of Col-1 and *gl1-1* seeds, placing 10-20 seeds in each pot. These plants will serve as controls for phenotypic observations.
3. Continue watering plants as described in Procedure 1 of Activity 1. After three weeks of growth, your teacher will provide the instructions related to the assignment at the end of this activity.

Using Peer Review to Improve Lab Report Assignments

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ABSTRACT

Introductory science students participate in peer review as a component of their final lab report assignment. The peer review activity is conducted during lab time at least two weeks before the final report is due. This activity is designed to increase student understanding of science as a process that includes peer review as well as the lab activity, and to provide feedback before the final assignment is submitted for grading. It can be used for any science laboratory course with large lab report assignments.

Key Words: pedagogy; STEM Education; critical thinking; feedback; assessment; term paper.

○ Introduction

Inquiry-based writing has been shown to increase students' scientific knowledge (Walker & Sampson, 2013). One ubiquitous example of inquiry-based writing is the lab report assignment, whose purpose is to learn and practice research article writing and demonstrate that learning to their lab instructors (Parkinson, 2017). The learning goals of lab reports are to encourage deeper understanding of science as a process and to demonstrate how to think in a scientific way, as opposed to considering science as lists of memorized facts and findings (Deiner et al., 2012). Lerner (2007) and Russell (2002) described the original purpose of lab reports as to prepare students to act, think, and write like real scientists. Toward this purpose, lab reports target development of scientific writing skills as well as scientific inquiry (Harris, 2009). Lab reports mimic the process of scientific inquiry and are formatted as scientific publications, though assessment is by instructors rather than peers. In the context of assignments, "peer review is understood to mean the educational arrangement in which students consider or evaluate the value, quality or success of work produced by their fellow students and provide each other with feedback" (Pearce et al., 2009, p. 3). Peer review has an important role in improving writing,

reading, and collaborating. It also helps lower or prevent writing errors, ensure accurate reporting, and improve grades (Colthorpe et al., 2014). In addition to improving individual grades, peer review also contributes to improving critical thinking and self-assessment skills, collaboration and communication skills, as well as increasing motivation, self-confidence, and independence (Table 1).

In 2013, a master's student interviewed environmental science and biology undergraduate students and lab instructors at George Mason University regarding lab report assignments (Kalaskas, 2013). His research identified some concerns:

1. Students may lack the epistemological background to understand lab reports as a process of doing science.
2. Students believe that the lab report is a schooling or academic genre that is assigned exclusively in science lab courses. This is opposed to the view that lab reports are an apprenticeship type assignment designed to teach students how to do science work.
3. Students suggested instructors make too many assumptions about what students know regarding lab report writing and most suggested that instructors intentionally distribute vague instructions.
4. Instructors report a lack of attention to detail is the reason students do poorly, which implies a lack of revision and editing.

In the past we found that few students asked questions during lab or attended faculty office hours. However, our course has participated in a peer mentoring program where undergraduate Learning Assistants (LAs) (see <https://laprogram.colorado.edu>) were available during lab time and held additional study sessions. These peer-led activities had higher participation than other available study aids. Given the LA program's popularity, which employs peer mentoring, and the findings of the 2013 survey, a peer review component was developed to improve student understanding of both the assignment and how peer review is used in the process of doing science. The purpose of

Table 1. Reasons for incorporating peer review for students to write and revise their laboratory report (modified from bulleted list in Pearce et al., 2009, p. 4).

Critical Thinking and Self-Assessment Skills	Motivation, Self-Confidence, and Independence	Collaboration and Communication Skills
<ul style="list-style-type: none"> • Building problem solving skills through identifying areas needing improvement and providing constructive suggestions (Dochy et al., 1999; Somervell, 1993) • Encouraging reflection and thereby promoting skills in self-assessment (Liu & Carless, 2006) • Engaging students actively in critical thinking, in applying criteria, in reflection, and through this, in learning transfer (Nicol et al., 2014) • Providing valuable experience and preparation for the professional workplace (Brindley & Scoffield, 1998; Biggs & Tang, 2007) • Enabling students not only to develop important high-order (generic) skills such as critical evaluation and communication, but also to develop important skills of self-assessment—arguably one of the most important goals of a higher education (Mulder et al., 2014b) • Including the ability to engage with and take ownership of evaluation criteria, to make informed judgements about the quality of the work of others, to formulate and articulate these judgments in written form, and, fundamentally, the ability to evaluate and improve one's own work based on these processes (Nicol et al., 2014) 	<ul style="list-style-type: none"> • Enhancing greater meta-cognitive self-awareness (Topping, 1998; Liu & Carless, 2006) • Increasing student motivation by fostering a sense of responsibility and ownership for their peers' learning (Dochy et al., 1999; Cheng & Warren, 1997) • Increasing interactivity self-confidence (Brindley & Scoffield, 1998) and empathy for others (Topping, 1998) • Engaging in peer review has a positive effect, not only on students' perceptions of peer learning and the value of peer feedback, but also on their academic outcomes (Mulder et al., 2014b) • Making significant improvements in the students' ability to write in science and to evaluate the quality of their peers' writing with a relatively high degree of accuracy (Walker & Sampson, 2013) • Promoting independent learning and reducing dependence on staff as "the experts" (Brindley & Scoffield, 1998; Dochy et al., 1999) 	<ul style="list-style-type: none"> • Sensitizing students to the different ways in which readers might interpret what they have written (Nichol et al., 2014) • Improving social and communication skills such as verbal or written communication, negotiation skills, diplomacy, and giving and accepting criticism (Topping et al., 2000) • Enhancing relationships in the group (Cheng & Warren, 1997) • Developing a collaborative and participatory learning environment (Fallows & Chandramohan, 2001) • Presenting a comparative process wherein students evaluate each peer assignment against an internal representation of their own work, where they use the feedback they generate for others to update their thinking about their own assignment (Nichol et al., 2014) • Alerting students to deficiencies or gaps in their work (Nichol et al., 2014)

peer-review is to provide students with (1) an incentive to scrutinize, evaluate, and understand the grading rubric, (2) an opportunity to learn from seeing examples of different lab reports, and (3) an opportunity for external review and time for revision. In this manuscript, we show our essential evaluation outcomes and process for using peer review in an environmental science course laboratory component. We use this information to provide readers with guidance for incorporating peer review into their laboratory assignments and courses.

○ How to Do It

Peer review is incorporated into an existing lab report assignment. We recommend the review be done in-class to avoid plagiarizing and to ensure the instructor is available for clarification.

○ Format

The day students are introduced to the lab report assignment, they are also introduced to the concept of peer review and provided with grading rubrics. Two weeks after completing the lab activity, students are required to bring two copies of their lab reports for in-class peer review. The instructor collects the lab reports and

gives two different reports to each student to grade using the grading rubric. So each student has received two reviews from different classmates. The rubric is intentionally detailed to help students identify the essential components of an outstanding report, with the idea that the review process builds each student reviewer's own writing ability. After receiving input and grades from their peers, students had one to two weeks to incorporate revisions before turning in a final copy to instructors.

Students had one hour to complete reviews, about 30 minutes per report. The entire assignment lasts about 90 minutes, including instruction, collection, and distribution of reports. This activity is done at the beginning of a lab period that normally does not take the full 2 hours 50 minutes. Assigning this task at the beginning of lab before the regular lab assignments reduces incentive to rush the work and leave early.

○ Instruction and Rubrics

Clear and detailed grading rubrics are necessary to avoid discrepancies between instructors and peer grading. We provide an outline of rubric components (Table 2); a copy of our rubric is available in the Online Supplemental Material_Rubric or can be downloaded

Table 2. Rubric components.

Section	Components / Subsections
Format	Complete, logical, and correct format of sections and labels
Introduction	Concise background, research questions, objectives, hypothesis
Methods	Thorough explanation and justification
Results	Appropriate summary with tables/figures
Discussion	Interprets results in context of hypothesis and background, draws conclusions, addresses uncertainty, and poses new questions

at goo.gl/bMxZf0, though some details are specific to the project in this course and will need to be modified for other lab projects. We strongly suggest that each component be given a description and assigned points based on course learning objectives. For each section and component, we specified the content and format required for assignment of full, partial, or no credit.

○ Grading

Grades received from peers did not count toward the final lab report grade. Peer grades should provide a sense of the final grade assessed with the grading rubric. The graded part of this assignment contributed to participation, based on three components described in the assignment as follows:

Full credit for the peer-review process will be given for a student who (1) is present on the day of peer review, (2) brings two hard copies of their formal lab report, and (3) reviews and grades (using the grading rubric) the formal lab reports of two peers in a thoughtful, thorough, conscientious manner.

Grading the peer reviews requires additional guidelines. Thoughtful, thorough, and conscientious reviews will provide explanation for scores given via the grading rubric. Tools such as Calibrated Peer Review (Robinson, 2001) also exist to assist with honing reviewing skills and establishing expectations for reviewers.

○ Student Responses

Two years after the initial student interviews, peer review was introduced during the Spring 2015 semester into 21 labs taught by eight lab instructors (graduate teaching assistants, GTA) with 337 undergraduate students enrolled. During the last week of the semester, an anonymous link was emailed via SurveyMonkey® to students and instructors, inviting them to complete a questionnaire. The purpose of the questionnaire was to evaluate students' and teachers' opinions regarding peer review. All eight instructors and 298 students (88.4% response rate) participated in the questionnaire (Online Supplemental Material_Questionnaire). Survey responses were anonymized, and students received extra credit points for participating. Most survey questions used 5-point Likert scale with responses ranging from "strongly agree" to "strongly disagree" or provided multiple selections with the option to select more than one response. Open-ended questions were grouped using thematic coding.

The two courses were considered part of a year-long curriculum, though there is no required order, and 64 percent of respondents reported having already taken one of the courses. Of the 298 respondents, 24 percent had previously completed a lab report, and 49 percent had previously participated in peer review as a class assignment. Overall, most students (60%; $n = 226$) rated the peer review process as a helpful aspect of lab report assignments, and 66 percent ($n = 230$) agreed or strongly agreed that the peer review component helped them understand the lab report assignment. Almost all instructors (88%; $n = 7$) agreed or strongly agreed that the peer review component helped improve students' understanding of the lab report assignment. Also, well over half of the instructors (75%; $n = 6$) agreed or strongly agreed that the peer review component helped students earn higher grades on the lab report assignment. Overall, we feel that this activity has improved both student learning experiences and grades. The peer review component will continue to be a part of the lab report in all our undergraduate environmental science courses. Responses varied regarding quality of the revision process, clarity of instructions, and time spent on the peer revision process. The quality of the revision process was a concern for 25 percent of the respondents, who stated the reviewers were inconsistent or did not provide adequate commentary. Approximately 15 percent (45) of the students and two GTAs thought more time should be spent on the peer revision component.

○ Reflection

Based on responses, the two major concerns are allocating sufficient time and the quality of review. Most students believe that their peers did not have enough knowledge to review and revise the lab reports. One way this issue can be resolved is by students practicing review with their instructors. This practice could include demonstration by the instructor and providing students with the same document for review, followed by discussion or presentation of review points by the instructor. This would not only provide the students with more knowledge about the review process, but it will also give them the confidence to correctly revise other's work. Assessing the actual feedback given by reviewers could also be critical for high quality reviewing (Ruegg, 2014), and other studies reveal concerns about review quality and equity in participating in peer review (Mulder et al., 2014a; Cheng et al., 2015). To address both time management and quality of review, we suggest more time for reviewing, explaining, and demonstrating peer review. More time could involve more in-class time or introducing the activity earlier in the semester to give students more time to revise their reports. Using a piecemeal strategy

could also address both time management and instructor involvement. In this strategy, the lab report assignment is split into sections, with each section completed and submitted on different dates. After submitting each section, the instructor returns it to students with comments and feedback. This provides students with input in an iterative process of addressing comments and revising before turning in the final report at the end of the semester. This strategy also makes the learning process less stressful for the students since not everything will be due at one time (Kalaskas, 2013).

○ Acknowledgments

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References

- Biggs, J., & Tang, C. (2007). *Teaching for quality learning at university* (3rd ed.). Berkshire, UK: Open University Press.
- Brindley, C., & Scofield, S. (1998). Peer assessment in undergraduate programmes. *Teaching in Higher Education*, 3(1), 79–87.
- Cheng, W., & Warren, M. (1997). Having second thoughts: Student perceptions before and after a peer assessment exercise. *Studies in Higher Education*, 22(2), 223–239.
- Cheng, K. H., Liang, J. C., & Tsai, C. C. (2015). Examining the role of feedback messages in undergraduate students' writing performance during an online peer assessment activity. *The Internet and Higher Education*, 25, 78–84.
- Colthorpe, K., Chen, X., & Zimbardi, K. (2014). Peer feedback enhances a "journal club" for undergraduate science students that develops oral communication and critical evaluation skills. *Journal of Learning Design*, 7(2), 105–119.
- Deiner, J. L., Newsome, D., & Samaroo, D. (2012, October 24). Directed self-inquiry: A scaffold for teaching laboratory report writing. *Journal of Chemical Education*, 89, 1511–1514.
- Dochy, F., Segers, M., & Sluijsmans, D. (1999). The use of self-, peer and co-assessment in higher education: A review. *Assessment & Evaluation in Higher Education*, 24(3), 331–351.
- Fallows, S., & Chandramohan, B. (2001). Multiple approaches to assessment: Reflections on use of tutor, peer and self-assessment. *Teaching in Higher Education*, 6(2), 229–246.
- Harris, M. A. (2009). Investigation into the effectiveness of an inquiry-based curriculum in an Introductory Biology laboratory (Unpublished doctoral dissertation). The University of Maine, Orono.

- Kalaskas, A. B. (2013). Science lab report writing in postsecondary education: Mediating teaching and learning strategies between students and instructors (Unpublished doctoral dissertation). George Mason University, Fairfax, VA.
- Lerner, N. (2007). Laboratory lessons for writing and science. *Written Communication*, 24(3), 191–222. doi: 10.1177/0741088307302765
- Liu, N., & Carless, D. (2006). Peer feedback: The learning element of peer assessment. *Teaching in Higher Education*, 11(3), 279–290.
- Mulder, R. A., Pearce, J. M., & Baik, C. (2014a). Peer review in higher education: Student perceptions before and after participation. *Active Learning in Higher Education*, 15(2), 157–171.
- Mulder, R., Baik, C., Naylor, R., & Pearce, J. (2014b). How does student peer review influence perceptions, engagement and academic outcomes? A case study. *Assessment & Evaluation in Higher Education*, 39(6), 657–677.
- Nicol, D., Thomson, A., & Breslin, C. (2014). Rethinking feedback practices in higher education: A peer review perspective. *Assessment & Evaluation in Higher Education*, 39(1), 102–122.
- Parkinson, J. (2017). The student laboratory report genre: A genre analysis. *English for Specific Purposes*, 45, 1–13.
- Pearce, J., Mulder, R., & Baik, C. (2009). Involving students in peer review: Case studies and practical strategies for university teaching. University of Melbourne, Center for the Study of Higher Education. Retrieved from <http://melbourne-cshe.unimelb.edu.au/resources/teaching-and-learning/peer-review>
- Robinson, R. (2001). Calibrated Peer Review™: An application to increase student reading, writing skills. *American Biology Teacher*, 63(7), 474–480.
- Ruegg, R. (2014). The effect of assessment of peer feedback on the quantity and quality of feedback given. *Papers in Language Testing and Assessment*, 3(1), 24–43.
- Russell, D. R. (2002). *Writing in the academic disciplines: A curricular history* (2nd ed.). Carbondale: Southern Illinois University Press.
- Somervell, H. (1993). Issues in assessment, enterprise and higher education: The case for self-, peer and collaborative assessment. *Assessment & Evaluation in Higher Education*, 18(3), 221–233.
- Topping, K. (1998). Peer assessment between students in colleges and universities. *Review of Educational Research*, 68(3), 249–276.
- Topping, K., Smith, E. F., Swanson, I., & Elliot, A. (2000). Formative peer assessment of academic writing between postgraduate students. *Assessment & Evaluation in Higher Education*, 25(2), 149–169.
- Walker, J. P., & Sampson, V. (2013). Argument-driven inquiry: Using the laboratory to improve undergraduates' science writing skills through meaningful science writing, peer-review, and revision. *Journal of Chemical Education*, 90(10), 1269–1274.

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ABSTRACT

Arts-related science activities provide unique opportunities to engage students' strengths and motivate different types of learners (Jolly, 2014). Incorporating arts into the discussion of gene expression and microbiology introduces students to a multidisciplinary approach to STEM and provides an opportunity to explore the use of science in different fields such as design, art, and industry. In this protocol extension students create living works of art on agar plates by "painting" with *E. coli* that express fluorescent proteins of various colors.

Key Words: painting; bacteria; *E. coli*; protein; gene expression; DNA.

○ Introduction

It is all too common for students to think of art and science as being entirely separate and unrelated. Incorporating arts into the discussion of gene expression and microbiology introduces students to a multidisciplinary approach to STEM and provides an opportunity to explore the use of science in different fields such as design, art, and industry. Arts-related science activities also provide unique opportunities to engage students' strengths and motivate different types of learners (Jolly, 2014). In this activity, students create living works of art on agar plates by "painting" with *E. coli* that express fluorescent proteins of various colors.

This activity is designed for grades 8–12 and supports students in making the connection between DNA and gene expression. However, it can also be modified for younger students by emphasizing the role of microorganisms such as bacteria in research and in their lives. Undergraduate audiences can learn about plasmid construction and bioinformatics by focusing on the DNA sequences of the plasmids involved.

Bacterial transformation is a powerful research tool as well as a classic classroom laboratory activity that allows students to experience and reflect on core disciplinary ideas in biology. This technique provides a simple and effective demonstration of how DNA contains information for the production of proteins and how

changes in DNA can alter the phenotype of an organism (HS-LS1.A, NGSS, 2013). At the Fred Hutchinson Cancer Research Center's Science Education Partnership (SEP), teachers have been using a set of fluorescent plasmids to practice basic bacterial transformation with their students. However, many teachers have extended their lessons by using the transformed bacteria to paint with, thereby reaching different learners and reinforcing the concept that genetic engineering can create a specific protein product.

In research, plasmid design is an essential tool of molecular biology. Engineered plasmids are used in pharmaceutical development and to study gene expression and disease pathology. The ability to add new genetic instructions into a cell and expect reliable and reproducible protein expression has led scientists to develop new tools like CRISPR Cas-9 for genome editing, and to create cellular human insulin factories using bacteria and yeast. Scientists creating new plasmid constructs often include genes for antibiotic resistance or fluorescence as a visual indication of positive bacterial transformation.

The plasmids used in this activity were designed by the Tsein Lab at the University of California San Diego (Figure 1). Transformed bacteria containing the fluorescent plasmids can be ordered from the nonprofit plasmid repository Addgene (see Table 1) (Shaner, 2004). The sequences for these plasmids are also available on the Addgene website. In the activity described in this article, students take advantage of the introduced fluorescence genes to create living paintings with bacteria, forming a more tangible connection between gene expression and phenotypic changes.

○ Preparation

It is not necessary to do a bacterial transformation in order to do the painting, since the bacteria arrive containing the plasmids already. The Addgene plasmids listed in Table 1 are sent in bacteria stabbed into an agar slant. The bacteria should be streaked for isolation onto an LB (Luria Bertani or Lysogeny Broth) agar plate containing ampicillin as soon as possible. After overnight incubation,

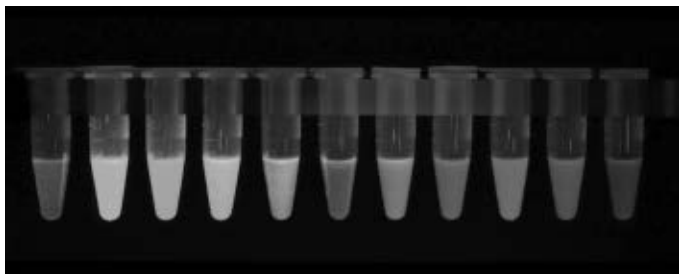


Figure 1. Composite of fluorescent cells from the Tsien Lab at the University of California, San Diego (<http://www.tsienlab.ucsd.edu/Images.htm>).

Table 1. Plasmids yielding fluorescent proteins, available from Addgene (www.addgene.org).

Plasmid Name	Visible Color	Color Under UV
pNCS mCherry, #91769	Dark Pink	Dark Pink
pNCS mHoneydew, #91760	Yellow/Lime	Lime
pNCS mTangerine, #91763	Pink	Orange/Pink
pNCS BFP, #91757	White	Blue
pNCS Venus (YFP), #91759	Yellow	Yellow/Lime

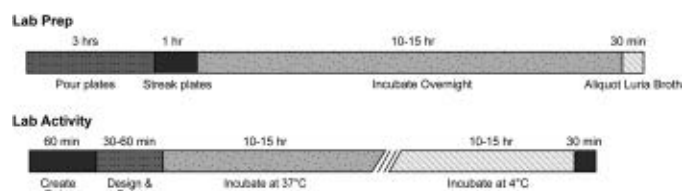


Figure 2. Timeline.

single colonies can be selected and used for creating the bacteria paint or prepped to isolate and purify the plasmid for use in a transformation protocol (Figure 2).

If you are interested in doing transformation as a lab activity, you will first need to do a “miniprep” to isolate the plasmids from the bacteria sent by Addgene. Commercial miniprep kits are available; most require high-speed centrifugation, so be sure to check the specific protocol beforehand. Students can then insert those plasmids into non-transformed bacteria using a transformation protocol (see Extra Resources). Calcium chloride and heat shock are used to introduce the plasmids into competent bacteria. K-12-safe *E. coli* strains JM101 and NEB5-alpha (available to teachers through New England BioLabs) can be used for all the plasmids listed here. Once transformed, the bacteria are plated on ampicillin agar plates and incubated overnight. The colonies of transformed *E. coli* will produce visible color and fluorescent color under UV light (Table 1). Over time, the colonies will become more vibrant as the proteins are continually produced within the cells. Students can then select successfully transformed colonies to use as their paints.

○ Materials

- Stock plates of transformed bacteria
- Sterile toothpicks
- Microcentrifuge tubes
- Micropipet & tips
- Sterile Luria Broth
- Sterile LB/amp plates (for stock)
- Sterile LB plates (for painting)
- Ampicillin
- Disinfectant (10% bleach or 70% alcohol)
- 37°C incubator (egg incubators work well)
- Autoclave — if making your own LB agar & Luria broth

○ Preparing the Paint and Plates

Pour LB/ampicillin stock plates and LB plates for students to use as canvases. Make at least two stock plates for each plasmid of the transformed bacteria by streaking LB/ampicillin plates to create isolated colonies. To create the medium for the bacteria paint, add ampicillin (100 mg/ml) to sterile Luria Broth at 1:1000 (1 μ l ampicillin to 1 ml of Luria Broth). Transfer ~500 μ l of the solution into microcentrifuge tubes. Store tubes in the refrigerator until ready to use. The ampicillin is light sensitive, so use your solution soon after preparation.

To create their paint, the students can select the best colony with a sterile toothpick and transfer it to a microtube of Luria Broth with ampicillin. They then suspend the colony in the Luria Broth using a pipet or vortexer, and incubate the tubes for 20–30 minutes at 37°C to allow the bacteria to multiply.

○ Painting with Transformed Bacteria

To plan their designs, students first make a template by tracing around the outside of an empty petri dish onto a piece of paper. Simple line drawings and block images work best. In general, images with recognizable shapes (flowers, mountains, neurons) and without backgrounds produce better results. Have students flip over their plates and tape their designs face-up onto the bottom so the image is visible through the agar. Discuss the limitations of bacterial paint, such as the inability to blend colors, the potential for contamination, and different growth rates that can occur on the same plate.

Toothpicks are the easiest way to paint the bacteria onto a plate. Students dip the rounded end of a sterile toothpick into a tube of bacteria-LB/ampicillin paint and apply it to their agar plate. Wooden toothpicks are cheap! If a tip appears jagged, select a new one. Encourage students to use new toothpicks often when painting with a particular color, and to always use a new toothpick when beginning a new color to avoid cross-contamination. The paint does not need to be heavy, and students should try to avoid tearing into or puncturing the agar. Cotton swabs generally take up too much of the paint solution, making precision difficult and greatly increasing the chances of contamination. Brushes can be used, but they should be rinsed in a 10 percent bleach solution and then in sterile water in between colors.

Place the plates agar side up in a darkened 37°C incubator overnight (wrap with aluminum foil if light is an issue). After 24 hours, the plates should have visible bacterial growth as their art comes to life. The bacteria will glow under UV light but should also express some color under normal light. Although colors will be visible after the overnight incubation, for better results place the plates in a refrigerator (4°C) for 24 hours after incubation. This allows the bacteria to express more of the fluorescent proteins, creating more vibrant colors. When the incubation time is completed, students can use cell phones to photograph their artwork. Teachers can provide a cardboard box to create a hood, as well as a digital camera and tripod to aid in photography. Students may need to experiment to find the best way to visualize fluorescence. A UV light under the plate will give different results than one held over the plate.

By using bacteria as paint, students can learn about gene expression and how plasmids are used by molecular biologists as a tool for harnessing a cell's genetic machinery. This extension to the transformation protocol is also an opportunity to further discuss the use of fluorescent proteins as a tag to help identify successful plasmid incorporation, or as an example of employing an organism to produce a desired protein. Incorporating art and science can be a useful mechanism for engaging different learners, introducing cross-disciplinary skill, and fostering creative and critical thinking in students.

○ Sample of Student Response to Activity

“The project created a wonderful example of real world application of genetics. It was fun to work hands-on with the bacteria and as a student who enjoys drawing, it was great to have an excuse to use my artistic skills in science class. It was so rewarding to spend the time working with the bacteria and then have the work produce a final result that was both entertaining, and memorable.” (Miranda, Class of 2020)

“I really enjoyed the bacteria art project because not only did it help me understand how bacteria can replicate and pass down the fluorescent protein DNA sequence, but it also helped wrap my head around the massive quantity of bacteria produced. It was also a very fun activity that allowed me to be creative while learning.” (Conor, Class of 2020)

“It was really fun not only drawing with bacteria (which is super cool and not many people can say they have done it) but also

an interesting experience making, and growing them. I think that it is a really fun way to teach students a complicated concept with many steps through an interactive and fun activity and really helped me understand it a lot better.” (Albert, Class of 2020)

○ Extra Resources

- Addgene Roger Tsien Repository (https://www.addgene.org/Roger_Tsien/)
- SEPGuides pFLO Transformation Protocol (<http://www.fredhutch.org/en/education-training/sep/teachers/labs-lessons.html>)
- ScienceBridge Transformation Resources (<http://sciencebridge.ucsd.edu/programs/labs/content-areas/transformation.html>)
- American Society for Microbiology, Agar Art Contest (<https://www.asm.org/index.php/public-outreach/agar-art>)
- New England BioLabs Inc. (<https://www.neb.com>)

References

- Jolly, A. (2014, November 18). STEM vs. STEAM: Do the Arts Belong? *Education Week: Teacher*. Retrieved from <http://www.edweek.org/tm/articles/2014/11/18/ctq-jolly-stem-vs-steam.html>
- NGSS Lead States. (2013). *Next generation science standards: For states, by states*. Washington, DC: Achieve, Inc.
- Shaner, N. C., Campbell, R. E., Steinbach, P. A., Giepmans, B. N. G., Palmer, A. E., & Tsien, R. Y. (2004). Improved monomeric red, orange and yellow fluorescent proteins derived from *Discosoma* sp. red fluorescent protein. *Nature Biotechnology*, 22(12), 1567–1572. <https://doi.org/10.1038/nbt1037>

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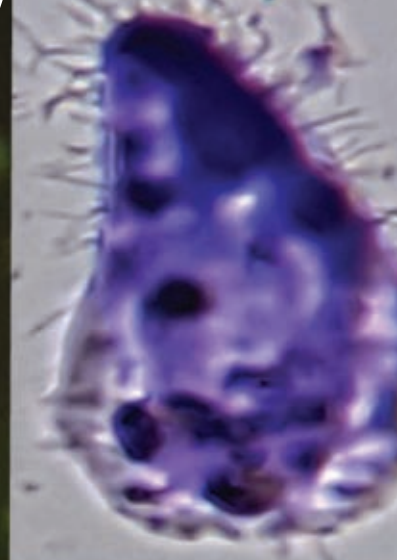
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Wright Stain and Fluorescence-
Based Methods in *Tetrahymena* for
Phenotypic AnalysisMARÍA DEL ROSARIO RAMÍREZ MATA,
ROBERT M. KAO**ABSTRACT**

In educational and research settings, *Tetrahymena* is an excellent model organism for engaging students to investigate function, morphology, structure, phagocytosis, and ciliary motion. Here, we present applications of Wright stain and Sytox green as useful low-cost tools for phenotypic analysis. We used heat-fixed *Tetrahymena* followed by Wright-stain-labeled organelles at different stages of its life cycle. In addition, a low concentration of Wright stain, at 1 percent (vol/vol), enabled visualization of filled vacuoles with stain in live *Tetrahymena*. Furthermore, we identified that Sytox green fluorescence labels not only nuclei of pre-incubated cultures of *Tetrahymena*, but also nuclei and some notable cytoplasmic staining after heat fixation. These applications can be used alongside inverted, battery-operated, bright field, fluorescence microscopes (Miller et al., 2010), as well as Cellcams (Martin & Shin, 2016) for acquiring images and time-lapse movies. In the future, these useful approaches can be applied broadly in many lab inquiry settings, such as toxicology and molecular genetics.

Key Words: *Tetrahymena*; development; Wright stain; Sytox green dye; phenotypic analysis; heat fixation; live cells.

○ Introduction

Tetrahymena is a model freshwater single-celled protozoan, ideal for investigating the molecular and cellular basis of cell morphology, organelle organization, structure, cell proliferation, and ciliary motion, and can be used to study cell shape, even rate of phagocytosis (Bozzone, 2000; Ruehle et al., 2016). This ciliate also possesses a diploid micronucleus (MIC), mostly silent transcriptionally, and two complete haploid genomes capable of both mitosis and meiosis (Orias, 1998). *Tetrahymena thermophila*, like other fresh water protozoa and cells of lower fresh water invertebrates, maintains its salt and water homeostasis via contractile vacuoles (Plattner, 2015). This enables the organism to maintain constant potassium and sodium concentrations over wide range of hypo-osmotic environmental

conditions. Moreover, investigations using *Tetrahymena thermophila* led to important discoveries and insights, such as telomeres and telomerase (Gilley & Blackburn, 1996; Chan & Blackburn, 2004), and ribozymes (Cech et al., 1981).

To visualize cellular organelle changes over *Tetrahymena* life cycle development (Figure 1), there are at least two chromogenic staining methods used before for phenotypic analysis: (1) silver preparation labels cortical basal bodies (Frankel, 2008); and (2) Giemsa stain labels micronucleus and macronucleus (Stone & Cameron, 1964; Gude et al., 1955). The reason for applying Wright stain was to visualize multiple cellular organelles. We wished to apply this stain, routinely used in medical laboratory settings, as a tool to explore and define multiple organelle labeling for phenotypic analysis. The traditional Wright stain dates from 1890s; it is also a modified version of the Romanowsky method. The original Wright stain was an alcohol-based solution of methylene blue and eosin Y, used primarily to stain peripheral blood smears, urine samples, and bone marrow aspirates to be examined under a light microscope (Wright, 1902). The acidic or basic nature of cellular structure determines their staining for the components of Wright's polychromatic dye.

We applied Wright stain as a method to label heat-fixed and live *Tetrahymena* as a tool used for phenotypic analysis. Sytox green is a high-affinity nucleic acid stain that easily penetrates cells with a compromised plasma membrane. In these cells, which are dying or undergoing cell death, Sytox green dye binds to DNA inside the nuclei, which brightly fluoresces green with over 500-fold enhancement. Stained cells will generally have bright green nuclei as well as some low-level cytoplasmic staining (Lebaron et al., 1998). In addition to using the compound light microscope, we utilized both the portable low-cost fluorescence microscope (Miller et al., 2010) for fluorescence detection, along with Cellcams (Martin & Shin, 2016) for videos. In the following sections, we provide step-by-step methods of Wright stain and Sytox green fluorescence that can be adapted to lab inquiry research and educational settings.

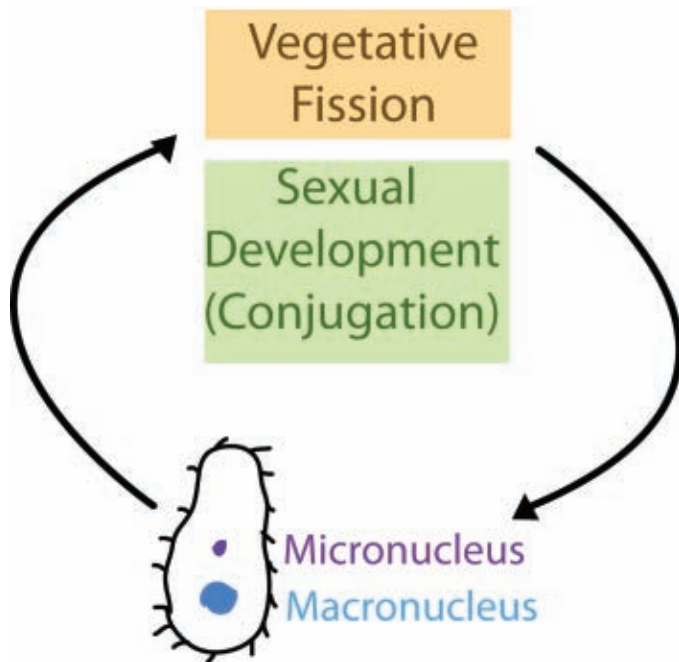


Figure 1. Life cycle of *Tetrahymena*. A micronucleus (purple) and macronucleus (blue) of the *Tetrahymena* undergo either vegetative asexual division under starved conditions, or sexual development (conjugation). Micronucleus is the diploid germline that is transcriptionally silent, and somatic macronucleus is polyploid and transcriptionally active (Chalker et al., 2013).

○ Materials

1. *Tetrahymena* standing (non-shaking) culture at room temperature, grown in 2% pentose peptone from Carolina Labs (*Tetrahymena* supplied with Phagocytosis and Vacuole Formation in *Tetrahymena* Kit, Catalogue Item # 131182B). Note: this kit also contains India ink.
2. Compound microscope (Olympus) with 4x, 10x, and 40x air objectives.
3. Portable battery-operated, low-cost, bright field, and fluorescence microscope (gift from Dr. Rebecca Richards-Kortum, Rice University; cited in Miller et al., 2010).
4. Alcohol lamp from Carolina Labs (Catalogue # 706604) and matches (Catalogue #12-075 from Fisher Scientific).
5. 10P, 20P, 100P, 200P, and 1000P hand-held pipettors (Gilson® Pipetman® Classic, Fisher Scientific). 10P (Catalogue # FA10002PG); 20P (Catalogue # FA10003MG); 100P (Catalogue # FA10004MG); 200P (Catalogue # FA10005PG); and 1000P (Catalogue # FA10006PG).
6. Eppendorf safe-lock micro centrifuge tubes (Part # 022363638 from Eppendorf).
7. Glass slides (Catalogue # 12-544-2, Fisher Scientific) and cover slips (Catalogue # 10-016-24, ThermoScientific). Note that glass slides were not pre-coated with gelatin, nor with poly-D lysine.

8. Wright stain (Fisher Scientific brand, SureStain™ Wright Stain, CS-432, opened 08/27/2015), placed in a glass bottle with dropper (Catalogue # 12-000-158, Fisher Scientific).
9. Methylene blue stain (Millipore Sigma R0310174), placed into a bottle with dropper (Catalogue # 12-000-158, Fisher Scientific).
10. Sytox green nucleic acid stain, 100μM stock solution diluted in distilled water (use stock SYTOX™ Green Nucleic Acid Stain, 5mM Solution, Invitrogen™ S7020).
11. Pentose peptone media, 2% (Stewart & Giannini, 2016).
12. Wash bottle with distilled water.
13. Stain rack holder (handmade, shown in Figure 3, panels A–C).
14. Parafilm paper (Parafilm M Sealing Film, #7315D11, Thomas Scientific).
15. Number 5 forceps (Model # IMS-JF5, Premium High Precision Jeweler Style Forceps, #5 Tweezers, Fine Point Tips, Stainless Steel, 4.5" L) used to make holes in parafilm to cover Eppendorf tubes.

○ Procedures

Heat-fixation procedure:

To preserve and adhere *Tetrahymena* cells onto the glass slide, use this step-by-step heat-fixation procedure:

1. Place 50 microliters of the sample in a glass slide.
2. Fix *Tetrahymena* onto the glass slide by intermittent exposure to heat using flame lamp onto the sample. For lab safety purposes, wear gloves and use tweezers to hold one end of the glass slide.
3. Pass the slide quickly through flame several times, sample side up, until completely dry. Allow 5 to 10 seconds to cool at room temperature away from the flame.
4. Carefully track the temperature by touching your arm with the glass slide to make sure it will not burn the sample.

Wright-staining procedure on heat-fixed *Tetrahymena* specimen:

1. Prepare the sample by pouring 25 microliters of untreated *Tetrahymena* placed on a glass slide.
2. Fix the sample using the heat-fixation procedure described above.
3. Place the slide sample side up on the staining rack.
4. Apply Wright stain to the slide using dropper bottles or pipettes.
5. Wait 5 seconds, then add an equal volume of distilled water.
6. Mix the stain and water, and allow it to incubate at room temperature for 5 minutes.
7. Pour the stain and water mixture off the slide.
8. The slide may be rinsed with distilled water until the stain is removed, or it may be washed using a wash bottle.
9. Wipe the back of the slide.

10. Dry the slide in a vertical position, on an absorbent surface (bibulous paper).
11. Examine cells using a compound microscope or bright field in low-cost portable microscope.

Wright-staining procedure on living *Tetrahymena* cultures:

1. Referring to Table 1, use either 20P or 100P or 200P micropipettor and add the appropriate solutions for the negative control, experimental groups (Wright stain at different concentrations ranging from 0.5%, 1%, and 5% vol/vol), and positive control using 1% (vol/vol) India ink diluted in distilled water.
2. When each component has been added and mixed, use a table-top centrifuge to pool all the solution at the bottom of the 1.5mL Eppendorf tube.
3. Use a parafilm and aluminum foil setup (Figure 2D–2G), and observe at 4 hours and 24 hours. Students can decide observation timepoints for their experiments. Tables 2–4 highlight example data that can be collected by students. Key frames from 1% (vol/vol) Wright stain from time-lapse experiment (Figures 3C–3E and 4).

○ Results

Wright stain in *Tetrahymena*

Wright stain allows visualization of different stages of *Tetrahymena* throughout its life cycle. It labels the cortical grooves, the oral cavity, and cilia (Figure 3A–3C). This stain can either be used in heat-fixed samples, or be modified to monitor cellular events in living cultures as mentioned below. Based on the collected data from the above pilot experiment in living cells, we determined that the 24-hour pre-incubation of 1% Wright stain (vol/vol) highlights the following organelles: (1) several food vacuoles and vesicles labeled in ranges of color from light violet to dark purple; (2) the activity of the contractile vacuole; (3) and ciliary motion.

Sytox green nucleic acid stain procedure (living cells):

1. Make a dilution of 20 μ M by mixing 5 μ L of Sytox green dye from the original stock concentration of 100 μ M with 20 μ L

of *Tetrahymena* (cultured for three days) to a final volume of 25 μ L. Follow the set-up of reagents listed in Table 5 with appropriate micropipettors.

2. Use No. 5 forceps to make seven holes into Parafilm paper, and cover the *Tetrahymena* culture in the Eppendorf tube. Let the sample incubate for three hours.

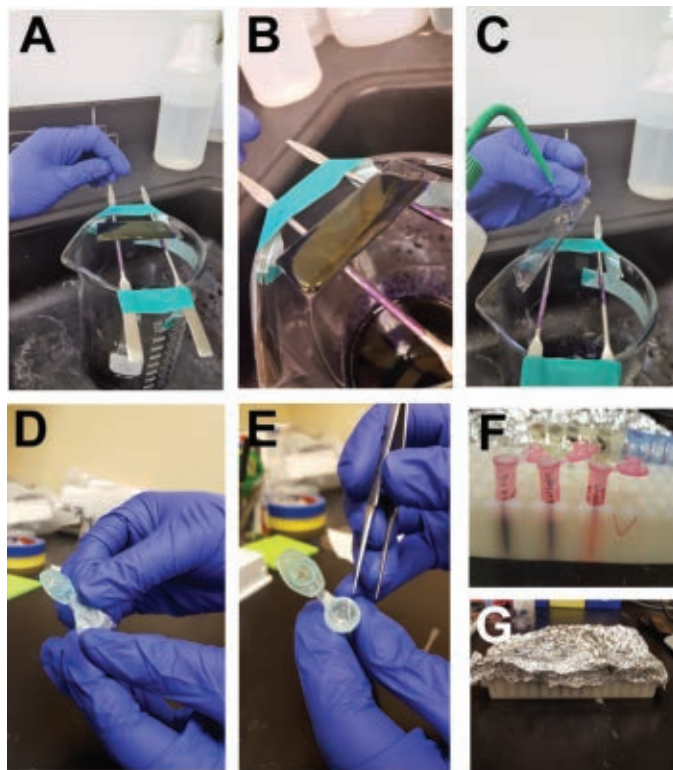


Figure 2. (A–C) Wright stain procedure. 1. Apply the dye on the sample (panel A). 2. Add water carefully and wait five minutes (panel B). 3. Rinse until all clear water from the side of the glass slide without touching the stained sample, and let it dry horizontally (panel C). **(D–G) Pre-incubation procedure.** 1. Place a piece of parafilm covering the Eppendorf tube (panel D). 2. Use No. 5 forceps to make seven holes on parafilm (panel E). 3. Let the samples incubate for a period at room temperature (panel F). 4. Cover the samples with foil (panel G).

Table 1. Pre-incubation of *Tetrahymena* in living cells using 0.5%, 1%, and 5% (vol/vol) of Wright stain concentrations.

	Negative control	Wright stain 0.5 % (vol/vol)	India ink 1% (vol/vol)	Wright stain 1% (vol/vol)	Wright stain 5% (vol/vol)
<i>Tetrahymena</i> culture	10 μ L	10 μ L	10 μ L	10 μ L	10 μ L
Wright stain 100%	—	2.5 μ L	—	5 μ L	25 μ L
India ink 100%	—	—	5 μ L	—	—
2% proteose peptone media	490 μ L	487.5 μ L	485 μ L	485 μ L	465 μ L
Total volume	500 μ L	500 μ L	500 μ L	500 μ L	500 μ L

Table 2. Wright stain procedure setup for living *Tetrahymena*.

	Negative control	Positive control	Experimental group
3 days cultured <i>Tetrahymena</i>	10 μL	10 μL	10 μL
2% proteose peptone media	490 μL	485 μL	485 μL
Wright stain 1% (vol/vol)	—	—	5 μL
India ink 1% (vol/vol)	—	5 μL	—
Total volume	500 μL	500 μL	500 μL

Table 3. Experiment 1: Experimental observations at 24 hours with different concentrations of Wright stain in *Tetrahymena* culture.

Group	Mobility	Labeling of vesicles	Colored precipitation in culture media	Cells undergoing conjugation
Negative control	+++	—	—	+
Wright stain 5% (vol/vol)	—	—	+++	—
Wright stain 1% (vol/vol)	+++	++	+	++
Wright stain 0.5% (vol/vol)	+++	—	—	+
India ink 1% (vol/vol)	++	+++	+	+

Qualitative analysis many (+++), some (++), very little (+), and none (—).

Table 4. Experiment 2: Experimental observations at 24 hours with 1% (vol/vol) Wright stain or 1% (vol/vol) India Ink in *Tetrahymena* culture.

Group	Cellular movement	Normal shape	Glassy shape	Labeled vesicles
Negative control	+++	+++	—	—
Positive control India ink 1% (vol/vol)	++	++	—	+++
Experimental group Wright stain 1% (vol/vol)	++	++	+	++

Qualitative analysis: large (+++), medium (++), and small (+). Negative or none (—).

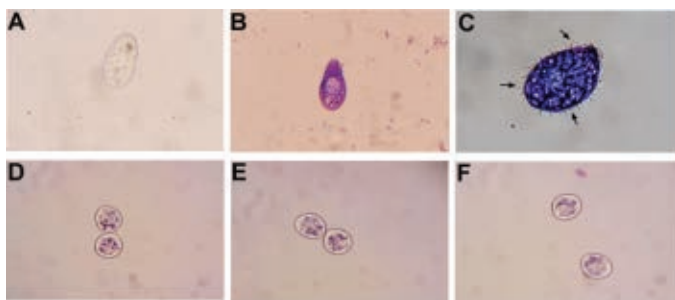


Figure 3. (A–C) Heat-fixed and Wright-stained *Tetrahymena* in living cells. (A) Heat-fixed *Tetrahymena* prior stain. (B) Wright-stained heat-fixed *Tetrahymena*. (C) Cilia (arrows) in heat-fixed *Tetrahymena*. (D–F) Time-lapse *Tetrahymena* cells undergoing conjugation in Wright stain preincubated for 24 hours in living cells.

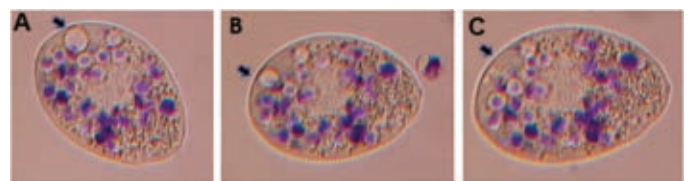


Figure 4. Wright stain 1% (vol/vol) incubated, active contractile vacuule. (A–C) Still frames from time-lapse experiment using CellCam to monitor contractile vacuule dynamics in 1% (vol/vol) *Tetrahymena* live culture. (A) *Tetrahymena* contractile vacuule (arrow). (B) Contracting vacuule (arrow). (C) Contractile vacuule releasing water (arrow).

Table 5. Pre-incubation setup of *Tetrahymena* with Sytox green fluorescence dye.

	Negative control untreated	Sytox green dye 20 μ M
<i>Tetrahymena</i> culture	25 μ L	25 μ L
Sytox green nucleic acid stain	—	10 μ L
2% proteose peptone media	25 μ L	15 μ L
Total volume	50 μ L	50 μ L

3. Use *Tetrahymena* untreated as a negative control, following the same incubation steps.
4. Place 20 μ L of the untreated *Tetrahymena* on a glass slide carefully; then place 20 μ L of the experimental group on a different glass slide, and cover it with a cover slip, making sure of not let the air bubbles to form on any of these samples.
5. Use an inverted battery-operated fluorescence microscope (Miller et al., 2010).

Sytox green nucleic acid stain procedure (heat-fixed cells):

1. Take 50 microliters of pre-diluted *Tetrahymena* in Sytox green already incubated for three hours (from the previous procedure).
2. Distribute it onto a glass slide
3. Heat-fix the sample carefully.
4. Examine by inverted low-cost fluorescence microscope (Miller et al., 2010).

Results of Sytox green fluorescent dye staining in *Tetrahymena*

After three hours of incubation, we observed that labeled nuclei in cells with plasma membrane were compromised, and several *Tetrahymena* cells were moving while observed with an inverted bright field fluorescence microscope (Figure 5A–5D). Heat-fixed pre-incubated *Tetrahymena* with Sytox green dye labels macronucleus (MAC) and some visible cytoplasmic stain (Figure 5E–5G).

○ Conclusion

In summary, we developed new, useful low-cost application tools for phenotypic analysis in *Tetrahymena*. In combination with Cellcams (Martin & Shin, 2016) and a portable low-cost fluorescence microscope (Miller et al., 2010), we demonstrated the utility of the following methods: heat fixation, Wright stain, and Sytox green fluorescence dye labeling. The versatility of the Wright stain in heat-fixed and live cell approaches allows the observation of cellular organelles, such as cilia, oral cavity, and some of the subcellular organelles (e.g., vacuoles and vesicles) during the life cycle of *Tetrahymena*. In future lab-inquiry and research settings, Wright stain and Sytox green dye staining approaches will provide a platform for phenotypic analysis of cellular

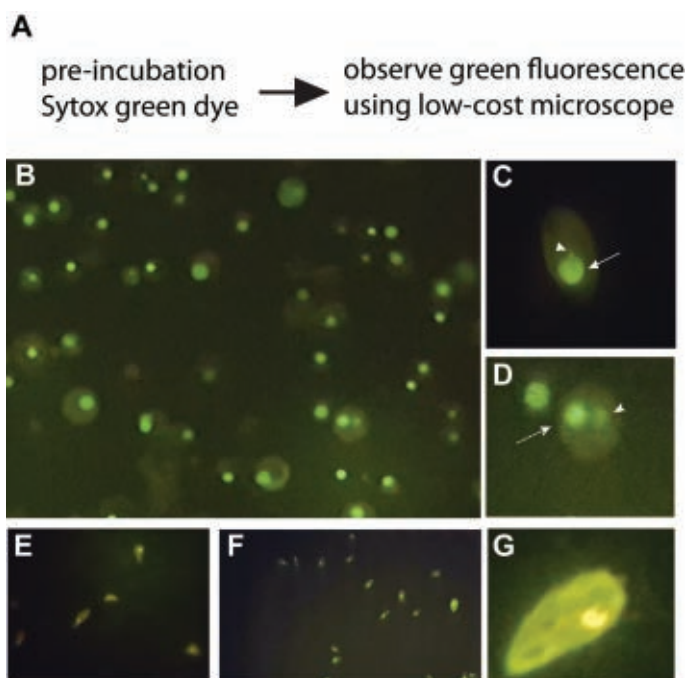


Figure 5. Sytox green fluorescence labeling of *Tetrahymena* in live and heat-fixed conditions using low-cost portable fluorescence microscope.

(A) Schematic of Sytox green dye pre-incubation experiment. (B–D) Pre-incubated *Tetrahymena thermophila* Sytox green in living cells. (E–G) Pre-incubated in Sytox green dye/heat-fixed *Tetrahymena thermophila*. (B) Low magnification view using 40x objective after 3 hours of Sytox green pre-incubation. (C) Higher magnification view of Sytox green labeled nuclei (macronucleus, arrow; abutting micronucleus, arrowhead). (D) Higher magnification of another cell with Sytox green labeled nuclei (arrow and arrowhead). (E–F) Low magnification views using 10x objective of heat-fixed, pre-incubated Sytox green dye. (G) Higher magnification view of Sytox green positive macronucleus and some general cytoplasmic staining.

decision-making processes in the changing microenvironment of *Tetrahymena*.

○ Acknowledgments

We thank Dr. Rebecca Richards-Kortum at Rice University for her gift of low-cost fluorescence microscopy; Dr. Terese Abreu Director of MLS program at Heritage University (HU) for her gift of Wright stain; Alejandra B. Cruz Adjunct English Teacher at HU for support in writing process; anonymous reviewers for their comments; and support through HU's NSF REU grant DBI #1460733.

References

- Bozzone, D. M. (2000). Investigating Phagocytosis in *Tetrahymena*. *American Biology Teacher*, 62(2), 136–139.
- Cech, T. R., Zaug, A. J., & Gravowsky, P. J. (1981). In vitro splicing of the ribosomal RNA precursor of *Tetrahymena*: Involvement of a

- guanosine nucleotide in the excision of the intervening sequence. *Cell*, 27, 487–496.
- Chalker, D. L., Meyer, E., & Mochizuki, K. (2013). Epigenetics of Ciliates. *Cold Spring Harbor Perspectives in Biology*. doi: 10.1101/cshperspect.a017764
- Chan, S. R., & Blackburn, E. H. (2004). Telomeres and Telomerase. *The Royal Society*, 359, 109–121.
- Frankel, J. (2008). What do genetic mutations tell us about the structural patterning of a complex single-celled organism? *Eukaryotic Cell*, 7(10), 1617–1639.
- Gilley, D., & Blackburn, E. H. (1996). Specific RNA residue interacts required for enzymatic functions of *Tetrahymena* telomerase. *Molecular & Cellular Biology*, 16(1): 66–75.
- Gude, W. D., Upton, A. C., & Odell, T. T. Jr. (1955). Giemsa staining of autoradiograms prepared with stripping film. *Stain Technology*, 30(4): 161–162.
- Lebaron, P., Catala, P., & Parthuisot, N. (1998). Effectiveness of SYTOX green stain for viability assessment. *American Society for Microbiology* PMID: PMC 106447.
- Martin, R., & Shin, S. (2016). Photo microscopy made easy by converting cell phones into “Cellcams.” *American Biology Teacher*, 78(1), 71–75.
- Miller, A. R., Davis, G. L., Oden, Z. M., Razavi, M. R., Fateh, A., Ghazanfari, M., . . . Richards-Kortum, R. (2010). Portable, battery-operated, low-cost, bright field and fluorescence microscope. *PLoS ONE*, 5(8), 1–3.
- Orias, E. (1998). Mapping the germ-line and somatic genomes of a ciliated protozoan, *Tetrahymena thermophila*. *Genome Research*, 8, 91–99.
- Plattner, H. (2015). The contractile vacuole complex of protist: New cues to function and biogenesis. *Critical Reviews in Microbiology*, 4(2), 218–227.
- Ruehle, M. D., Orias, E., & Pearson, C. G. (2016). *Tetrahymena* as a unicellular model eukaryote: Genetic and genome tools. *Genetics Society of America*, 203, 649–665.
- Stewart, C., & Giannini, J. (2016). Analyzing *Tetrahymena* movement with an inexpensive & engaging inquiry lab. *American Biology Teacher*, 78(9): 764–771. <https://doi.org/10.1525/abt.2016.78.9.764>
- Stone, G. E., & Cameron, I. L. (1964). Methods for using *Tetrahymena* in studies of the normal cell cycle. *Methods in Cell Biology*, 1, 127–140.
- Wright, J. H. (1902). A rapid method for the differential staining of blood films and malarial parasites. *Journal of Medical Research*, 7(1): 138–144.

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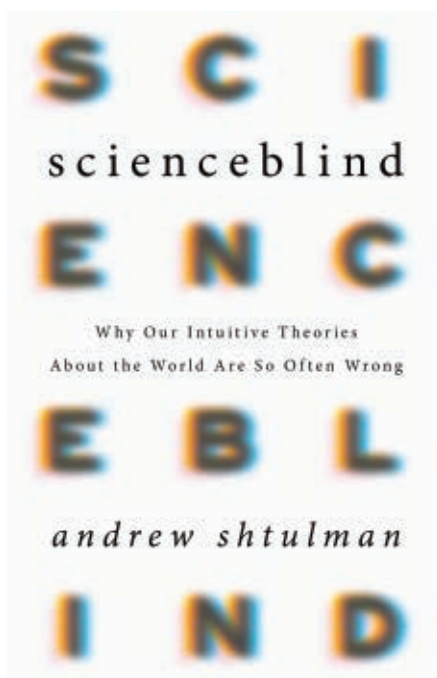
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PUBLIC PERCEPTIONS OF SCIENCE

Scienceblind: Why Our Intuitive Theories About the World Are So Wrong. By Andrew Shtulman. 2017. Basic Books. (ISBN: 9780465053940). 311 pp., illus. Hardcover \$30.00.

Science denial has been a major issue of concern in recent years. Many factors have been proposed as the cause of science denial, including political ideology, religious ideology, and purveyors of misinformation and doubt like antievolutionists, anti-vaccination proponents, and climate change skeptics. In *Scienceblind*, Andrew Shtulman, associate professor of psychology and cognitive science at Occidental College, discusses yet another factor that contributes to skepticism about established science. Shtulman provides an overview of intuitive theories about the world that develop early in life, have been consistently found in people of all ages, across many different cultures,

and throughout history. These intuitive theories can pose barriers for students trying to learn many of the well-established, but counterintuitive, theories central to the biological and physical sciences. Shtulman argues that, “To get the world right we need to do more than just change our beliefs; we need to change the very concepts that articulate those beliefs. That is, to get the world right, we cannot simply refine our intuitive theories; we must dismantle them and rebuild them from their foundations” (p. 5). This poses particular challenges for designing science instruction.

Scienceblind provides a very readable introduction to a vast literature on science misconceptions that stretches back to the early 1980s. The book opens with a chapter entitled “Why we get the world wrong,” providing an overview of the concept of intuitive theories. Most of the remainder of the book is divided into two parts, one summarizing research on intuitive theories in the physical sciences and the other, the biological sciences. An example of an intuitive theory of adaptation familiar to biologists is the inheritance of acquired characteristics where species evolve due to environmental pressures that cause a need for change, and all individuals in the population simultaneously respond to this need by adapting their anatomy, physiology, or behavior in order to survive. The well-established scientific theory is, of course, Darwin’s theory of natural selection, which is a two-step process involving the generation of random variation followed by selection. In Darwin’s theory many individuals die without having successfully passed on their genes to the next generation. Only a select few successfully reproduce. This causes the population to evolve, not the individual organisms.

Intuitive theories are grounded partly in innate expectations and partly in concepts that emerge early in a child’s development. By school age these foundational concepts form students’ common-sense intuitions about the world, and when scientific ideas clash with them, the common-sense ideas

usually win out (Bloom & Weisberg, 2007). For example, essentialism is a foundational belief that objects have a set of observable characteristics determined by an immutable underlying nature that cannot be seen, but that gives the object its identity. Essentialism is important for learning concepts, but can interfere with learning natural selection. Students who view evolutionary change through an essentialist bias see evolution as the simultaneous transformation of the essence of all individuals in a population, rather than as the survival and reproductive success of only a select few organisms from each generation.

In addition to the intuitive theory of adaptation, the section of the book on intuitive biological theories also includes chapters on life, growth, inheritance, illness, and ancestry. A careful reading of the chapter on ancestry will help the reader understand why nonscientists continue to challenge evolution by asking, “If humans evolved from chimpanzees, then why are chimpanzees still around.” The section of the book dealing with intuitive theories of the physical world also contains chapters that may be of interest to biologists. For example, the chapter on intuitive theories of energy may be relevant for helping students to understand energy transformations at the cellular and molecular level. There is also a chapter that addresses intuitive theories of the earth (continental drift) and climate that may interest biologists.

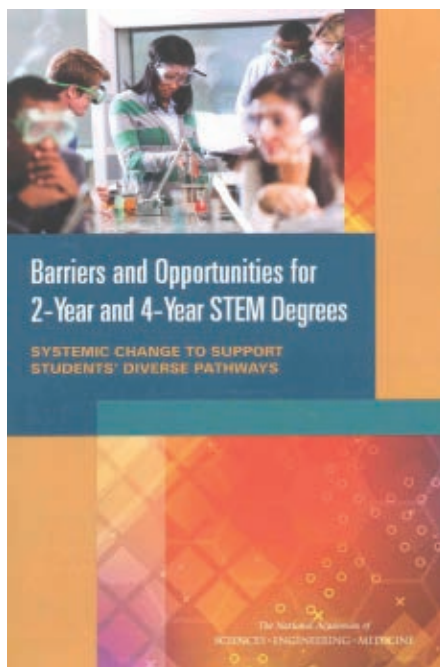
The book closes with a chapter entitled “How to get the world right,” where Shtulman discusses some educational implications of our knowledge of intuitive theories. He concludes that science denial is unavoidable. Grounded in innate expectations and our earliest attempts to understand causal relationships in the world, intuitive theories are coherent and robust.

But there is hope. Many of the chapters on the various intuitive theories discuss educational interventions that have been successful in helping students overcome the barrier that an

intuitive theory can impose to learning a well-established scientific theory. Shtulman writes, “Any educator who wants to help students confront and correct their intuitive theories needs to tailor his or her instruction to those theories” (p. 245). The key is to guide students through an evaluation of the intuitive theory and its well-established scientific counterpart. Students need a clear demonstration of how the intuitive theory fails to adequately explain the phenomenon in question, followed by a clear demonstration of how the scientific theory adequately explains the phenomenon. *Scienceblind* is a book that all science teachers should read, if only to sample the chapters relevant to their discipline, whether it be biology, chemistry, physics, or earth science. The ideas in this book have important implications for designing instruction and planning both formative and summative assessments that will challenge students to confront their intuitive theories and rebuild their understanding of the world. *Scienceblind* provides a fine illustration of how cognitive science can inform the practice of science teaching, just as the biological sciences inform the practice of medicine.



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experience? The authors go a step further and identify several common barriers to STEM education success: poor advising; not prepared for rigor; stereotypes from faculty or peers; unwelcome environments; and uninspired teaching.

Early on in the book, we are re-acquainted with some hopeful facts and figures that are likely familiar. For instance, those holding STEM degrees have higher salaries and lower levels of unemployment than other fields. Also, the pay gap between male and female workers is less for those holding STEM degrees than other fields. And in the classroom, with much praise to the *Vision and Change Call to Action*, best practices such as active learning, group work, and feedback from instructors have shown improvements in the learning and culture in STEM classrooms. The first chapter also points out some tidbits that may be less widely known. Did you know that most people with STEM degrees are not working in STEM fields? And that there are more minority and single parent students pursuing STEM degrees than ever before? Interestingly, in active learning environments, the achievement gap between black and white students is decreasing. Furthermore, the achievement gap between first-generation and traditional students was eliminated altogether!

But after that, the picture starts to get a bit grim, particularly for two-year college STEM students. Two-year college students make up over 40 percent of the total undergraduate population, yet two-year college STEM students are switching out of STEM majors at higher rates than their four-year college counterparts. Unfortunately, the authors did not provide any reasons for this attrition or possible solutions. However, they did provide several

excuses: it is natural that some students would switch out, and perhaps students are switching to majors that are more suited to their perceived abilities.

This is in direct contradiction to an earlier statement in the book that we (students, advisors, instructors, administrators) should not base students' chance of STEM success on their perceived natural abilities. I thought they made a good point in this section, as they mention that perceived abilities are most likely attributed to early exposure to science and math. Since it is likely that students in low socio-economic environments for their primary and secondary education would not have early exposure opportunities, they would never even have the chance to build their abilities in science and math aptitude.

The most interesting aspect of the book is the discussion of the role that the “culture of science” plays in pushing students out of STEM. The idea that some students are naturally inclined toward science are outdated, but still largely perpetuated. The lack of visible scientists of color has a negative impact on the perceptions of students of color. The highly competitive nature of introductory science courses and the rigid structure of course sequencing delays movement through the majors. Institutions do not incentivize and rarely support the use of best practices and professional development. Even if institutions invested in this area, this is unlikely to make a large impact, because these practices have been shown to be most effective in introductory-level courses, which are widely taught by part-time instructors who typically don't have access to institutional professional development programs.

In the end, the authors assert that research shows that STEM classrooms can be unwelcoming. But they also make it clear that there is not enough data to really understand the effect of barriers or best practices in STEM education. If you consider yourself an engaged instructor and use best practices in science education in your classrooms and labs, this is probably not the book for you. However, if you are looking for data to support funding and programming for STEM retention and success programs on your campus, or if you are an administrator who would like to support or promote such efforts, then this book has all the data you need to build a strong case for the work ahead.



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Reference

Bloom, P., & Weisberg, D. S. (2007). Childhood origins of adult resistance to science. *Science* 316, 996–997.

SCIENCE PATHWAYS IN EDUCATION

Barriers and Opportunities for 2-Year and 4-Year STEM Degrees: Systemic Change to Support Students' Diverse Pathways. By National Academies of Sciences, Engineering, and Medicine. 2016. (ISBN: 978-0-309-37357-9). Paperback. \$54.00.

Barriers and Opportunities for 2-Year and 4-Year STEM Degrees is generated from many white papers compiled on a multitude of factors affecting undergraduate STEM student success. The data extracted from these white papers illuminates the numerous influences that can affect STEM degree attainment. The book begins with two overarching questions: (1) Why do so many students who start out pursuing STEM lose interest before degree completion, and (2) How can we improve the quality of the STEM educational



Curators: Behind the Scenes of Natural History Museums. By Lance Grande. 2017. University of Chicago Press, Chicago, IL. (ISBN: 978-0-226-19275). Hardcover \$35.00.

Have you ever wondered how museum curators get into the business, what they do day to day, and how they manage such great collections of materials? If you answered yes to any of these questions, then this is the book for you. Lance Grande takes us on his journey from humble beginnings to that fateful day when a friend gave him a preserved fish fossil that began his love of collections. That gift set him on a path even he likely could not have anticipated. Suddenly the curiosity that had been in him as a

child was reawakened, and he shifted majors to Geology. From that day forward he was hooked (so to speak) and began working at the Natural History Museum in Chicago as a part of his studies. He knew, after three years of working there, that his goal was to become curator of the museum. This book provides some autobiographical account of his pathway, historical telling of museum milestones, and a candid introduction to different types of people he has worked with over the years.

Although this book is an interesting read, and I would recommend for people interested in a behind-the-scenes telling of what it means to be a curator (from lawsuits of *T. Rex* skeletons to attending gem shows and carrying home four-pound gold nuggets), I believe the deeper connection comes from Lance Grande's personal narrative. His humble blue collar origins, which lead to his being a first-generation college student who worked to support his own education, can be an inspiration to students, if they are looking in the right place. In addition, he tells a story of his interest being sparked outside the classroom initially, and seeking out those who could help him identify that passion rather than waiting for someone to come to him. His ultimate career path was not even on his radar when he set out to go to college. Through self-determination, one fateful fish fossil, and a desire to know, he wound up in the Geology Department at the University of Chicago. It is a testament to finding new paths and new interests and seeking out those who can help rather than settling for the known.

Each chapter of the book could be taken separately as needed, as I think most of the information would be of little use to the high school

classroom per se. However, the read is quite informative, and the author goes into detail on background data on the many different facets of his job that would never occur to those without museum experience. I believe high school students would find the stories compelling as a part of required readings, but as chapters; as a whole, they may lose interest. College students in the fields of Biology, Natural History, Geology, and the like might find the stories more interesting on a broader level, and the information does lend itself more to the unseen side of museum curation.

Overall, I would recommend this book as a supplement to natural studies.



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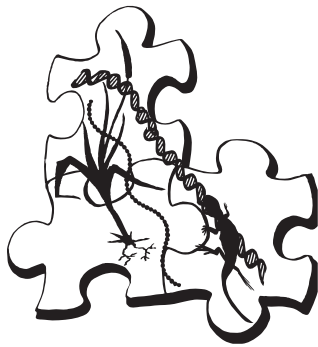
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Look for the BioClub logo to indicate recommended articles for NABT BioClub members. If you are interested in forming a chapter of the NABT BioClub, contact NABT at office@nabt.org.

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Cracking Cancer (DVD; 2017; 44 minutes; Bullfrog Films; <http://www.bullfrogfilms.com/catalog/crac.html>)

Practical applications of genomics can be seen in Bullfrog Films' recent release, *Cracking Cancer*. The riveting stories of cancer victims—some who survive, some who do not—grip viewers as they learn about the science behind some of the latest cancer-fighting techniques. In particular, viewers learn about a British cancer agency called Personalized OncoGenomics. The scientists working there carry out clinical trials on cancer patients in ways unique to each individual by sequencing the patients'

particular cancers. Honors and advanced placement students would benefit from watching this video both in affirming their understanding of genetics and in generating new understanding around biotechnologies.

Dr. Janessa Laskin appears on camera to describe the process by which they identify treatment for their patients. In short, a biopsy is made of a patient's tumor. The sample is genetically sequenced. DNA from one of the patient's normal cells is also sequenced. The two sequences are then compared, and the research scientists and doctors look for mutations that make the two sequences different. They specifically look for mutations on oncogenes (i.e., genes associated with cancer development). By understanding the specific ways in which the genes are different, they are able to propose unique treatments. Some of the treatment is unprecedented, such as the use of diabetes medication to block a growth factor.

Not every story has a happy ending. Viewers will meet survivors, and they will

get to know others who eventually succumbed to their illness. In some cases, they will hear the story directly from the deceased person and find out later that the treatment failed to work. In that sense *Cracking Cancer* offers an authentic look at both the advantages and shortcomings of biotechnology. But rather than inspire discouragement, this film may motivate students to consider cancer research careers. The cast of scientists is as diverse as the treatments they provide.

While this film could compliment a classroom lesson, the producers have not developed curriculum to support that kind of integration. Educators looking to use the film in meaningful ways will have to develop their own film-related activities. Moreover, the scientific content of the film, while not overwhelming, is better suited for upper-level high school biology courses.

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