VOL. 84 | NO. 1
JANUARY 2022

THE AMERICAN BIOLOGY TEACHER

Theme Issue: Mendel and Genetics

Gregor Mendel

Pisum sativum

NABT
National Association of Biology Teachers

Biology Lab Books

- **Grow your Program – We have helped schools double their program size.**
- **Cut your Budget – Our labs don't waste your resources.**
- **Affordable for Students – Our books are a fraction of the price of others.**
- **Proven Quality - Labs tested for 30 years across the country.**

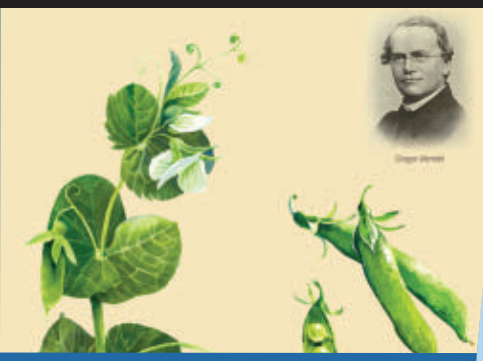
Biology, Human Biology, Human Physiology and more!

Complimentary copies are available for schools looking to adopt.
Email us today!
sales@biologylabbooks.com

View Our Products:
www.biologylabbooks.com

VOL. 84 | NO. 1
JANUARY 2022

THE AMERICAN BIOLOGY TEACHER



About Our Cover

This issue of *The American Biology Teacher* is dedicated to Gregor Mendel as we celebrate his 200th birthday this year.

Pictured on our cover is a drawing of *Pisum sativum*, the common garden pea, accompanied by an image of Gregor sometime after 1847. The garden pea was well suited to Mendel's investigations as it allowed for control over fertilization so that he could be sure of a given strain's parentage. The garden pea also demonstrates many easily discernible phenotypic traits, each controlled by a single gene that assorts independently during meiosis, including seed shape, flower color, seed-coat tint, pod shape, pod color, plant height, and flower location. As discussed in this issue's lead article, *Still Learning from Gregor Mendel after 200 Years*, Mendel's choice to study these traits of *P. sativum* was likely due to a combination of both luck and scientific insight, vindicating Pasteur's aphorism that "chance favors the prepared mind." The fundamental laws of inheritance that Mendel discovered through his meticulous investigations using this humble model organism were ultimately found to apply to all living things and ultimately laid the groundwork for the entire field of genetics.

Special thanks to graphic designer Michelle Finney, who created this special cover to honor Mendel and his groundbreaking work with peas that led to the modern science of genetics.

Contents

Erratum:

The ABT editorial office has been notified of an error in the order of the authors listed in the article "An Inquiry-Based Lab Activity to Investigate Potential Effects of Arbuscular Mycorrhizal Fungi on Seed Germination" published in the October 2021 issue of ABT (83.8). The correct order of authors is Lori Wollerman Nelson, KC Cifizzari, Tanya E. Cheeke.

THE AMERICAN BIOLOGY TEACHER

Printed by The Sheridan Press, on Forest Stewardship Council®–certified paper

The American Biology Teacher (ISSN 0002-7685, e-ISSN 1938-4211) is published nine times a year (monthly except June and July, Nov/Dec combined) for the National Association of Biology Teachers in collaboration with University of California Press, 155 Grand Avenue, Suite 400. Oakland, CA 94612-3764. Periodicals postage paid at Oakland, CA, and additional mailing offices. POSTMASTER: Send address changes to ABT, University of California Press, 155 Grand Avenue, Suite 400. Oakland, CA 94612-3764. E-mail: customerservice@ucpress.edu.

The American Biology Teacher is the official peer-reviewed journal of the National Association of Biology Teachers. All manuscripts must be submitted online at http://mc.manuscriptcentral.com/ucpress-abt.

Requirements for submissions can be found in the ABT Authors & Photographers Guidelines located online at https://nabt.org/files/galleries/ABT_Guidelines_July_2020.pdf.

The comments and opinions expressed in this publication are not attributed to and do not necessarily represent the views of the National Association of Biology Teachers. Announcements and advertisements in this journal for products do not imply endorsement of or by NABT.

Membership Information

NABT membership is $79 a year for full membership and $149 for organizational membership in the United States, Canada, and Mexico; $150 for full membership elsewhere. $48 of annual membership dues applies to a subscription of *The American Biology Teacher*. Student, adjunct faculty and early career teacher memberships are $35 and retired membership is $60 ($35 online only) if that person has been an NABT member for 10 years or more and is retired.

Subscriptions are offered on a calendar-year basis; members and subscribers receive all issues in the volume for the year in which their subscription is entered.

See http://abt.ucpress.edu/content/subscriptions-and-single-issues for single issue and institutional subscription orders, back issues from volume 69 forward, and claims information. Domestic claims for nonreceipt of issues should be made within 90 days of the mail date; overseas claims within 180 days. Mail dates can be checked at: http://www.ucpress.edu/journals.php?p=release. UC Press does not begin accepting claims for an issue until thirty (30) days after the mail date. For back issues before volume 69, contact NABT at publication@nabt.org.

Inquiries about advertising can be sent to abt@networkmediapartners.com. For complete abstracting and indexing coverage for the journal, visit http://abt.ucpress.edu. All other inquiries can be directed to customerservice@ucpress.edu.

For more information, visit **www.nabt.org**

NABT
National Association of Biology Teachers

Colichrome™ MF ECC - Kit

- **10 TESTS/BOTTLE**
- **RESULTS IN 24 HOURS OR LESS**
- **ACCURATE AND EASY TO UNDERSTAND**
- **E.COLI (DARK BLUE DOTS) COLIFORM (DARK PINK/RED DOTS)**

Visit www.rothbioscience.com for more information

OFFERS MEMBRANE FILTRATION PRODUCTS FOR:

- COLIFORM
- E.COLI
- ENTEROCOCCUS
- SALMONELLA
- LISTERIA
- LACTIC ACID BACTERIA

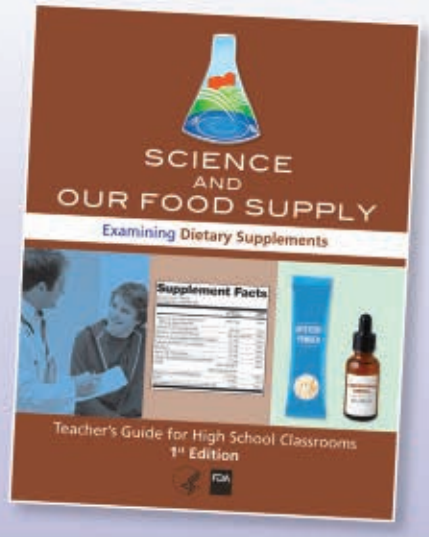
ROTH BIOSCIENCE, LLC

ROTH BIOSCIENCE, LLC Tel: (574)533-3351 HELLO@ROTHBIOSCIENCE.COM

Science and Our Food Supply

Examining Dietary Supplements

High School Edition



Millions of Americans take dietary supplements, and that number continues to rise. However, dietary supplement labeling can be confusing or misleading. Teens are particularly vulnerable targets for misunderstanding what dietary supplements are and are often unaware of the potential benefits and adverse effects they can have on their bodies. This curriculum is designed to empower students to evaluate the accuracy and credibility of information they see and hear about dietary supplements.

As dietary supplements attract interest among teenagers with perceived or real weight issues, sports performance goals, or overall appearance concerns, it is important that they have the right educational tools to help them understand whether to use a particular dietary supplement. The content and activities in *Science and Our Food Supply: Examining Dietary Supplements* are designed to intrigue and stimulate student curiosity and help them to make healthy decisions.

Your students will learn about:

- The Supplements Facts label
- Limited government oversight of dietary supplements
- Adverse event reporting
- Multivitamins
- Live microbials (commonly referred to as "probiotics")
- Athletic performance enhancement/bodybuilding products
- Dietary supplement advertising
- Caffeine and energy products
- Botanicals

Check out other topics in the *Science and Our Food Supply* series...

Food Safety

Nutrition

Biotechnology

www.fda.gov/teachsciencewithfood

FDA

President's Commentary

Chris Monsour

Teaching in a Time of COVID

As they say, the only constant in life is change. I am not sure who first said this, but I have certainly been uttering this phrase more now than ever as I try to make sense of what we have collectively been through as teachers specifically and in society generally. As we approach the two-year mark of living in COVID World, I would be remiss if my first message to you was not about how proud I am of all of us. I am proud of those who have stuck it out in the face of what seems to be insurmountable odds. There have been many days when I sat in the car staring at the building where I have been teaching for the past 23 years convincing myself that today will be better. If I can just get in the door, it will be better without pretending that online teaching is just as effective as the intimacy of face-to-face instruction. It will be better because I will be able to do science with my students. We will be able to make mistakes in the lab, discuss data, and I will be able to ask my students in person to tell me more, to ask what they would like to explore further, and engage in real conversation about science and life. It will be better because I won't have to ask them to put their thoughts in a chat or to turn on their camera if they can. Finally, it will be better because I will be doing what I have been doing for over half my life, I will be teaching kids about biology.

I have determined that *Teaching in a Time of COVID* will be the title of my first book if someone doesn't claim it first. But let's be optimistic. As we reflect on the past two years, I suspect we can all agree that there have been some changes for the better as it relates to biology teaching during this difficult and occasionally impossible task. I know I have been forced to take a hard look at what I am teaching and why I am teaching it. I hate to admit this, but until the period of forced introspection I had not taken a hard enough look at my teaching. I was in the same place for more than two decades and had developed what I thought was a good AP program and other effective and engaging science classes, but closer scrutiny is always useful.

Then, like many of you, I had little more than a weekend to create virtual versions of my classes. At first, we were told it was only for three weeks, so I told myself, I got this. I just need to tweak what I have done for many years, so no problem. But then everything changed. Our governor announced that we would be virtual for the rest of the year, and I remember sitting down at my dining room table, which had become my desk, putting my head in my hands, and questioning how I was going to do this. Many of my students have been with me every year since they were freshman, and now I wouldn't see them graduate. How would these kids take the AP exam or the end-of-course biology test? And so it went. Like many of you, I had questions but no answers.

But I knew I had to figure it out. When I had to create experiences for my students in an online world, I had to cut to make sure it was manageable not only the students but also for me. I constantly reviewed my goals, revised, and revisited the content. At times, I became my worst enemy. I realized that I was trying to recreate what happened in the classroom, but no matter what I planned it could not be the same. I was simply not getting the same results from the students. Those who were normally overachievers and vocal seemed lost while those who were usually silent in class seemed to do well in our technology-mediated, distanced classrooms. I began to see this as a huge unplanned and unexpected experiment in science education. Some of the greatest discoveries are serendipitous, and perhaps the past two years has taught us some useful lessons. I now know that what I would do in the face-to-face classroom was no longer viable virtually. However, I also learned that teaching at a distance is not the same as face-to-face, and to those who suggest otherwise, I say show me the evidence. The barrage of new apps and techniques for online learning are interesting and may have limited use, but nothing can replace the engaging experience of being in a classroom with a skilled and caring teacher.

I am sure those reading this have similar stories and have potentially learned these and other lessons, and we should share them. However, the most important lesson is that even after two years, we are a community of educators here to support each other. We educators can and should join our medical providers and other first responders as heroes of the pandemic. We have risen to the challenge and have continued to serve as sources of valid information about vaccines, immunization, the challenge of mutation, and even trust in science. We will continue to come together as a community and promote high-quality life science instruction as we support each other, our students, and our communities.

CHRIS MONSOUR (Chris_Monsour@tiffincityschools.org) is a high school biology teacher at Tiffin Columbian High School, Tiffin, OH 44883.

DOI: https://doi.org/10.1525/abt.2022.84.1.5

INSPIRING
ENCOURAGING
SUPPORTING

Nominate a teacher for a **2022** NABT award.

Submit your nomination online before March 15th

https://nabt.org/Awards-NABT-Award-Nomination-Form

NABT
National Association of Biology Teachers

Guest Commentary

Joseph D. McInerney

Honoring Gregor Mendel

This theme issue, devoted to genetics, acknowledges the centrality of that discipline, in conjunction with evolution, to our understanding of the history and nature of life on earth. Gregor Mendel and Charles Darwin, the recognized founders of their deeply intertwined fields, are—or should be—inseparable in biology education.

Although Darwin's work ultimately provided biology with its enduring conceptual basis—evolution by natural selection—he died unable to answer two central questions: Where does the biological variation central to differential selection reside, and how is it transmitted from one generation to the next? As James P. Evans details in his feature article in this issue, Mendel, whose 200th birthday occurs in July of this year, provided those answers, demonstrating the particulate nature of inheritance and the principles of segregation and independent assortment.

Unfortunately, Mendel's work escaped the attention of his contemporaries, including Darwin, attracting notice only with the rediscovery of his now-classic 1865 paper in 1900. The growth of genetics as a central biological discipline followed in short order, and the new field ultimately was joined to evolutionary biology by extensive mathematical formulations in population genetics. The resulting "modern synthesis of evolution" occurred in the 1930s and 1940s and continues to serve, with amendments, as a framework for research in biology and biomedicine. So central is that framework to the life sciences that in 1973 this journal published Theodosius Dobzhansky's now-famous article declaring that "nothing in biology makes sense except in the light of evolution." Genetics provides much of the integral supporting structure for evolution theory, including the mechanisms for the variation that is fuel for the fires of selection and for the smooth transmission of information that maintains biological continuity within species.

Genetics is the study of inherited biological variation, though in some four decades of my asking diverse audiences—students, teachers, health professionals, the public—to define the subject, the concept of variation has arisen only rarely without prompting. Perhaps that lack of recognition is a function of the way we approach genetics in formal educational settings and in the media broadly defined. In those settings, the focus generally is structures and processes—chromosomes, meiosis, DNA, genes, replication, genomes, sequencing—all while failing to emphasize the underlying messages of the discipline.

Recently, as the world has struggled to understand and control COVID-19, the concept of biological variation has come to the fore for the public in inescapable and often worrying updates on the variants of SARS CoV-2. In the September 2020 *ABT* Guest Commentary, Gordon Uno discussed the opportunities COVID-19 provides to address genetic variation and evolution in the classroom, as we track the evolution of this virus in real time. Uno's observation is apt, and we have traversed about 120 years from the rediscovery of Mendel's work on the "factors" carrying variation to the extraordinary ability to elaborate variation at the level of individual DNA bases, where it is encoded in a universal, digital information molecule. The recent literature carries the news that a large team of scientists has filled in the previously intractable gaps in the human genome and in the process has uncovered more than two million new indications of human genetic variation.

Advances in sequencing technology also have generated the genomic sequences of many other species, and their individual and collective analyses affirm that, as Darwin demonstrated, all of life shares a single history and is related by descent with modification. The unity of life was long evident from previous work in fields such as comparative anatomy and embryology, but access to complete genetic sequences has made our understanding of the underlying relationships even more certain, in some cases even allowing revisions in systematics and taxonomy.

As important as Mendelian genetics is, we have, unfortunately, allowed the venerable priest's original formulations to become a bit of an impediment in our instruction, which often emphasizes single-gene disorders because they illustrate Mendelian inheritance. That focus ignores the reality that most human traits, including many of the world's leading causes of morbidity and mortality, result from the interactions of multiple genes with environmental variables. Indeed, a 2009 article by Michael Dougherty in the *American Journal of Human Genetics* proposed that we begin genetics instruction with examples of complex, quantitative traits, rather than traditional single-gene characters.

In any case, genetics encompasses much more than genetic disease, and the diverse articles in this special issue demonstrate the pervasive nature of genetics and the questions its concepts and tools allow scientists to pursue. The amount of genetics knowledge now available challenges us not to get lost in the ever-accumulating details as we teach. Darwin knew that variation is the rule, not the exception, in the living world. Mendel helped us to understand how variation is transmitted. We should constantly remind students that the structures and processes we describe as we teach genetics exist to harbor, preserve, and transmit biological variation, the lifeblood of any species.

JOSEPH D. MCINERNEY was on the staff of the Biological Sciences Curriculum Study for 22 years and was its director from 1985–1999. He was president of NABT in 1991. e-mail: mcinerney.joseph1@gmail.com.

DOI: https://doi.org/10.1525/abt.2022.84.1.7

FEATURE ARTICLE

Still Learning from Gregor Mendel after 200 Years

JAMES P. EVANS

Abstract

Although unrecognized for his scientific achievements during his life, Gregor Mendel pioneered our modern understanding of the gene, work that shaped the field of genetics and advances in biology and medicine. The field that he set in motion 200 years ago lies at the center of current ethical debates about the future of humanity, the limits of science, and how best to employ our knowledge for betterment of the human condition. Mendel's personal life also offers lessons, especially for those of us engaged in teaching future generations.

Key Words: *Mendel; history of medicine; history of science; genetics; Darwin; philosophy of science.*

○ Heredity

I am the family face;
Flesh perishes, I live on,
Projecting trait and trace
Through time to times anon,
And leaping from place to place
Over oblivion.

The years-heired feature that can
In curve and voice and eye
Despise the human span
Of durance—that is I;
The eternal thing in man,
That heeds no call to die.

—Thomas Hardy, 1917

○ Introduction

In this strikingly prescient poem, the famous English author Thomas Hardy elegantly articulated the central elements of a profound mystery—what is the mechanism of heredity? What explains why a daughter looks like her mother or a son's gesture so uncannily echoes that of his long-dead father? How does such information "leap" from one generation to another, heeding no "call to die"? Shortly before Hardy penned these lines, the scientific work of Gregor Mendel, a previously obscure Augustinian priest, was rediscovered, propelling him to posthumous fame and providing an answer to this age-old mystery.

This July 20, we celebrate Mendel's 200th birthday (Mendel Museum, 2021). Although unrecognized for his scientific achievements during his life, Mendel pioneered the modern conception of the gene, anticipating the field of genetics and the astounding advances in biology and medicine that we are now witnessing. Recognizing these advances and Mendel's unsung genius would be reason enough to celebrate his birth. But we can still learn from Gregor Mendel. For genetics now lies at the center of thorny ethical debates about the future of humanity, the limits of science, and how (or whether) to employ our knowledge for betterment of the human condition. And on a more personal note, Mendel's life—his struggles, anxieties, and hopes (both requited and unrequited)—offer us lessons that may be less grand but are perhaps more intimately applicable to our own lives, especially for those of us engaged in teaching future generations.

> *"Mendel's results pointed to a radically different notion—that the units of inheritance persisted unchanged in a 'particulate' manner and could reappear, unchanged, in subsequent generations."*

○ A Brief Biographical Sketch

Johann Mendel (who only later took the name of Gregor upon entering the priesthood) was born to a modest farming family in the small agricultural village of Heinzendorf (now Hynčice in the Czech Republic). His father, Anton, like many farmers, was interested in the creation of hybrids to improve the qualities of fruit trees, presaging Gregor's

The American Biology Teacher, Vol. 84, No. 1, pp. 8–13, ISSN 0002-7685, electronic ISSN 1938-4211. DOI: https://doi.org/10.1525/abt.2022.84.1.8.

own lifelong fascination with hybridization. Mendel's mother, Rosine, was a devoted mother who, like his sisters, doted on the young man. A few central themes emerge from Mendel's early life—a keen intellect, a disinclination toward farming, and periods of incapacity that saw him bedridden for months at a time (Henig, 2001). For those who wish for more detail about Mendel's life, see the biographies by Robin Marantz Henig, *The Monk in the Garden* (2001), and Simon Mawer, *Gregor Mendel: Planting the Seeds of Genetics* (2005).

Mendel's intellect was recognized and fostered by his parents, who, although of limited means, managed to send him to a "gymnasium," or secondary school, in the nearby city of Troppau (now Opava, in the Czech Republic). Before graduating at the age of 17, he abruptly returned home, taking to his bed for months. From our vantage point, it seems almost certain that Mendel was suffering from what would be the first of several episodes of severe clinical depression. Ironically, given his scientific legacy, it is possible that Mendel inherited from his melancholic father a predisposition to depression, a condition now known to have a strong genetic component (Mullins & Lewis, 2017). Possible genetic predisposition aside, the 17-year-old was also under considerable stress as, due to his family's meager resources, it was necessary for him to support himself while maintaining academic standing. Moreover, as the eldest son Gregor was expected to take over the family farm, a prospect that held no appeal for him. Ultimately, he spent four months in bed, cared for by his mother and 10-year-old sister, Theresia, who encouraged him during what must have been a difficult time for the entire family. Consistent with current knowledge about clinical depression, which often lifts after several months, Mendel reentered the gymnasium and graduated in 1840.

In the fall of 1840 Mendel enrolled in the Philosophical Institute of the University of Olomouc, meant as a stepping-stone to students aspiring to a university education. Young Gregor supported himself by tutoring, but as a German speaker, he struggled to attract students in this Czech-speaking city. In 1841, hungry and feeling defeated, he fled home and again was bedridden for a year. It was difficult for him to see a way forward—dreams of an academic life seemed unreachable, he still faced expectations that he would take over the family farm, and he was likely crippled with feelings of inadequacy and anxiety. However, once again his family rallied round. His older sister and her husband agreed to take over the farm, relieving Mendel of this daunting obligation, and his 12-year-old sister, Theresia, loaned Gregor her dowry to finance his studies. It is difficult for us today to appreciate the magnitude of Theresia's generosity since her life's prospects were directly tied to her ability to marry well. Putting her future at risk to help her 19-year-old brother, incapacitated by what must have seemed a perplexing malady, was an act of immense love and selflessness (Historical Figures, 2018). Mendel never forgot her generosity; he and Theresia maintained a loving relationship throughout his life, and later he helped to support her children, two of whom became physicians.

Bolstered by his family's support, Mendel returned to Olomouc to complete his studies in philosophy and physics. However, his road remained challenging, and by 1843 it became clear that even with Theresia's dowry he simply could not afford university. Would his and his family's struggles be in vain? At this critical juncture his physics professor, Friedrich Franz, made a life-changing suggestion to Mendel (Hasan, 2004). Franz, a priest, pointed out that the Augustinian Order of the Catholic Church placed great value on intellectual pursuits and that the priesthood could offer Mendel a viable path to a life of learning, research, and teaching. Thus, at the age of 21 Mendel traveled to Brno (now in the Czech Republic) and entered the Abbey of St. Thomas to begin training as a priest.

Although Mendel found a degree of stability in the monastery and a mentor in Abbot Cyril Napp, his path remained difficult. After five years of study Mendel was ordained a priest in 1847—only to realize that he was wracked with anxiety when called upon to perform his official duties, especially giving last rights or comforting the sick. Once again it seems that Mendel was plunged into debilitating depression, this time being rescued by Abbot Napp, who realized that while Mendel was decidedly not "priest material," he was an excellent teacher. Napp petitioned the bishop to allow Mendel to become a teacher of Greek, math, and physics to local high school students, fulfilling an obligation of the Augustinian Order and providing a viable way forward for Mendel.

Mendel proved to be a popular teacher, and his success compelled him to seek full teaching certification in 1850 (Richter, 2015). However, Mendel suffered from devastating test anxiety; he performed poorly in the written certification exam and disastrously in the oral component. The examiners failed him but, recognizing his passion and earnestness, suggested he obtain further schooling at the University of Vienna. After Abbot Napp successfully pleaded with the bishop for dispensation, Mendel began classes at the age of 29, fulfilling his long-standing dream of attending university.

Mendel's two years at university were tremendously formative. He studied under Christian Doppler (of the Doppler effect); Andreas Von Ettingshausen, who provided Mendel with a rigorous mathematical education; and Franz Unger, a botanist who introduced Mendel to his own experiments in which he had used the garden pea (!) as a model to study the transmission of hereditary traits. Thus, equipped with rigorous training in subjects that in retrospect can only be seen as stunningly fortuitous, the 31-year-old monk headed back to St. Thomas where he began breeding mice to investigate coat-color transmission. However, Mendel's bishop was aghast that one of his priests would engage in research involving sex and forbade this line of research. So, in 1854 Mendel switched his research to the garden pea, demonstrating his sly sense of humor along the way by remarking, "You see, the bishop did not understand that plants also have sex" (Henig, 2001).

○ Mendel's Research

Although the transmission of traits from parents to offspring was well know, the mechanisms involved were utterly mysterious in the 19th century. This profound mystery, coupled with its obvious practical importance in agriculture, made it an appealing focus of study for an ambitious young scientist. While others had studied trait transmission in plants, Mendel employed several previously neglected strategies. Critically, he spent two years ensuring that his parental stocks "bred true" for the traits he would investigate (in today's parlance, ensuring that they were homozygous for the traits of interest) (Edelson, 1999). Another innovation was his focus on seven specific traits of *Pisum sativum* (including flower color and seed shape) that turned out to assort independently during meiosis (being, as we now understand, distant from one another in the *P. sativum* genome). Although it is often assumed that Mendel was "lucky" in the traits he chose to study, it is more likely that his choices were practical and insightful. During the years he was establishing breeding stocks, he likely noted which traits gave the most consistent results and thus focused on those. The classical view of the scientific method often posits a simplistic notion

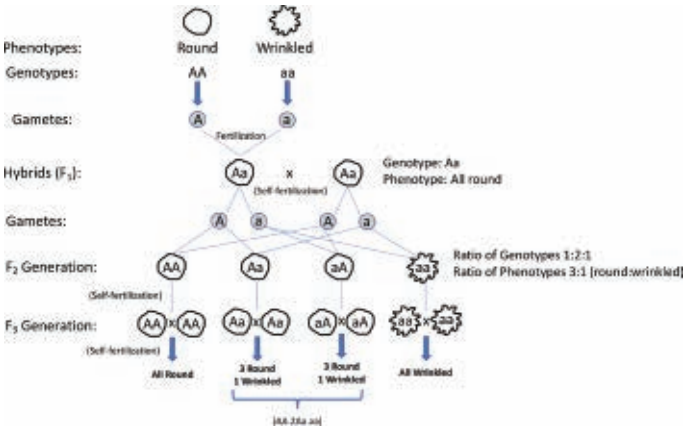


Figure 1. An example of Mendel's first experiment in the transmission of seed shape, in *Pisum sativum*. After painstaking work to ensure that his parental stocks "bred true," he crossed plants that each produced only round or only wrinkled (a trait also known as angular) seeds to yield the "F_1" generation. All peas in the F_1 generation were round—the wrinkled trait had "disappeared." However, in the 7324 peas of the next "F_2" generation, while the majority (5474) were round, 1850 of the F_2 peas were wrinkled—the trait that had been in the grandparental generation suddenly reappeared, with a ratio of three round to one wrinkled pea. The underlying "element" encoding the wrinkled trait had not vanished at all but had only been somehow "hidden" while in the F_1 generation. It should be noted that accusations by statistician Ronald Fisher in 1936 that Mendel was guilty of falsifying his data have not been upheld by subsequent investigation: reproduction of Mendel's experiments and reanalysis of Fisher's accusations conclusively demonstrate no bias (Monaghan & Corcos, 1985; Novitski, 2004; Hartl & Fairbanks, 2007).

that observation leads to hypotheses in that rigid order. However, scientists typically pursue observations and hypotheses in a synergistic manner, formulating hypotheses while simultaneously searching for observations that support (or disprove) them. Thus, Mendel was likely formulating the hypothesis of independent segregation while feeling his way forward in his research, thereby influencing the traits he would ultimately choose to study.

Mendel spent nine years crossing and examining some 300,000 peas, observing multiple traits and analyzing what it all meant (Henig, 2001). At that time the leading view of heredity was the notion of "blending inheritance"—that progeny are a mixture of parental traits. However, Mendel's results pointed to a radically different notion—that the units of inheritance persisted unchanged in a "particulate" manner and could reappear, unchanged, in subsequent generations.

Mendel's patience and keen mathematical mind led him to formulate his now-famous laws of inheritance. Today we would summarize them as follows:

1. Each inherited trait is governed by an underlying element (gene), which can exist in alternate forms (alleles) that lead to different visible traits (phenotypes).
2. Genes for different traits are inherited independently of one another.
3. Each individual plant or animal possesses two sets of genes, one set inherited from each parent.
4. Genes remain unaltered from generation to generation.
5. Alleles of genes can be dominant or recessive; a recessive trait will be displayed only if an individual inherits two recessive alleles.

Mendel could only anticipate our modern understanding of the gene, simply recognizing that his "elements" were somehow responsible for the traits that were ultimately manifested. Although major modifications of Mendel's laws would occur with the description of, for example, mutations and genetic linkage, Mendel's insights continue to undergird our understanding of heredity. It is for good reason that even today, from middle school to medical school, we teach Mendelian genetics to describe the transmission of traits and diseases that are dictated by single genes.

Mendel's subsequent studies of other species (including beans, snapdragons, and maize) generally confirmed his conclusions, leading him to remark that "the law of development discovered for *Pisum* applies also to the hybrids of other plants" (Henig, 2001). But while Mendel recognized the general applicability of his conclusions, the rest of the world failed to. He presented his work to the Natural History Society of Brno in 1865 and sent 40 reprints of his paper to preeminent scientists of the day (Mendel, 1866). Sadly, the only scientist who didn't ignore his results at the time, Carl Nägeli, failed to understand them.

While Mendel evinced interest in the natural world to the end of his days, by 1868 his biological research ended. In that year his beloved Abbot Napp died and Mendel was elected as the next abbot of St. Thomas, effectively ending his research career due to new responsibilities. Mendel famously enjoyed the splendid food of St. Thomas, and his obvious weight gain over the years, coupled with his failing eyesight and deteriorating kidney

Parallel Currents of Thought in 19th Century Science

Mendel may have been influenced considerably by the work of contemporaries in physics and chemistry who were then establishing the first atomic theories, research the monk would have been well aware of due to his university studies. The finding of whole-number ratios in both atomic weights and the combinatorial chemical properties of elements were critical clues to the structure of matter (Scientific Odyssey, 2015). In a parallel manner, Mendel recognized that his finding of a whole-number ratio of round to wrinkled peas in the F_2 generation strongly hinted at fundamental "rules" underlying the mechanism of inheritance. Further hinting at the influence of those investigating the structure of matter on Mendel was his choice of the German word for "element" to describe his genetic factors. Just as chemists were discovering that the elements of the periodic table remained unaltered throughout chemical reactions, appearing in different forms depending only on their combinations, Mendel's "elements" were likewise not destroyed or altered when passing from generation to generation, simply revealing different properties when combined in various ways. Alas, we will likely never know details of Mendel's reasoning since his personal papers were burned by the abbot who succeeded him upon his death (Carlson, 2004).

function, indicates that it was likely that he suffered from uncontrolled Type II diabetes. He died of renal failure on January 6, 1884, at the age of 61.

○ Lessons from Mendel

Delayed Recognition

As is common knowledge (and perhaps part of Mendel's allure—after all, who hasn't felt unappreciated?), Mendel received no contemporary recognition for the formulation of laws that would explain an age-old mystery of life and undergird a new science. Indeed, it would be almost 40 years after his original publication that his work was rediscovered.

What explains the decades in which Mendel's work was neglected? One likely explanation is that Mendel's work represented a "premature" scientific discovery (Stent, 1972), a discovery that cannot be contextualized in terms of contemporary understanding and is simply ahead of its time. Other examples of such "premature" discoveries include Copernicus's heliocentric model of the universe and Oswald Avery's identification of DNA as the genetic material. Premature discoveries are typically neglected (at best) or ridiculed (at worst) by contemporaries, until further developments make them comprehensible and they finally enter the scientific mainstream. As Mendel was arguably the first biologist to pursue rigorous mathematical analysis of data, his contemporaries were woefully ill-equipped to appreciate his statistical arguments and thus ignored them. Also contributing to the obscurity of his work, Mendel was a monk living outside the scientific establishment, without ample time to devote to his research and its promotion due to teaching and administrative responsibilities. Finally, Mendel was not inclined to self-promotion, being a shy man immersed in a religious community that frowned upon vanity. Taking all this into consideration, it may be less a wonder that Mendel's work was ignored than that he ultimately received credit for it.

We can learn clear lessons from the historic neglect of Mendel's work. We must make conscious efforts to be open to new ideas, even when they don't comport with our preconceived notions. We need to be receptive to novel cross-disciplinary approaches (such as Mendel's application of mathematics to biology). And critically we must disregard humble origins of an idea and judge it on its merits. In our modern world of pre-prints, peer review, and the internet, one might think that the playing field is level—but science is not yet free of bias, hostility toward novel notions, class considerations, or a tendency to look askance at outsiders with new ideas.

Mendel & Darwin

The delay in appreciating Mendel's work leads to a compelling historical "what if" scenario: imagining a meeting of Charles Darwin and Gregor Mendel. These contemporaries had much in common—both were scientifically curious, both were seeking basic laws that would explain fundamental scientific mysteries and, unbeknownst to Darwin, the work of Mendel solved many problems that plagued Darwin's theory of evolution by natural selection.

Darwin's theory is the bedrock upon which all biology is founded. But during Darwin's life, ignorance regarding the mechanism of inheritance was a profound impediment to the theory's acceptance. The current prevailing notion of blending inheritance was incompatible with Darwin's theory since in this model new, advantageous traits would be quickly diluted and lost from a population. Darwin's theory required a particulate mechanism of inheritance, in which the factors that control traits persist through generations—precisely what Mendel demonstrated to be the case a mere six years after *The Origin of Species* was published.

Mendel's copy of *The Origin of Species* is heavily annotated with marginal notes that show he understood the strong support his work lent to Darwin's theory (Evans, 2021). And it was not for lack of trying by Mendel that his ideas were unappreciated by Darwin: Mendel sent Darwin a copy of his paper—where it appears to have remained unread in Darwin's library. We know this because at that time many books and manuscripts needed to be cut open at the top and sides to be read. Sadly, Darwin's copy of Mendel's paper was found uncut, indicating that it had never been opened. Had Darwin or one of his supporters been capable of appreciating Mendel's work, many contemporary objections to evolutionary theory would have been swept aside and the "modern synthesis" combining genetics and evolution may have occurred earlier (Lorenzano, 2011). Again, Mendel's work was "premature"—Darwin and his contemporaries were not inclined toward mathematical analysis and were simply ill-equipped to see that his work offered a central pillar of support to the theory of evolution.

Finally, from a personal standpoint, it seems a pity that Darwin and Mendel never met. Beyond their mutual scientific and professional interests, they were both humble, friendly, and curious men. I suspect they would have enjoyed one another's company greatly.

Heeding Science

The field of genetics has improved the human condition in countless ways, from agricultural to medical advances. However, the aftermath of Mendel's rediscovery also tells a cautionary tale. Trofim Lysenko, who directed the USSR's Institute of Genetics in the 1940s, decided that Mendel's laws were antithetical to Communist ideology, formulating his own (evidence-free) theory of "environmentally acquired inheritance." His ideologically motivated attacks on science, backed by the power of a totalitarian state, led to the persecution, imprisonment, and even death of dissenting scientists. More broadly, this embrace of state-sponsored pseudoscience led to the implementation of devastating agricultural practices responsible for famines that killed millions of Soviet citizens (Kean, 2017). Extension of these ignorant but ideologically "correct" notions to China in 1958 led, in part, to the Great Chinese Famine of 1959–62.

Mendel's chilly reception by those blinded by ideology is highly relevant today. The world's response to the COVID pandemic reminds us that science and evidence matter. When a society abandons reliance on fact and ideology eclipses evidence, we court disaster. One need look no further than today's efforts by some to deny overwhelming evidence of human-induced climate change because scientific reality threatens their political beliefs and business interests. We engage in willful ignorance at our own peril.

Genetics in Medicine—Promise and Peril

It is remarkable how quickly after the rediscovery of Mendel's work in 1900 that it was found to apply not just to garden peas but to humans when, in 1902, Archibald Garrod observed that transmission of the human disease alkaptonuria conformed to Mendelian laws (Prasad & Galbraith, 2005). In the ensuing century medical

geneticists described over 7000 diseases that are caused by changes in a single gene and therefore demonstrate Mendelian transmission (OMIM). Going beyond single-gene disorders, we now recognize that almost every human disease has at least some genetic component, usually from the contribution of variants in numerous genes, each with a small effect.

In the 1940s Avery and coworkers demonstrated that the chemical at the heart of Mendel's "elements" was DNA (ironically another example of a premature scientific discovery) (Avery, 1944). In 1953 Watson and Crick elucidated DNA's double helical structure, relying significantly on the research of Rosalind Franklin (who, like Mendel, did not receive sufficient credit during her lifetime) (Watson & Crick, 1953).

A century after the rediscovery of Mendel's work, the international effort to sequence the human genome was declared complete (International Human Genome Sequencing Consortium, 2001), and today the tools of molecular genetics are routinely used to investigate every malady to which humans are subject.

Genetics has been particularly successful in the realm of public health. Every child on earth born in a developed country undergoes newborn screening, through which countless lives have been improved or saved; soon we will likely see similar, routine genetic analysis of adults to identify those at high risk of severe but preventable disease (Evans et al., 2013). Genome-scale sequencing of individual patients has become a powerful diagnostic tool, and we now stand poised on an era in which we will routinely manipulate Mendel's elements through gene therapy to treat a host of devastating diseases (Dunbar et al., 2018).

Mendel would have been deeply gratified to see the benefits to human health that were anticipated by work he carried out in his monastery garden with the humble garden pea. However, this gentle monk might also caution us that uncritical application of knowledge can be a double-edged sword as our burgeoning ability to manipulate the human genome presents difficult ethical dilemmas. Most might agree that the ability to genetically tweak an individual to eradicate a serious disease such as sickle cell anemia is desirable. But what about parents who wish to have a baby who is taller, smarter—or lighter skinned? From Nazi Germany to the US, we witnessed chilling abuses of human rights during the eugenics era, which began distressingly quickly after the rediscovery of Mendel's work (Bashford, 2010). How much more room for misapplication and outright evil is now afforded by our growing power over the human genome? This is not to argue that we should eschew research designed to better understand our world. But humans have a long history of (ab)using scientific knowledge, and Mendel might well caution us to tread carefully as our increasing knowledge gives us the power to change the genetics of individuals, future generations, and ultimately our species itself.

Mendel—Student and Teacher

Mendel played many roles throughout his life, including priest, abbot, brother, and scientific researcher. But of all his roles, few were as prominent as those of student and teacher. Just as Mendel's research remains relevant today, his life as a student and teacher holds invaluable lessons for us.

As a student, Mendel was influenced repeatedly by teachers and mentors who helped him overcome overwhelming obstacles. His professor Friedrich Franz stepped in during one such crisis, guiding Mendel toward the monastery and enabling an academic life otherwise unobtainable for this young man of limited means. Likewise, the supportive Abbot Napp recognized both Mendel's weaknesses and strengths, seeing that while Mendel was a poor priest, he was an excellent teacher, helping find a path that coincided with his talents rather than trying to force him into a preconceived role for which he was ill-suited.

When Mendel twice failed to obtain full teaching certification, the very same panel of examiners who unanimously failed him paved the way for study at the University of Vienna, transforming failure into an opportunity that would change Mendel's life—and the history of science. At university, Mendel's mentors profoundly influenced his subsequent research career by emphasizing combinatorial mathematics and the use of the garden pea as a model organism.

Mendel's crippling test anxiety, which on multiple occasions threatened to derail his future, serves as a lesson to those of us who are charged with assessing and mentoring students. While rigorous assessment is critical to education, blind adherence to testing formats that ignore individual variation seem destined to throw otherwise promising students on the academic scrap heap. Mendel's examiners recognized that he had earnest passion and they worked to foster his potential. They saw Mendel as an individual worthy of another chance, helping him find a way forward that coincided with his strengths rather than focusing on his limitations. It is highly unlikely that his teachers suspected that his work could change the course of science—it is impossible for anyone to see that clearly into the future. Rather, a teacher's role is not necessarily to identify genius but to foster each student in ways that harness and promote their strengths. By doing so we may indeed occasionally (sometimes unknowingly) foster genius—but regardless we will routinely improve individual lives and propel human knowledge forward.

Mendel's financial struggles are highly relevant today as we cope with a higher educational system that is increasingly out of reach to those with limited means. Mendel was brilliant and hard-working. But he was also lucky to have a generous family who sacrificed to provide for his education and a monastic path that enabled him to earn a university degree. How many other students throughout history who might have advanced human knowledge were thwarted because they couldn't afford schooling? We are attracted to the story of the genius who overcomes great odds. But even genius cannot blossom without help. We do ourselves and society a grave disservice if we do not facilitate accessible, quality education for all.

Finally, Mendel's lifelong struggles with depression and anxiety are both poignant and instructive. The unstinting support provided to the young man by family and compassionate teachers is remarkable, especially in an era when mental health struggles were often seen as moral failings rather than medical disorders. That Mendel was not stigmatized—but rather encouraged and loved—allowed him to rise above these profound obstacles and not only contribute great things to the world but also find a path in life that allowed for personal satisfaction and a measure of happiness. Interestingly, the same can be said of Charles Darwin, who struggled with anxiety throughout his life and yet, with the support of friends, mentors, and a loving family, would also go on to greatness. We now live in a world that recognizes mental health as a medical condition (with treatments undreamt of in the days of Mendel and Darwin), but all too often continue to stigmatize those suffering from these cruel diseases. It is vital that we recognize when our students are suffering and offer them assistance and kindness to allow them to rise above such challenges.

○ Conclusion

As we celebrate Gregor Mendel's birthday, we may see his life in somewhat tragic terms because his work was unrecognized by contemporaries. However, that would be doing him a disservice. His was a full life with loving family, friends, fulfilling work, and the joys derived from a life of learning. What more can one really ask from this world? Gustav von Niessl, a contemporary and friend of Mendel's who lived to see his posthumous fame, reported that during his years of anonymity Mendel was fond of telling his friends, "My time will come" (Henig, 2001). Whether this appealing anecdote is true or not, it is instructive to examine lines from a poem Mendel wrote as an adolescent:

May the might of destiny grant me

The supreme ecstasy of earthly joy,

The highest goal of earthly ecstasy,

That of seeing, when I arise from the tomb,

My art thriving peacefully

Among those who are to come after me.

This poem is usually interpreted to illustrate Mendel's longing for (what was ultimately to be unrequited) fame. However, I am struck that at the root of his adolescent fantasy there was a more mature aspiration—not a shallow version of worldly fame but ultimately a hope that he might contribute something of value to this world. Indeed, his poem seems to eerily presage just the sort of posthumous recognition that would be his.

Surely if Mendel were able to glimpse our world and how his work has blossomed, he would be joyous indeed.

References

Avery, O.T., MacLeod, C.M. & McCarty, M. (1944). Studies on the chemical nature of the substance inducing transformation of pneumococcal types—induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III. *Journal of Experimental Medicine*, *79*(2), 137–58.

Bashford, A. & Levine, P. (2010). *The Oxford Handbook of the History of Eugenics*. Oxford: Oxford University Press.

Carlson, E.A. (2004). Doubts about Mendel's integrity are exaggerated. In *Mendel's Legacy: The Origins of Classical Genetics* (pp. 48–49). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Dunbar, C.E., High, K.A., Joung, J.K., Kohn, D.B., Ozawa, K. & Sadelain, M. (2018). Gene therapy comes of age. *Science*, *359*(6372), eaan4672. https://doi.org/10.1126/science.aan4672.

Edelson, E. (1999). *Gregor Mendel and the Roots of Genetics*. Oxford: Oxford University Press.

Evans, J.P. (2021). Author's personal examination of Mendel's copy.

Evans, J.P., Berg, J.S., Olshan, A.F., Magnuson, T. & Rimer, B.K. (2013). We screen newborns, don't we?: realizing the promise of public health genomics. *Genetics in Medicine*, *15*, 332. https://dx.doi.org/10.1038%2Fgim.2013.11.

Hartl, D.L. & Fairbanks, D.J. (2007). Mud sticks: on the alleged falsification of Mendel's data. *Genetics*, *175*(3), 975–79. https://doi.org/10.1093/genetics/175.3.975.

Hasan, H. (2004). *Mendel and the Laws of Genetics*. Rosen Publishing Group.

Henig, R.M. (2001). *The Monk in the Garden: The Lost and Found Genius of Gregor Mendel, the Father of Genetics*. Boston: Houghton Mifflin.

Historical Figures. (2018). Gregor Mendel: get to know the father of genetics [podcast]. Spotify.

International Human Genome Sequencing Consortium. (2001). Initial sequencing and analysis of the human genome. *Nature*, *409*, 860–921.

Kean, S. (2017, December 19). The Soviet Era's deadliest scientist is regaining popularity in Russia. *The Atlantic*.

Lorenzano, P. (2011). What would have happened if Darwin had known Mendel (or Mendel's work)?. *History and Philosophy of the Life Sciences*, *33*(1), 3–49.

Mendel, J.G. (1866). *Versuche über Pflanzenhybriden* (pp. 3–47). Abhandlungen. (English translation: Druery, C.T., Bateson, William (1901). Experiments in plant hybridization. *Journal of the Royal Horticultural Society*, *26*, 1–32.)

Mendel Museum. (2021). *Gregor Johann Mendel*. https://mendelmuseum.muni.cz/en/about-the-museum/gregor-johann-mendel.

Monaghan, F. & Corcos, A. (1985). Chi-square and Mendel's experiments: where's the bias?. *Journal of Heredity*, *76*(4), 307–309. https://doi.org/10.1093/oxfordjournals.jhered.a110099.

Mullins, N. & Lewis, C. (2017). Genetics of depression: progress at last. *Current Psychiatry Reports*, *19*, 43. https://doi.org/10.1007/s11920-017-0803-9.

Novitski, C.E. (2004). On Fisher's criticism of Mendel's results with the garden pea. *Genetics*, *166*(3), 1133–36. https://doi.org/10.1534/genetics.166.3.1133.

Online Mendelian Inheritance in Man. (2021). https://www.omim.org.

Prasad, C. & Galbraith, P. (2005). Sir Archibald Garrod and Alkaptonuria—story of metabolic genetics. *Clinical Genetics*, *68*(3), 199–203. https://doi.org/10.1111/j.1399-0004.2005.00487.x.

Richter, C. (2015). Remembering Johann Gregor Mendel: a human, a Catholic priest, an Augustinian monk, and abbot. *Molecular Genetics & Genomic Medicine*, *3*(6), 483–85. https://doi.org/10.1002/mgg3.186.

Scientific Odyssey. (2015). Episode 2.7. The Periodic Atom [podcast]. https://thescientificodyssey.libsyn.com/episode-27-the-periodic-atom.

Stent, G.S. (1972). Prematurity and uniqueness in scientific discovery. *Scientific American*, *227*(6), 84–93. https://doi.org/10.1038/scientificamerican1272-84.

Watson, J. & Crick, F. (1953). Molecular structure of nucleic acids: a structure for deoxyribose nucleic acid. *Nature*, *171*(4356), 737–38. https://doi.org/10.1038/171737a0.

JAMES P. EVANS, MD, PhD, is the retired Bryson Professor of Genetics and Medicine at The University of North Carolina, Chapel Hill, NC; email: jamesphilipevans@gmail.com.

FEATURE ARTICLE

Recognizing Mendel: Rediscovery, Discovery, or Neither?

Erik Zevenhuizen

Abstract

In 1900, three botanists claimed they had found regularities in inheritance, which soon would be known as Mendel's Laws, without knowing the work of Gregor Mendel or of each other. Their claims of independent (re)discovery have been thoroughly studied during the past decades, with various outcomes. The case is still of interest today as it offers an interesting example of how science is done.

Key Words: *history of science (biology); scientific competition; heredity; genetics; Gregor Mendel; Hugo de Vries; Carl Correns; Erich von Tschermak.*

In the April 1900 issue of the *Berichte der Deutschen Botanischen Gesellschaft* (reports of the German Botanical Society), Hugo de Vries (1848–1935), professor of botany at the University of Amsterdam, presented a paper in which he described regularities in inheritance in plants he had discovered during the previous years. However, he was not the first to publish them, as he confessed. Gregor Mendel had described them 35 years earlier. De Vries had become aware of this after he had deduced the rules himself, in part because Mendel's paper reporting his work with garden peas (*Pisum*) was only very rarely referred to. With his own results at hand, obtained from crossings of over a dozen species, De Vries concluded that the rules Mendel found have 'allgemeine Gültigkeit' (general validity). Hence the title of his paper: *Das Spaltungsgesetz der Bastarde* (the law of segregation in hybrids). The issue in the following month contained a paper by Carl Correns (1864–1933), lecturer at the University of Tübingen. Triggered by De Vries's announcement, he claimed that he had recently found the rules as well, while equally being unaware of Mendel's results, but had read the paper since then also. The same claim was made again in June by Erich von Tschermak (1871–1962) from Esslingen, near Vienna. These events soon came to be known as "the rediscovery of Mendel's laws," and 1900 therefore is often called the birth year of the science of genetics.

> *"To fully grasp the chain of events we must view the work of the three rediscoverers as three independent stories, each of which eventually merged with the story of Mendel's discovery and finally all three with each other."*

When we speak of the rediscovery of Mendel's laws in 1900, we do so in hindsight. The very word *rediscovery* implies that the three "rediscoverers" only came in second. But looking backward, knowing how the story ended, is—as a rule—not the proper way to understand how things went. This is certainly true in the case of the rediscovery. All three rediscoverers arrived at their conclusions independently, that is, being unaware of the findings of Mendel and of each other. All three initially thought they had made a unique discovery, and a very important one too. To fully grasp the chain of events we must view the work of the three rediscoverers as three independent stories, each of which eventually merged with the story of Mendel's discovery and finally all three with each other.

For reconstructing past events that lie beyond living memory, the historian relies on archival material like letters, diaries, and notes on the one hand and relies on contemporary literature on the other. Often, there is a vexing lack of sources, and many questions must remain unanswered. Fortunately, in the case reported here the work of the rediscoverers before they published their rediscovery is well documented and provides us with a good insight into the intellectual context in which their separate rediscoveries took place.

Correns started in 1894 to investigate *xenia*, the phenomenon in which fruits and seeds of a fertilized mother plant show characters of the pollen-giving father plant. He kept records of his crossings in notebooks, which incidentally show that this was only one of the many subjects he studied during the 1890s. He crossed several species, among them *Lilium*, *Pisum*, and *Zea mays*. It was only in the latter that he could attribute the

The American Biology Teacher, Vol. 84, No. 1, pp. 14–15, ISSN 0002-7685, electronic ISSN 1938-4211. DOI: https://doi.org/10.1525/abt.2022.84.1.14.

form and color of the seeds to the xenia effect. The ratios he found with *Pisum* taught him the laws of segregation and recombination. Tschermak started crossing experiments in 1898 while working in a nursery in Ghent, investigating differences in fruit development after self- and cross-fertilization. He initially worked with *Cheiranthus cheiri* (the common wallflower) but soon switched to *Pisum*. This fast-growing species suited him better as he had limited time. He continued his cultures the next year on an estate of the Habsburg imperial family where he had become a gardener. From the ratios obtained that season, he deducted the laws of inheritance. Hugo de Vries started crossing experiments in at least 1892 and eventually cultivated dozens of species (interestingly enough, not *Pisum*). In the late 1880s he had set up an extensive research program to collect experimental evidence for Charles Darwin's theory of heredity, his "provisional hypothesis of pangenesis." According to pangenesis (McComas, 2012), hereditary characters are bound to macromolecules which behave independently from each other. Variation (and hence evolution) is caused by the increase and decrease of the hereditary particles on the one hand and the emergence of new particles on the other. De Vries studied the presumed existence and independent behavior of the particles through breeding of aberrant traits (so-called monstrosities), selection, and crossing. He managed to transfer single characters from one species to another. He concluded that the ratios he obtained followed the laws of probability, which he had applied in the interpretation of his selection experiments since 1890.

So, to reconstruct the three individual stories of the rediscoverers, the sources are quite helpful to the researcher. But when it comes to the merger of the rediscoverers' stories with Mendel's story, we are less lucky. All three rediscoverers published their results after reading Mendel's paper, giving him due credit for his priority. Earlier publications and archival material that can show us when our protagonists read Mendel's paper and consequently lost their independence are scarce. This even leads to the question whether there were indeed three discoveries, or just two, or one, or none.

Tschermak read the paper in one of the last months of 1899 when he was preparing a thesis to receive the right to lecture in an Austrian university in which he described his experiments of the previous years. Correns, by his own account, read the paper "a few weeks" after he had figured out an explanation for the results of his breeding experiments in October or November 1899. It must have been just a few weeks because in a paper he wrote in December 1899, he mentioned Mendel's work. However, an entry in one of Correns' notebooks dated April 16, 1896, contains an extensive reference to Mendel's paper. It seems that Correns soon forgot the paper after reading it for the first time, not knowing how to apply Mendel's results to his own investigation. But it might have been lurking at the back of his mind and, unconsciously, contributing to the explanation he worked out a few years later. In a letter from 1901 or 1902 De Vries wrote that he had found a reference to Mendel's paper in an American publication from 1892 and had looked it up "a few years later." He repeated this story in 1925 and 1930. But somewhere during the 1910s or 1920s, he told one of his students that a colleague from the Netherlands had sent him an offprint of the paper just before he intended to publish his results. The offprint was found in this colleague's library in 1935 after his death, but whether he already owned it in 1900 cannot be established, nor whether he ever sent it to De Vries.

Since the 1950s a host of researchers have investigated the three claims to independent (re)discovery. Publications were interpreted and reinterpretated (and misinterpreted), and archives were scrutinized to find new sources. Apart from getting the events and their chronology straight, the question was asked how the rediscoverers' views related to those of Mendel. Some concluded that none of them can be called a (re)discoverer because their interpretations were markedly different from those originally offered by Mendel. Measured by modern standards they certainly do not qualify as Mendelians, but of course, this goes even for Mendel. Paired factors, genotype, and phenotype are not mentioned in his paper, and chromosomes and genes were still things of the future, yet all are key elements of classic Mendelian genetics, so Mendel himself was not truly a Mendelian.

Over the years, all three men were dethroned and reinstated several times by scholars. The number of books and papers discussing the matter has grown to an impressive number, which is still increasing. Fascinating and intriguing as they are, showing expert scholarship and ingenious detective work, one cannot help wondering sometimes whether we should simply stick to what Correns said in 1925 when he was questioned about the events: "I do not lay too much weight upon the re-discovery itself. According to my opinion, it was important that the Mendelian laws should finally be known and verified. Whether it happened by their being independently found anew or through the fact that someone first read the memoir of Mendel and then repeated the experiments, is, however, at bottom, an indifferent matter for science."

On the other hand, and on a higher level, the story of the rediscovery provides us with another historic example of how science develops. It very seldom goes in a straight line progressing from ignorance toward "the truth." Instead, the scientist's way is often a bumpy ride on a winding road with unexpected and unpredictable twists and turns and dead ends, with competition and *jalousie de métier* (sometimes leading to collisions) from other travelers. This story and others like it may help students to gain a better understanding of how science is done, and to better appreciate its fascinating, challenging, and alluring nature.

References

Corcos, A.F. & Monaghan, F.V. (1990). "Mendel's work and its rediscovery: a new perspective. *Plant Sciences*, *9*, 197–212.

McComas, W.F. (2012). Darwin's invention: inheritance and the "mad dream" of pangenesis. *American Biology Teacher*, *74*, 86–91.

Olby, R.C. (1985). *Origins of Mendelism*. Chicago and London: University of Chicago Press.

Rheinberger, H.-J. (1995). When did Carl Correns read Gregor Mendel's paper?. *Isis*, *86*, 612–16.

Roberts, H.F. (1929). *Plant hybridization before Mendel*. Princeton: Princeton University Press.

Simunek, M., Hossfeld, U. & Wissemann, V. (2011). Rediscovery revisited—the cooperation of Erich and Armin van Tschermak-Seysenegg in the context of the "rediscovery" of Mendel's laws in 1899–1901. *Plant Biology*, *13*, 835–41.

Zevenhuizen, E.J.A. (1998). The hereditary statistics of Hugo de Vries. *Acta Botanica Neerlandica*, *47*, 427–63.

ERIK ZEVENHUIZEN studied history at the University of Amsterdam, specializing in the history of biology, and received his PhD with a biography of Dutch botanist Hugo de Vries (1848–1935). He is currently a guest researcher at the Artis Library of the University of Amsterdam; e-mail: e.zevenhuizen@uva.nl.

INQUIRY & INVESTIGATION

Introducing Evolution of the Human Lactase Gene using an Online Interactive Activity

Merav Siani, Anat Yarden

Anetlanda/Shutterstock.com

Abstract

Human evolution is a sensitive and controversial topic, which might explain why it is not included in science curricula or textbooks in many countries. We prepared an online student-centered human evolution activity dealing with lactose tolerance. In constructing the activity, we considered the following design principles: a medical issue connected to students' lives, a noncontentious topic of human evolution, and a one-step genetic example that can be demonstrated by basic bioinformatics tools. The activity consists of four units dealing with the activity of the enzyme lactase in our small intestine, the differences in lactose tolerance in people from different origins, the genetic foundation of lactose tolerance, and an extension unit dealing with the control of lactase gene expression. The activity was experienced by a pilot group of approximately 100 students, preservice and in-service teachers who showed great interest in the genetics of a trait that has undergone evolutionary changes. We noted the need for a teacher as mediator while students perform the activity. We suggest using the activity in the context of evolution, genetics, or when teaching about systems of the human body, either all units in succession or as a modular activity.

Key Words: *human evolution; lactose tolerance; bioinformatics tools; online student-centered activity; high school.*

○ Introduction

Human evolution is a controversial topic worldwide. Those who accept evolution are willing to do so for both animals and plants. However, theologians claim that the status of humans is different because they have a soul and moral order in their societies; thus, theologians do not accept the notion of an evolutionary process for humans (Webb, 2002). Moreover, a recent study among Israeli science teachers showed that one of the topics that is most unknown to them is human evolution (Siani & Yarden, 2021). These findings, among many others, show us that human evolution is a very sensitive and controversial issue, which might explain why it is not included in the science curricula and textbooks in many countries, including the (Next Generation Science Standards, 2013).

> *"Educational stakeholders spoke about the need for learning materials that include evidence of evolution as a way of avoiding theological tensions."*

An approach led by Briana Pobiner (Pobiner, 2012, 2016; Pobiner et al., 2018) shows the advantage of dealing with human examples when teaching evolution. This approach claims that focusing on examples from the field of human evolution may provide an enjoyable, engaging, and effective way to help students overcome their lack of enthusiasm for the study of evolutionary concepts and processes (Pobiner et al., 2018). Furthermore, integrating convincing examples of personal and concrete functions of evolution that are relevant to students' lives might increase their motivation to study and remember evolutionary concepts (Borgerding et al., 2015; Heddy & Sinatra, 2013; Pobiner, 2012, 2016).

Previously interviewed educational stakeholders spoke about the need for learning materials that include evidence of evolution as a way of avoiding theological tensions (Siani & Yarden, 2020). Thus we decided to construct an online activity that deals with human evolution while taking into consideration the following design principles:

- A medical issue that is connected to nearly every student or his/her family's life
- A noncontentious topic of human evolution that will not raise protests from different sectors of the population
- A human evolution example that occurred in the not too distant past
- An unambiguous genetic *frame story* that includes a simple, one-step genetic mutation that affects a known trait
- An example that exposes students to basic bioinformatics tools through which students can gain a glimpse of authentic science that deals with genetic evidence of evolution

The American Biology Teacher, Vol. 84, No. 1, pp. 16–21, ISSN 0002-7685, electronic ISSN 1938-4211. DOI: https://doi.org/10.1525/abt.2022.84.1.16.

○ Activity Description

The activity consists of four units. Here we describe the main items in each unit.

The whole activity is open free of charge at https://petel.weizmann.ac.il/biology/login/signup.php?key=T6518373X&lang=en.

Information regarding the PeTeL (Personalized Teaching and Learning) environment in which this activity is included is at https://stwww1.weizmann.ac.il/petel/home-en.

1. What is the lactase enzyme?

The aim of this unit is to expose students to the normal activity of the enzyme lactase and the consequences when the enzyme does not work. The unit includes:

a. A diagram of lactase activity and a short description of the fact that humanity has undergone changes since the *agricultural revolution*, when humans started to domesticate plants and animals. It is only with the latter that they began to consume dairy products and the need arose to break down the sugar lactose (found in milk) throughout the individual's lifetime.

b. A short clip "Lactose - What Is Lactose - What Is Lactose Intolerance" (Whats Up Dude, 2017). Throughout the clip, there are short questions that the students must answer in order to continue with the clip (Figure 1).

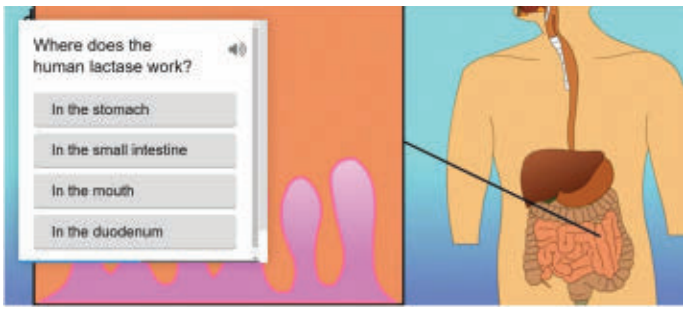


Figure 1. A question in the clip "Lactose - What Is Lactose - What Is Lactose Intolerance."

c. A diagram of the small and large intestines. By clicking on the purple hot spots, the students can see the process occurring in each part of the digestive system (Figure 2).

An exercise to summarize the knowledge that has been obtained from this unit (Figure 3).

2. Can I drink milk?

The aims of this unit are to reveal the fact that in some populations lactase activity decreases after weaning and to explain the advantage of this trait in some regions of the world.

a. A graph showing the reduction of lactase activity in people who are lactose intolerant in comparison to those who are lactose tolerant. The information is obtained by clicking on the purple hot spots (Figure 4).

b. An interactive Google map that gathers the origin of the students' families, showing that lactose tolerance is a trait that is connected to one's origin, as depicted on the map (Figure 5) (Ségurel & Bon, 2017).

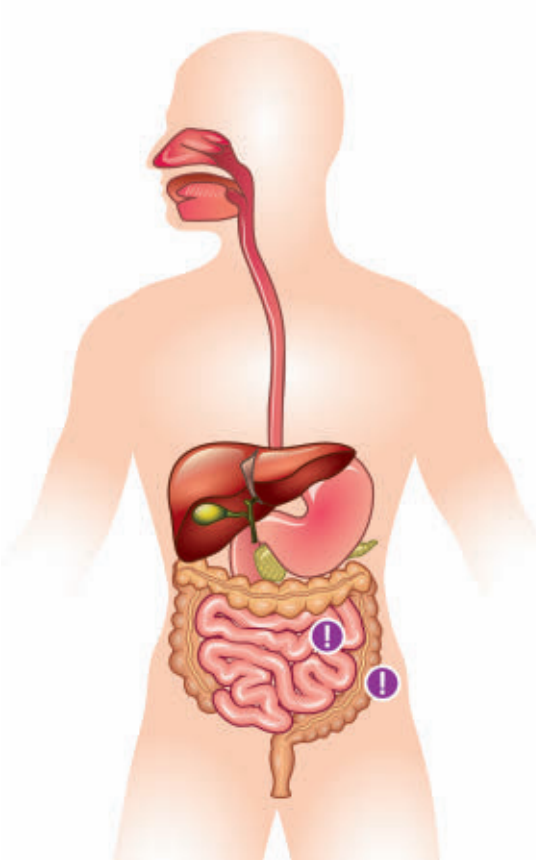


Figure 2. An interactive picture of the digestive system. A color version of this figure can be found with the online article. Image source: La Gorda/Shutterstock.com.

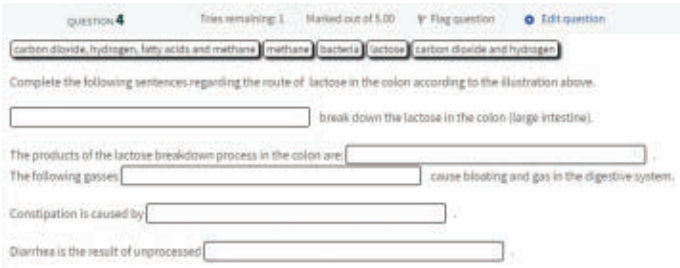


Figure 3. An exercise to summarize the knowledge obtained from Unit 1.

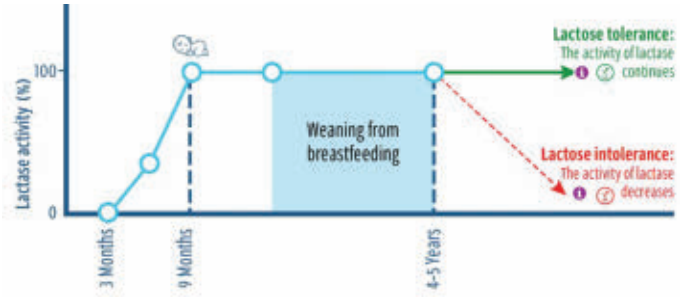


Figure 4. A graph showing the lactase activity in people who are lactose intolerant and tolerant.

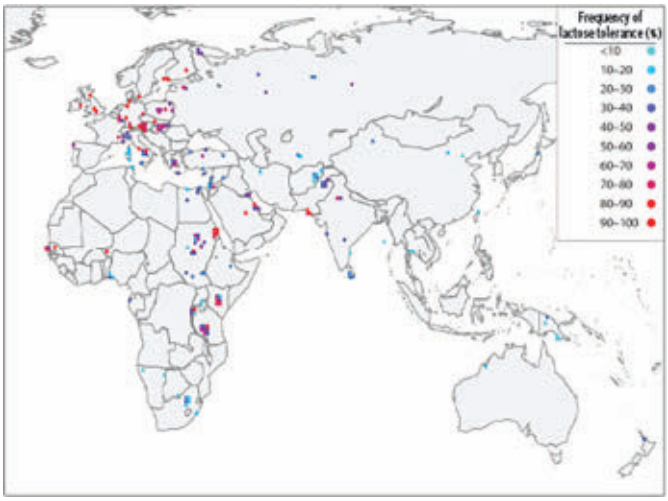


Figure 5. A map showing the frequency of lactose tolerance around the world. A color version of this figure can be found with the online article.

c. One of the advantages for lactose tolerance, as a possible explanation for why the trait has been preserved, is that lactose can substitute for calcium absorption (Camara-Martos & Amaro-Lopez, 2002). Substances are fermented by the bacterial flora in the colon, while lactose and other organic acids such as acetate, propionate, and butyrate lower the pH and make the calcium more soluble. The solubility of calcium improves its absorption (Trinidad et al., 1993). Since infants cannot produce vitamin D, we can understand why most mammalian milks contain lactose (Wacker & Holick, 2013). Thus, according to this hypothesis, the ability to tolerate lactose in the gut would enhance calcium absorption and protect against bone malformities in the context of insufficient UVB light to synthesize vitamin D. This would further help explain why some European dairying populations have high frequencies of lactose tolerance and make extensive use of high-lactose dairy products. Students are asked to drag the phrases into the figure according to the information that they have read (Figure 6).

3. The genetic foundation of lactose tolerance

The aim of this unit is to use bioinformatics tools to reveal the positive mutation that has led to lactose tolerance.

a. The students get detailed instructions on using *pairwise sequence alignment* in the Clustal Omega tool, available at https://www.ebi.ac.uk/Tools/psa/emboss_needle/ (Figure 7).

By using the DNA pairing tool and comparing DNA 1 (in Appendix 1, without the mutation) to DNA 2 (in Appendix 2, with the mutation), the students can see the exact location of the transition point mutation. A few questions summarize this part of the unit. Examples are demonstrated in Figure 8.

b. To search for the location of the lactase mutation in the human genome, the students get instructions on using the tool Nucleotide BLAST (available at https://blast.ncbi.nlm.nih.gov/Blast.cgi). The results show that the mutation does not occur in the lactase gene but rather in the MCM6 gene, where the enhancer region of the lactase gene is situated;

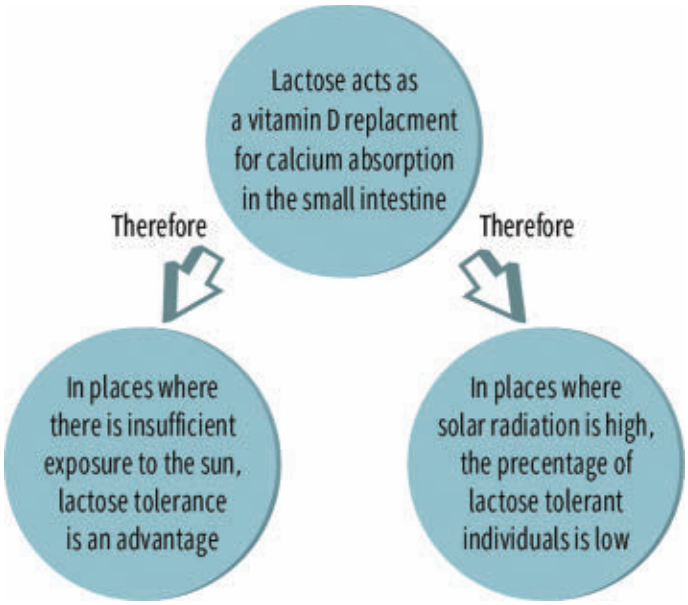


Figure 6. An exercise summarizing the advantage of lactose tolerance.

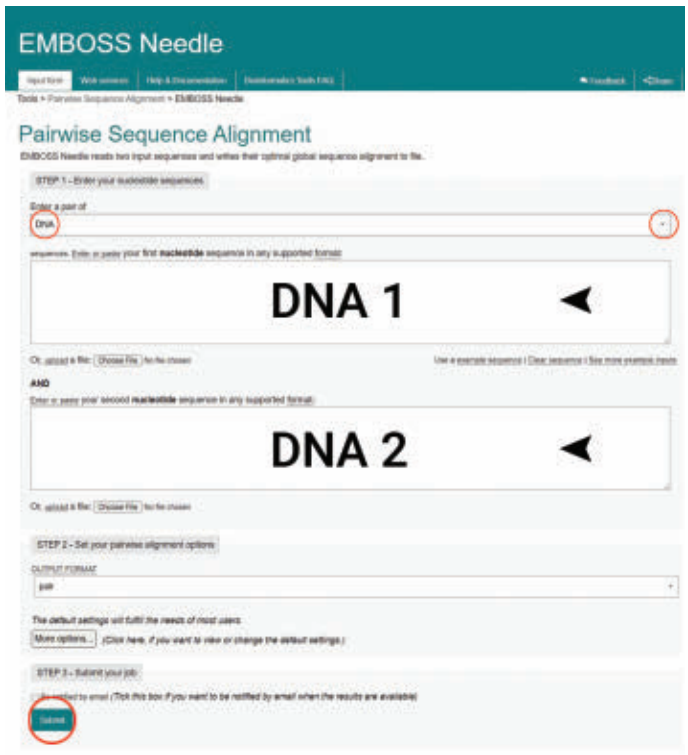


Figure 7. Instructions for using the Clustal Omega tool.

both of these genes are on chromosome 2. An activity summarizes the unit (Figure 9).

4. The control of lactase gene expression

The last unit, which is an extension unit, is intended for students who have studied control of gene expression. The aim of the unit is to teach the genetic mechanism governing the

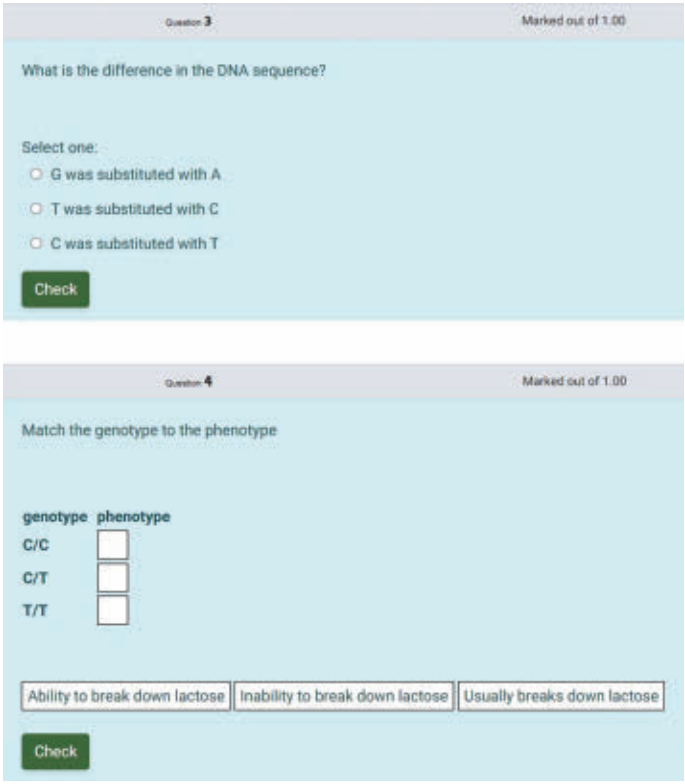


Figure 8. Exercises to summarize the comparison of DNA with and without the mutation.

mutated gene's enhancement, as well as to learn more about the site on the MCM6 gene where the mutation occurs.

a. The students are presented with written information along with a scheme depicting the genetic mechanism of lactase gene enhancement due to the mutation (Figure 10).
b. Students are asked to fill in the scheme presented in Figure 11.
c. Since the mutation occurs in the MCM6 gene, why does it not damage the function of the MCM6 gene? The students are referred to the MCM6 gene in the Genome Browser bioinformatics tool (Figure 12; https://genome.ucsc.edu/cgi-bin/hgTracks?db=hg38&lastVirtModeType=default&lastVirtModeExtraState=&virtModeType=default&virtMode=0&nonVirtPosition=&position=chr2%3A135839626%2D135876443&hgsid=1215176811_3HENOSVE5PalxeQKHAOthyP0xZfv). A few questions, such as the one at the bottom of Figure 12, assist the students to focus on the relevant parts of the information shown in the bioinformatics tool and find out

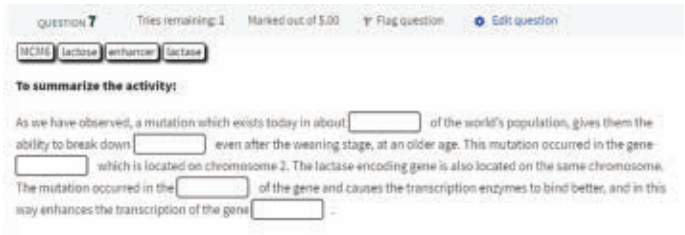


Figure 9. An activity to summarize Unit 3.

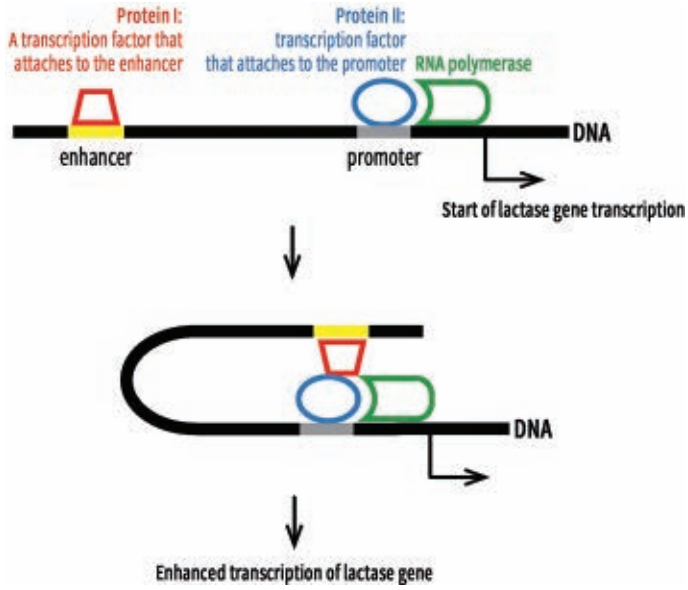


Figure 10. A scheme depicting the genetic mechanism of lactase gene enhancement due to the mutation.

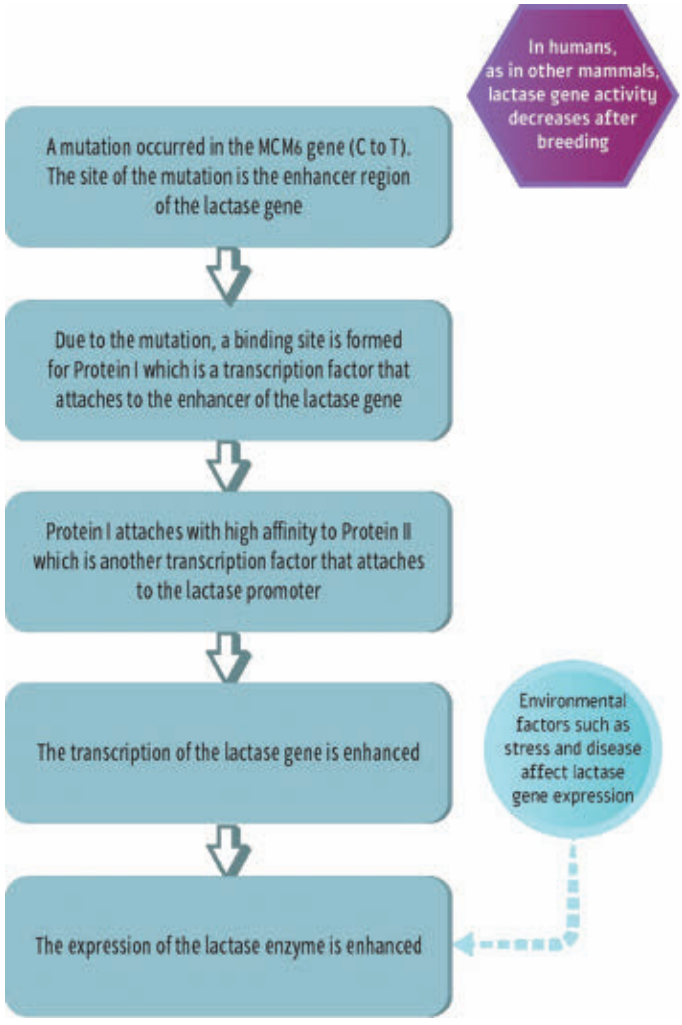


Figure 11. A scheme summarizing the genetic mechanism of lactase gene enhancement due to the mutation.

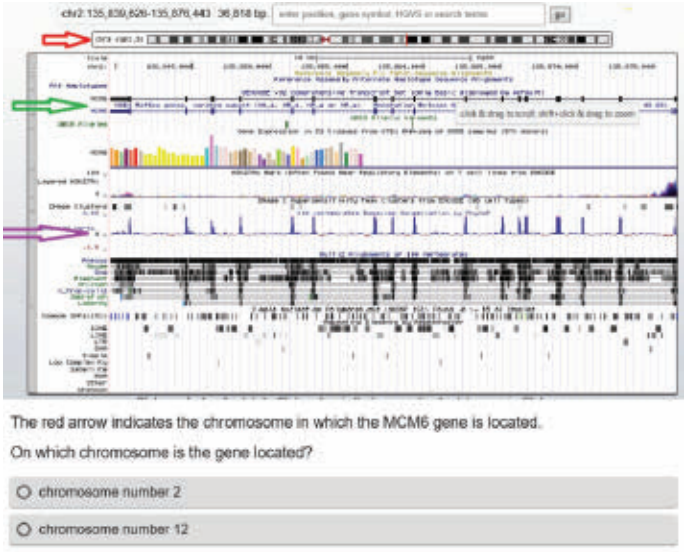


Figure 12. The information shown in the Genome Browser tool relating to the MCM6 gene and an example of a question that follows it. A color version of this figure can be found with the online article.

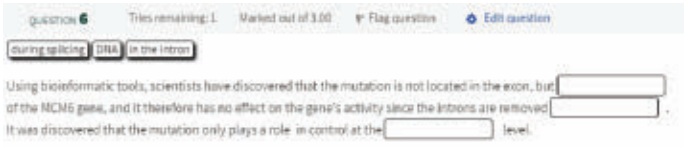


Figure 13. An exercise to summarize Unit 4.

that the mutation occurs in the intron. The students discover along the unit that the intron is removed during gene splicing, and there is therefore no damage to MCM6 gene function.

d. An exercise summarizes the unit (Figure 13).

e. A clip that summarizes the whole activity with questions interspersed throughout the clip (HHMI BioInteractive, 2014).

○ Experiencing the Activity in Schools

The activity has been experienced by approximately 100 Israeli students as well as 10 biology preservice teachers, 11 in-service teachers, and 13 science education researchers. These pilot groups enabled us to improve the activity according to their feedback. So far, most of the students have only completed Units 1 and 2 because they are in the 9th grade and have not yet studied genetics. At the end of Unit 2, they were asked, "What would you like to research and discover about the genetics of the lactase-encoding gene?" The students' answers reflected a keen interest in the topic, which seems to be relevant to their lives:

- "I wonder if the change in the lactase gene that leads to the tolerance trait is minimal (one nucleotide replacement), completely different, or not different at all. I am interested in knowing the genetic difference between lactose-intolerant adults and intolerant children. Is the change in the same gene?" (L. M.)
- "How does lactose tolerance develop, and will everyone be fully lactose-tolerant in a few years?" (Y. S.)
- "I am interested to know if the gene for lactase has changed during evolution." (S. Y.)

These quotes and others show the students' interest in continuing to explore the genetics of this gene. The first two units are therefore a good trigger to continue exploring the genetic change that has occurred.

The preservice teachers completed the whole activity. Their feedback regarding the bioinformatics activity (Units 3 and 4) encouraged us to continue and expose more students to the whole activity:

- "You see the sequences in front of your eyes, and the changes that have taken place. It really illustrates what's going on. It's not just theoretical learning." (O.L.)
- "The use of bioinformatics tools helped me understand how the lactase gene is inherited by understanding where the mutation occurs and that the exchange is between C and T." (R.Y.)

We also realized the importance of a teacher as mediator in this activity. The preservice teachers performed the activity online with no mediation or support, except for technical support. Maybe that is why we heard this other voice:

- "The activity did not help me so much because I did not understand how to interpret the results I received. I just didn't understand what I had to do with the information received." (S.T.)

This response demonstrated the need for a teacher who would help the students grasp the full significance of the bioinformatics results as well as understand the options for application of bioinformatics for evolution research.

○ Recommendations for Use of the Activity

The activity can be used for teaching not only evolution but also many other topics in the biology syllabus because it deals with a genetic disorder in humans based on enzyme activity. As such, it can be learned as part of the unit on the digestive system dealing with digestion in the small or large intestine, mutations in the field of genetics, or control of gene expression and genetic engineering.

Use of the activity can be modular. The teacher can either use all of the units of the activity in succession or use the first two units to introduce the lactase enzyme and lactose tolerance (Units 1 and 2), and then later on the teacher can familiarize the students with the genetic basis of lactose tolerance (Unit 3); finally, at the appropriate time in the syllabus, the teacher can acquaint the students with control of lactase gene expression (Unit 4). In addition to its modularity, parts of the activity can be performed as either homework or schoolwork, depending on the availability of computers at the school for each student. Whether applied successively or not, the activity is ready for use and handy for the teacher who wishes to diversify his or her teaching tools. However, a teacher must serve as mediator for some parts of the activity, especially when using the bioinformatics tools in Units 3 and 4 and when students are

using these tools for the first time. In addition, since there are a few forum-style questions (Unit 1 Question 2, Unit 2 Question 4, Unit 4 Question 2) in the activity, where the students are asked to express their opinions, the teacher has the option to project the answers in front of the whole class and hold a class debate.

References

Borgerding, L.A., Klein, V.A., Ghosh, R. & Eibel, A. (2015). Student teachers' approaches to teaching biological evolution. *Journal of Science Teacher Education*, *26*(4), 371–92. https://doi.org/10.1007/s10972-015-9428-1.

Camara-Martos, F. & Amaro-Lopez, M.A. (2002). Influence of dietary factors on calcium bioavailability. *Biological Trace Element Research*, *89*(1), 43–52. https://doi.org/10.1385/BTER:89:1:43.

Genome Browser https://genome.ucsc.edu/cgi-bin/hgTracks?db=hg38&lastVirtModeType=default&lastVirtModeExtraState=&virtModeType=default&virtMode=0&nonVirtPosition=&position=chr2%3A135839626%2D135876443&hgsid=1215176811_3HENOSVE5PalxeQKHAOthyP0xZfv. Retrieved on September 1, 2021.

Heddy, B.C. & Sinatra, G.M. (2013). Transforming misconceptions: using transformative experience to promote positive affect and conceptual change in students learning about biological evolution. *Science Education*, *97*(5), 723–44. https://doi.org/10.1002/sce.21072.

HHMI BioInteractive. (2014). *The Evolution of Lactose Tolerance* [Video]. YouTube. https://www.youtube.com/watch?v=MA9bol1qTuk.

Next Generation Science Standards. (2013). *Topic Arrangements of the Next Generation Science Standards*. https://www.nextgenscience.org.

Pobiner, B. (2016). Accepting, understanding, teaching, and learning (human) evolution: obstacles and opportunities. *American Journal of Physical Anthropology*, 159, 232–74. https://doi.org/10.1002/ajpa.22910.

Pobiner, B., Beardsley, P.M., Bertka, C.M. & Watson, W.A. (2018). Using human case studies to teach evolution in high school A.P. biology classrooms. *Evolution: Education and Outreach*, *11*(1), 3. https://doi.org/10.1186/s12052-018-0077-7.

Pobiner, B.L. (2012). Use human examples to teach evolution. *The American Biology Teacher*, *74*(2), 71–72. https://doi.org/10.1525/abt.2012.74.2.2.

Ségurel, L. & Bon, C. (2017). On the evolution of lactase persistence in humans. *Annual Review of Genomics and Human Genetics*, *18*(1), 297–319. https://doi.org/10.1146/annurev-genom-091416-035340.

Siani, M. & Yarden, A. (2020). "Evolution? I don't believe in it": theological tensions surrounding the implementation of evolution in the Israeli curricula. *Science and Education*, *29*(2), 411–41. https://doi.org/10.1007/s11191-020-00109-7.

Siani, M. & Yarden, A. (2021). "I think that teachers do not teach evolution because it is complicated": difficulties in teaching and learning evolution in Israel. *International Journal of Science and Mathematics Education*, 1–21. https://doi.org/10.1007/s10763-021-10179-w.

Trinidad, T.P., Wolever, T.M. & Thompson, L.U. (1993). Interactive effects of calcium and short chain fatty acids on absorption in the distal colon of man. *Nutrition Research*, *13*(4), 417–25. https://doi.org/10.1016/S0271-5317(05)80706-7.

Wacker, M. & Holick, M.F. (2013). Sunlight and vitamin D: a global perspective for health. *Dermato-Endocrinology*, *5*, 51–108. https://doi.org/10.4161/derm.24494.

Webb, G.E. (2002). *The Evolution Controversy in America*. Lexington, KY: University Press of Kentucky. https://www.google.com/books/edition/The_Evolution_Controversy_in_America/OqYeBgAAQBAJ?hl.

Whats Up Dude. (2017). *Lactose - What Is Lactose - What Is Lactose Intolerance* [Video]. YouTube. https://www.youtube.com/watch?v=g6A_DwHkzGs.

MERAV SIANI is a postdoctoral fellow in the Department of Science Teaching at Weizmann Institute of Science, Rehovot, Israel, and head of the Department of Science Teaching at Herzog College, Alon Shvut, Israel; e-mail: sianimerav@gmail.com. ANAT YARDEN is head of the Life-Sciences Group and head of the Department of Science Teaching at Weizmann Institute of Science, Rehovot, Israel; e-mail: anat.yarden@weizmann.ac.il.

○ Appendix 1

GCAATTATGGGTGACTGGATAGGAGCACCTTACGTCCCGAGTTTCTTGTTAGATTTTTAG

TTTGTAATATGTTTACGTTGGATTCCTCCTCTCAAGGAAACTCCGGTCCCCGATGTAATA

GAATAGACATAACGGTCGCGTCTCCGGATGATCATGTAACATCCCAGATTCATGTAAAAA

○ Appendix 2

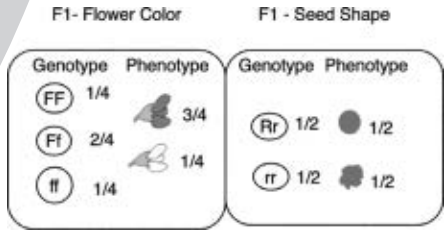
GCAATTATGGGTGACTGGATAGGAGCACCTTACGTCCCGAGTTTCTTGTTAGATTTTTAG

TTTGTAATATGTTTACGTTGGATTCCTCCTCTCAAGGAAACTCCGGTCCCTGATGTAATA

GAATAGACATAACGGTCGCGTCTCCGGATGATCATGTAACATCCCAGATTCATGTAAAAA

INQUIRY & INVESTIGATION

Inheritance Patterns: Probability Rules & Probability Trees



Umadevi Garimella, Nesrin Sahin

Abstract

Educators usually teach the Mendelian inheritance model using Punnett squares to determine the probability of an offspring having a particular genotype and phenotype. To find the probability of an outcome of a particular cross, students need to understand the underlying biological concepts of these visual representations. However, this approach becomes more complex for cases with three or more characters and shies away from the authentic integration of mathematical and biological concepts. Therefore it is crucial for students to use mathematical algorithms that Mendel used to understand and solve inheritance problems. In this paper, we propose relating two simple probability rules to the laws of inheritance and using a probability tree diagram to predict the combined frequency of traits in the offspring of crosses. We validate the proposed probability rules for various examples.

Key Words: *Mendel; laws of inheritance; law of independent assortment; law of segregation; probability; addition or sum rule; multiplication or product rule.*

○ Introduction

Many science educators strongly support the integration of mathematics and life sciences (Duffus & Olifer, 2010; Labov et al., 2010; NRC, 2012; Salsberg, 2009). Additionally, in the reformed undergraduate science courses, educators expect students to reason and to build biological knowledge by using various mathematical representations (Matthews et al., 2010). Similar changes have been proposed in K–12 settings to promote STEM education (Common Core Standards Initiative, 2010; NRC, 2012). However, in life science courses, teachers often opt for easy and noncontextual methods for quick solutions, which can often undermine mathematical concepts (Garfield & Ahlgren, 1988; Liu & Thompson, 2007). A good example is the probability concepts in Mendelian genetics. This topic is being taught using the Punnett square method, a nonmathematical and easily understandable matrix to demonstrate Mendel's laws of inheritance. Freshman biology majors, in spite of exposure to genetics since primary grades, have difficulty understanding basic concepts (Batzli et al., 2014; Bowling et al., 2008; Colon-Berlingeri & Burrowes, 2011; Shaw et al., 2008; Stewart, 1982). A Punnett square is a checkerboard of squares with the gametes from each parent arranged along the top and sides of the grid. Each box of the grid represents the union of the gametes in the corresponding row and column, which show all possible genotype combinations of offspring that can result from a random crossing. Though the Punnett square method is an excellent visual method for predicting the probability of possible combinations of genotypes for monohybrid or dihybrid crosses, it has some limitations. First, the Punnett square gets complicated as the combination of traits grow exponentially with an increasing number of characteristics. The matrix for a monohybrid cross is 4 (2 × 2), for a dihybrid cross it is 16 (4 × 4), for a trihybrid cross it is 64 (8 × 8), and so on. Second, there are steps in the Punnett square method that can lead to mistakes. For example, one has to set up a matrix, find all possible combinations by visually counting the desired phenotype, and then calculate the frequency. Third, as the process becomes complex, students lose the connection between the process and concepts and tend to solve the problem in a nonmeaningful way.

Providing structured opportunities for students to apply probability rules and algorithms to biological concepts will allow them to experience the interconnectedness of the STEM fields. In this work, we propose a methodology that implements two rules of probability to create probability tree diagrams for students to understand Mendelian genetics and show the effectiveness of this activity when dealing with more complex scenarios.

○ Prior Knowledge & Background Information

Some working knowledge of the genetic basis for inheritance will be helpful. It is also important that students understand that Mendel's laws and the rules of probability are applied to single-gene traits that are on different chromosomes. The probability rules involve addition and multiplication of fractions; therefore, a quick review

The American Biology Teacher, Vol. 84, No. 1, pp. 22–27, ISSN 0002-7685, electronic ISSN 1938-4211. DOI: https://doi.org/10.1525/abt.2022.84.1.22.

of these concepts is recommended. Table 1 includes a summary of Mendel's laws of inheritance and probability rules relevant to the proposed activities.

○ Overview of the Activity

This activity is designed to converge statistics with biology. Students in this investigation will have an opportunity to use probability rules in the context of Mendel's laws of inheritance and visually represent all potential outcomes and their respective likelihoods by drawing a probability tree. A tree diagram is a special type of graph that is constructed to visually represent possible combinations of two or more events that are random and mutually exclusive while minimizing mathematical errors.

The proposed lesson consists of two modules with one extension. Module 1 will focus on monohybrid crosses to allow students to work with one character as they get familiar with the algorithm of implementing probability rules to predict the results of a cross. Module 2 will deal with more complex dihybrid crosses. Students will use the algorithms learned in the first activity to predict the inheritance patterns of two characters. The Extension Module 3 includes a challenge for students to study a trihybrid cross. The garden pea plant found in traditional textbooks will be used as a model organism in this lesson. The lesson presented here is intended for two 50-minute class periods. The extension activity can be given as a homework problem.

Instructors can choose to modify the pace and depth of the content depending on their time frame, comprehension level, and class size. Mendel's inheritance and probability are middle school and high school concepts in the Next Generation Science Standards and the Common Core State Standards. The modular nature of the lesson allows easy adaption to the grade level. At the middle school level, teachers can use Module 1, however both Modules 1 and 2 can be implemented for high school and introductory college courses. The lesson can be extended to non-Mendelian inheritance such as codominance, complete dominance, and multiple alleles for more advanced students.

The process of predicting the outcome of a cross is divided into three steps. In each step the relevant biological principles, probability rules, and the steps in creating graphical representations, such as tree diagrams, are discussed.

Step 1: Gamete formation. This step involves predicting the types and probabilities of gametes that can form from each parent. The law of segregation, or the separation of the allelic pair with only one of the allelic pairs passing to the gametes, will be applied. In crosses involving two or more characteristics, the law of independent assortment, which states that allelic pairs of different characters assort independently of each other, will be applied. Since each character is inherited independently, the inheritance pattern of the allelic pairs of each character will be calculated separately. For easy visualization of the outcomes, results will be represented as a pattern of branches with probabilities labeled on the branches and types of gametes at the ends of the branches.

Step 2: Fertilization. In this step the combined probabilities of the offspring genotypes and phenotypes are predicted. Sexual reproduction involves a random fertilization of a gametes from each parent. Gamete formation and fertilization are consecutive, independent, and mutually exclusive processes, and therefore the multiplication and addition rules of probability can be applied. A probability tree diagram will be constructed by connecting the branches from step 1.

Step 3: Outcome. The final step deals with calculating the outcome of the genetic cross by using the multiplication and/or addition rules of probability. The genotypes, the combination of alleles of a specific gene, and phenotypes, or any observable characteristics, of the offspring are calculated by multiplying the allele combinations and the frequencies along the branches. If there are branches with the same results, the frequencies of those branches are added.

○ Implementation of the activity

The instructor should guide the entire class through the first monohybrid cross. After this demonstration, students should organize and work in small groups. Student groups will be guided by a worksheet that details the steps for each mechanism (provided as Supplemental Material with the online version of this article). The parent generation is labeled P, and the offspring generation is labeled F.

Module 1: One-Character Inheritance or Monohybrid Crosses

Students will study the inheritance pattern of a single-trait seed shape. Two possible crosses will be presented to the students.

Monohybrid cross 1: This is an instructor-led whole-class activity. Students will be presented with a cross between a heterozygous P-Female strain, with a different form of alleles (Rr), and a homozygous recessive P-Male strain, with the same form of alleles (rr). Students will be asked to draw the shape of the seeds and

Table 1. A summary of Mendel's laws of inheritance and multiple event probabilities rules relevant to the proposed activities.

Laws and Rules	Description
Law of segregation	During gamete formation, each pair of alleles segregate unchanged and pass into two different gametes, so that each gamete (egg or sperm) receives only one allele of a pair.
Law of independent assortment	Each allelic pair separates independently during gamete formation, and therefore traits are passed on to offspring independent of each other.
Probability rule of addition	The probability of either of two mutually exclusive events occurring is equal to the sum of their individual probabilities. P(A) or P(B) = P(A) + P(B)
Probability rule of multiplication	The probability of two independent events both occurring is the product of their individual probabilities. P(A) and P(B) = P(A) × P(B)

label the appropriate genotype (Figure 1). Students should notice that the parent genotype is diploid and that the shape of the seed depends on the two alleles.

In step 1, students are to draw branches to visually represent the frequency and types of gametes that can form from each parent based on the law of segregation and the rules of probability. A heterozygous (Rr) P-Female will produce two types of gametes, each with 1/2 probability. A homozygous recessive (rr) P-Male will produce only one type of gamete (r) with a probability of 1 (Figure 1).

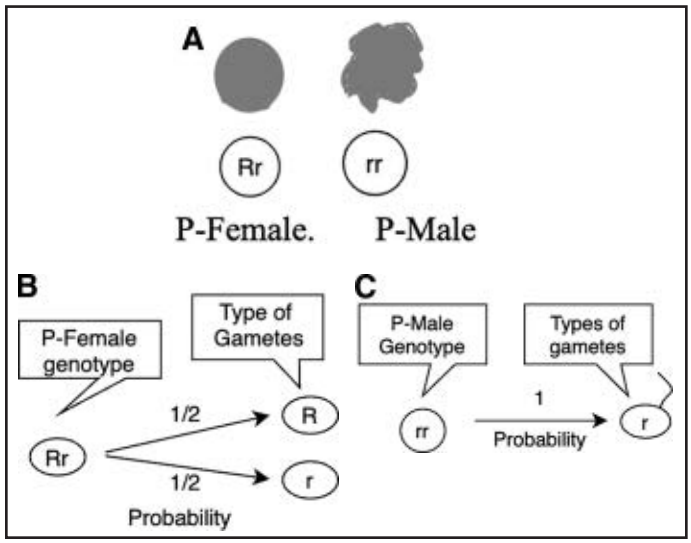


Figure 1. Genotype and phenotype of the parents and the predicted types and frequencies of gametes formed from each parent. (**A**) Parents. (**B**) Gametes of P-Female. (**C**) Gametes of P-Male.

In step 2, the possible combination of gametes to form the zygote and their frequencies is predicted by drawing a tree diagram representing the gamete of P-Male as the primary branches. The secondary branches, representing P-Female, are added to the ends of each primary branch. Reversing the primary and secondary branches yields the same results. With two types of eggs and one type of sperm, offspring with two genotypes, each with a probability of 1/2, will result from the cross (Figure 2).

In step 3, the outcome of the cross is predicted by multiplying the alleles and the probabilities along each branch to predict the frequency and possible offspring genotype and phenotype. The addition rule does not apply in this case because there are no branches with identical results. Since there are two types of gamete combinations, 1/2 of the offspring will have heterozygous round seeds (Rr) and 1/2 of the offspring will have homozygous wrinkled seeds (rr).

Monohybrid cross 2: In this example a cross between two heterozygous parent strains is conducted. Students break into small groups for this activity. Each group will work through the worksheet on their own by following the three steps. Students will identify the parents' phenotype and genotype to determine the types and probabilities of gametes for each parent. Both the P-Female and the P-Male plants are heterozygous (Rr), so the frequencies and types of gametes for both parents will be the same. Two types of eggs and two type of sperms will form, and the probability will be 1/2 for each gamete (Figure 4).

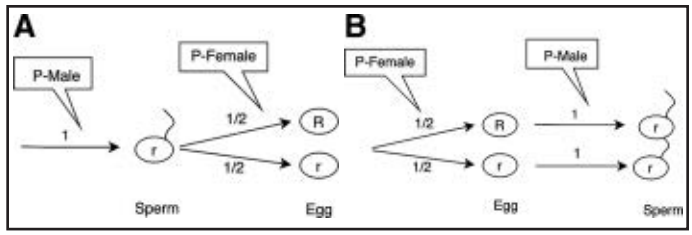


Figure 2. Representation of possible combinations of gametes using a probability tree diagram. (**A**) Probability tree with P-Male as primary branch followed by P-Female branch. (**B**) Probability tree with P-Female as primary branch followed by P-Male branch.

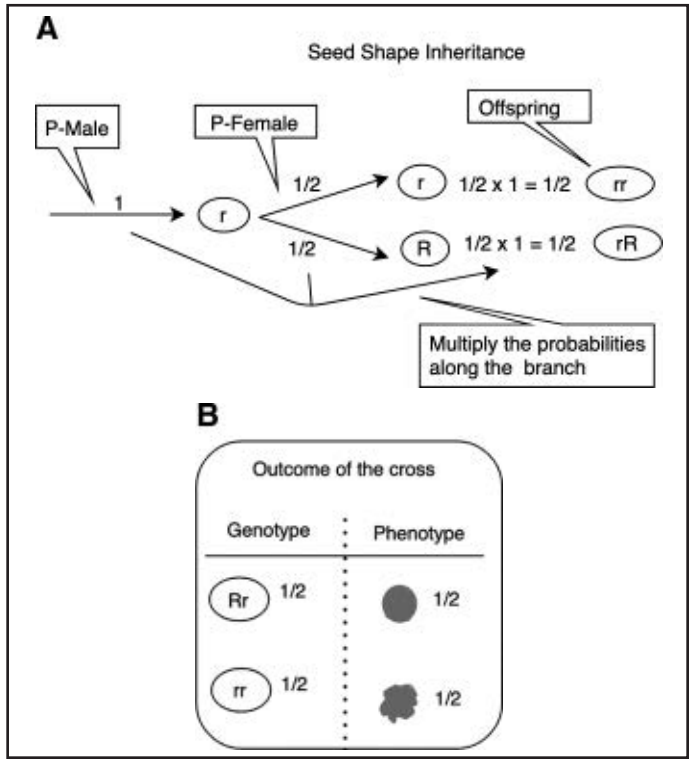


Figure 3. Outcome of the cross. (**A**) Tree diagram predicting the combined outcomes of the offspring genotypes. (**B**) The predicted genotype (heterozygous condition is represented in traditional form with the dominant allele followed by the recessive allele) and phenotype of the monohybrid cross 1.

Students will draw a tree diagram with two branches for P-Female and two branches for P-Male and calculate the outcomes. At this point the instructor should direct the students to look at the probability tree in Figure 5 and apply the addition rule, P(A or B) = P(A) + P(B). Students will add the probabilities of the branches with the same outcomes (Figure 5).

Module 2: Two-Character Inheritance or a Dihybrid Cross

In this module students will work with two traits, flower color and seed shape, to predict the possible combinations of traits in the offspring (F) generation.

This is a cross between a P-Female strain with heterozygous genotype for both purple flowers and round seeds (FfRr) and a

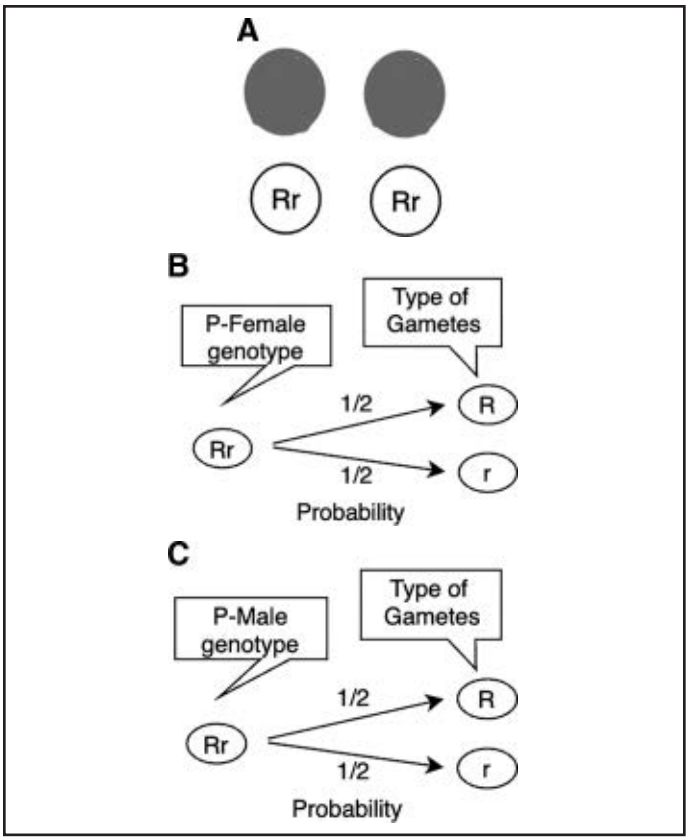


Figure 4. The genotype and phenotype of the parents and the prediction of the types and frequencies of gametes formed from each parent. (**A**) Parents of monohybrid cross 2. (**B**) Gametes of P-Female. (**C**) Gametes of P-Male.

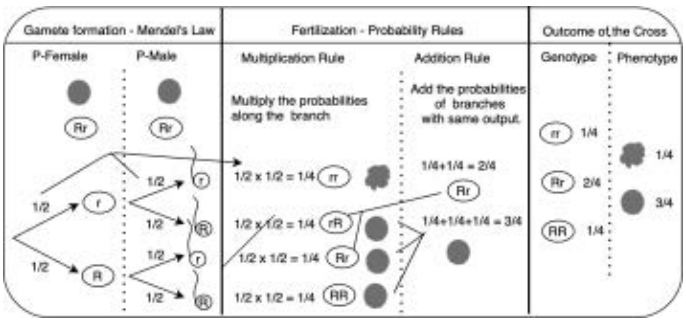


Figure 5. Calculation of the combined outcomes of the offspring genotypes using tree diagrams. Note that the fractions are not simplified, to keep the denominator the same.

P-Male strain with heterozygous purple flowers and homozygous wrinkled seeds (Ffrr). Students are asked to draw the phenotype and the genotype of P-Female and P-Male strains. Based on the law of independent assortment, the inheritance pattern of the allelic pairs of each character will be calculated separately (Figure 6A). Students will follow the steps learned in the monohybrid cross and work with each character separately.

Students will construct a tree diagram and calculate the probabilities of inheritance for each trait. (An example is provided in the Appendix). Once the inheritance pattern of each trait is determined (Figure 6), students will calculate the combined inheritance pattern for two traits by using the multiplication rule.

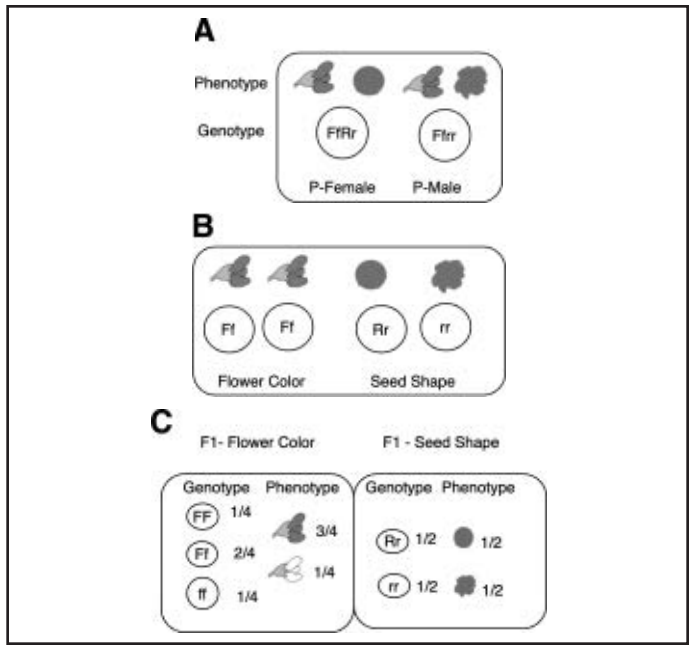


Figure 6. Dihybrid cross. (**A**) The genotype and phenotype of each parent. (**B**) The genotype and phenotype of separated traits for each parent. (**C**) Inheritance pattern of flower color and seed shape.

$$P(A \text{ and } B) = P(A) \times P(B)$$

$$P(\text{flower color and seed shape}) = P(\text{flower color}) \times P(\text{seed shape})$$

Example 1. What is the probability of having an offspring with purple flowers and round seeds?

$$P(\text{purple flower and round seeds}) = P(\text{purple flowers}) \times P(\text{round seeds})$$

$$= 3/4 \times 1/2 = 3/8$$

Example 2. What is the probability of having an offspring with ffrr genotype?

$$P(ffrr) = P(ff) \times P(rr)$$

$$= 1/4 \times 1/2 = 1/8$$

Extension Module 3—Three-Character or Trihybrid Crosses

In this example we will consider a cross between two parents with three traits: plant height, flower color, and seed shape. Both parents are tall with purple flowers and round seeds. They are heterozygous for all three traits; P-Female: Tt and P-Male: Tt.

Students will separate each trait for both parents based on the law of independent assortment. They will follow the three steps and calculate the probability and the inheritance pattern of the allelic pairs for each trait. Since both parents are heterozygous for all three traits, the frequencies and the inheritance patterns of all three traits will be the same (Figure 7). Using the inheritance pattern of each trait students will calculate the frequencies and types of combinations of the genotype or phenotype of the offspring using multiplication rules.

$$P(A \text{ and } B \text{ and } C) = P(A) \times P(B) \times P(C)$$

$$P(\text{height and flower color and seed shape}) = P(\text{height}) \times P(\text{flower color}) \times P(\text{seed shape})$$

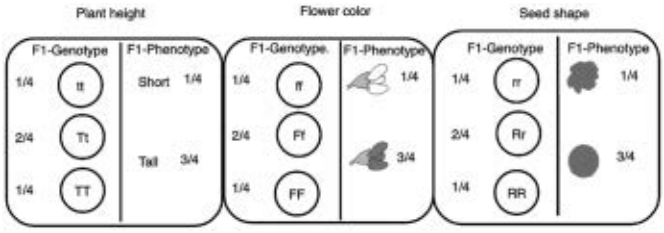


Figure 7. The inheritance pattern of each trait in a trihybrid cross.

Example 1: What is the probability of having a short offspring that produces purple flowers and round seeds?

$$P(\text{short, purple flower and round seeds}) = P(\text{short}) \times P(\text{purple}) \times P(\text{round})$$
$$= 1/4 \times 3/4 \times 3/4 = 9/64$$

Example 2: Find the probability of forming an offspring with genotype TtFFrr

$$P(\text{TtFFrr}) = P(\text{Tt}) \times P(\text{FF}) \times P(\text{rr})$$
$$= 2/4 \times 1/4 \times 1/4 = 1/32 \text{ or } 2/64$$

The three-step process presented in this paper can be applied to crosses or crossings of multiple traits, incomplete dominance, codominance, and traits with multiple alleles.

○ Discussions

The activity was implemented in a science concept class for middle grade preservice teachers. After completing the module, students were comfortable relating the mathematical steps with biological concepts. Students had a better understanding of the three sequential steps, as each step included the relevant biological principles, probability rules, and the steps in creating graphical representations or the tree diagram. For example, in step 1 students used the principle of segregation to visually represent (branching) the probabilities of different types of gamete formations, and when studying two or more traits together, students used the principle of independent assortment to separate and predict possible gametes for each trait. One observed hurdle was the students' lack of confidence with mathematical concepts. Students struggled to figure out when to add or multiply fractions while calculating the outcomes of a cross (step 3). We recommend reviewing the rules of probability and the probability tree diagrams using the coin toss method before the activity. Students stated that the review helped them understand and perform well in completing the module.

As we make a concerted effort to erase the boundaries between math and science, educators must select the most commonly used mathematical formulas in the sciences and carefully develop and implement tasks that allow students to make connections between science concepts and their mathematical representations. Probability tree diagrams, a graphical representation, can reinforce the probability rules and promote understanding of Mendel's inheritance patterns by minimizing errors. The traditional approach emphasizes easy-to-use matrices by skipping the mathematical steps whereas the probability rule-based approach works with fractions. Unlike the traditional Punnett square approach that can become more complex as students derive probabilities for cases with more traits, the probability rule-based approach remains simple with three easy-to-follow steps. Once students are comfortable with using the rules of probability, instructors can then extend the probability rule-based approach for di- and trihybrid crosses. The lesson can also be extended to non-Mendelian inheritance such as codominance, complete dominance, and multiple alleles for more advanced students.

References

Batzli, J. M., Smith, A. R., Williams, P. H., McGee, S. A., Dósa, K. & Pfammatter, J. (2014). Beyond Punnett squares: student word association and explanations of phenotypic variation through an integrative quantitative genetics unit investigating anthocyanin inheritance and expression in *Brassica rapa* fast plants. *CBE—Life Sciences Education*, *13*(3), 410–424. https://doi.org/10.1187/cbe.13-12-0232.

Bowling, B. V., Huether, C. A., Wang, L., Myers, M. F., Markle, G. C., Dean, G. E., Acra, E. E., Wray, F. P. & Jacob, G. A. (2008). Genetic literacy of undergraduate non–science majors and the impact of introductory biology and genetics courses. *BioScience*, *58*(7), 654–60. https://doi.org/10.1641/B580712.

Colon-Berlingeri, M. & Burrowes, P. A. (2011). Teaching biology through statistics: application of statistical methods in genetics and zoology courses. *CBE-Life Sciences Education*, *10*(3), 259–67. https://doi.org/10.1187/cbe.10-11-0137.

Common Core State Standards Initiative. (2010). National Governors Association Center for Best Practices & Council of Chief State School Officers. http://www.corestandards.org.

Duffus, D. & Olifer, A. (2010). Introductory life science mathematics and quantitative neuroscience courses. *CBE-Life Sciences Education*, *9*(3), 370–377.

Garfield, J. & Ahlgren, A. (1988). Difficulties in learning basic concepts in probability and statistics: implications for research. *Journal for Research in Mathematics Education*, *19*(1), 44–63.

Labov, J. B., Reid, A. H. & Yamamoto, K. R. (2010). Integrated biology and undergraduate science education: a new biology education for the twenty-first century? *CBE Life Sciences Education*, *9*(1), 10–16. https://doi.org/10.1187/cbe.09-12-0092.

Liu, Y. & Thompson, P. (2007). Teachers' understanding of probability. *Cognition and Instruction*, *25*(2), 113–60.

Matthews, K. E., Adams, P. & Goos, M. (2010). Using the Principles of *BIO2010* to develop an introductory, interdisciplinary course for biology students. *CBE—Life Sciences Education*, *9*(3), 290–97. https://doi.org/10.1187/cbe.10-03-0034.

NRC, N. R. (2012). *A Framework for K-12 Science Education: Practices, Crosscutting Concepts, and Core Ideas*. National Academies Press. https://doi.org/10.17226/13165.

Salsberg, E. (2009). *2009 State Physician Workforce Data Book*, 60. Association of American Medical Colleges. https://www.aamc.org/media/8396/download.

Shaw, K. R. M., Horne, K. V., Zhang, H. & Boughman, J. (2008). Essay contest reveals misconceptions of high school students in genetics content. *Genetics*, *178*(3), 1157–68. https://doi.org/10.1534/genetics.107.084194.

Stewart, J. (1982). Difficulties experienced by high school students when learning basic mendelian genetics. *American Biology Teacher*, *44*(2), 80–89.

○ Appendix: Probability Tree

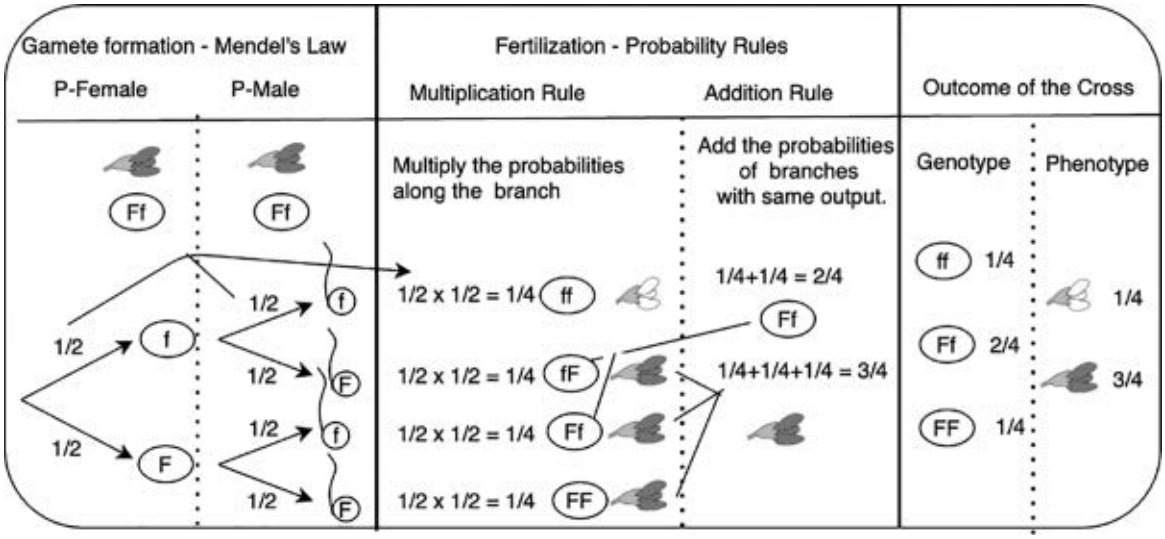


Figure A1. Construction of inheritance patterns of flower color using a probability tree.

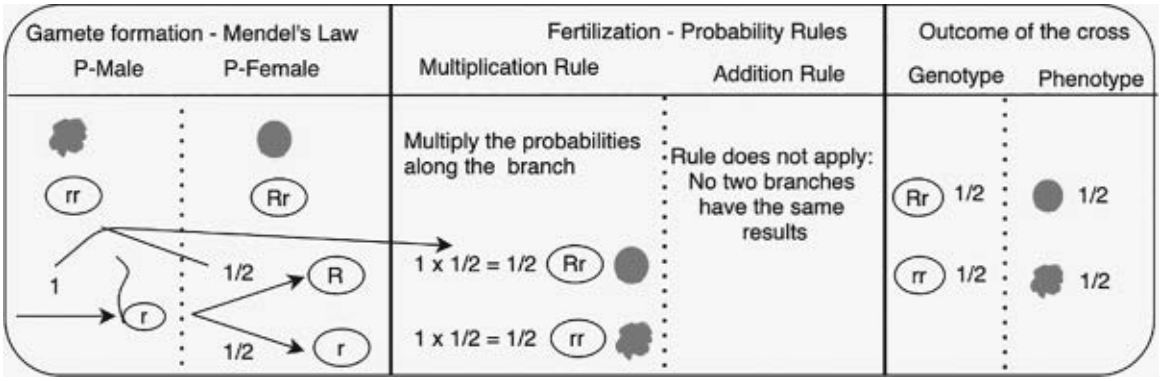


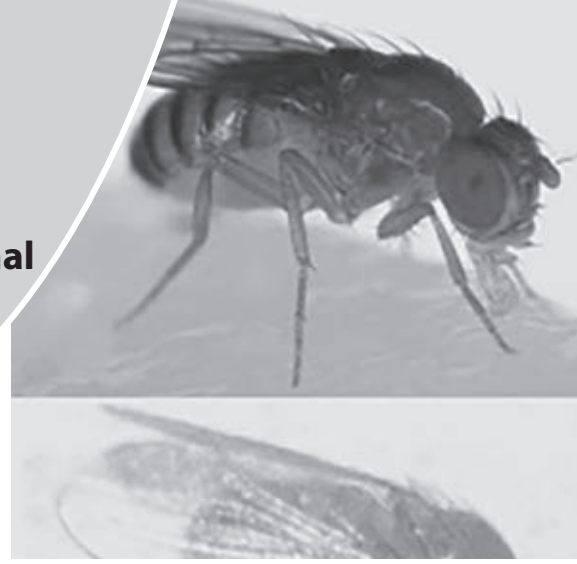
Figure A2. Construction of inheritance patterns of seed shape using a probability tree.

INQUIRY & INVESTIGATION

Investigating Causal Genetic Variation in the *yellow* Gene of *Drosophila melanogaster* as a Means of Teaching Foundational Molecular Genetic Concepts & Techniques

RECOMMENDED FOR AP Biology

Robert B. Page, Matt Crook



Abstract

How genetic variation influences phenotypic variation is of importance to many biological disciplines, including evolutionary biology, biomedicine, and agriculture. Nevertheless, students frequently struggle to make connections across levels of biological organization, which can make it challenging to facilitate understanding of how nucleotide variation gives rise to organismal variation. At the same time, biology students are now expected to gain early experience with cornerstone techniques from molecular biology, so that these skills can be reinforced and expanded upon. Here we describe a five-to-seven-week sequencing project that examines genetic and phenotypic variation in wild-type and yellow-bodied fruit flies and, in the process, exposes students to several foundational techniques in molecular biology. In addition, students analyze partial yellow *gene sequences from PCR products using the freely available bioinformatics suite UGENE and in doing so are introduced to core bioinformatics skills. The entire project is framed around the axiom that if the* yellow *gene controls phenotypic differences in body color between wild-type and yellow-bodied flies, it should be possible to identify causal variation in* yellow *sequences from wild-type versus yellow-bodied flies. This project relies on guided inquiry and can be used in 1000- or 2000-level molecular biology courses and advanced high school laboratories.*

Key Words: *bioinformatics; causal sequence variant; DNA sequencing; Drosophila melanogaster; guided inquiry; yellow body.*

○ Introduction

The causal relationship between nucleotide variation in DNA and phenotypic variation at the organismal level is among the most fundamental phenomena in all biology. It is essential to biological evolution (Futuyma, 1998), plant and animal breeding programs (Walsh and Lynch, 1998), genetic engineering (Copeland et al., 2001; Doudna & Charpentier, 2014), the risk and occurrence of disease (Botstein & Risch, 2003; Santoro et al., 2016), and many other features of biological systems. However, teaching this foundational concept to lower-division biology students is often challenging, and there is evidence that ***biological variation*** is a threshold concept (sensu Meyer & Land, 2003) that students must grasp in order to deepen their understandings of other biological processes, such as natural selection (Ross et al., 2010; Walck-Shannon et al., 2019). In addition, a firm understanding of causal genetic variation requires integration of knowledge across topics that span several levels of biological organization, which can also be challenging for students to understand and instructors to convey (National Research Council, 2009; American Association for the Advancement of Science, 2011). While lower-division students are struggling with foundational biological concepts, they also face pressure to gain exposure to knowledge and skills that will give them a competitive advantage with professional/graduate programs and the job market (Page et al., 2018). Indeed, it is now standard practice to expose lower-division college students to cornerstone techniques from molecular biology, such as DNA isolation, electrophoresis, and Sanger sequencing, as early exposure to these methods serves as a foundation that can be built upon in upper-division courses. Moreover, given the role that high-throughput techniques play in modern life sciences research, it is also important to expose students to biological databases and computerized analysis of biological data early in their careers (e.g., Hoatling et al., 2018).

> *"Our objectives with this series of modules are to teach the link between phenotype and molecular genetics and to introduce students to the skills they need to succeed in upper division classes and the workplace."*

The common fruit fly, *Drosophila melanogaster*, is one of the most time-honored model organisms in genetics and has played a role in many seminal discoveries including gene linkage (Morgan, 1911), sex-linkage (Morgan & Bridges, 1916), and the roles of homeobox genes in animal development (Kaufman et al., 1980; Nüsslein-Volhard & Wieschaus, 1980). As such, its genome is

The American Biology Teacher, Vol. 84, No. 1, pp. 28–32, ISSN 0002-7685, electronic ISSN 1938-4211. DOI: https://doi.org/10.1525/abt.2022.84.1.28.

thoroughly characterized, as are a large number of phenotypic and genetic variants. Indeed, much of this information is accessible through the fruit fly research community's dedicated database, FlyBase (https://flybase.org). Of the many varieties of *D. melanogaster* that have been described, one of the best known is the non-Mendelian X-linked trait yellow body, which was originally described by Morgan & Bridges in 1916. The *yellow* gene is pleiotropic, and loss of function leads to decreased pigmentation, viability, and male copulation success (Wilson et al., 1976). *Yellow* encodes a dopamine conversion enzyme (Heinze et al., 2017; Wittkopp et al., 2002; Xu et al., 2011) and has a readily available loss of function allele (y^1) which is an A-to-C transversion that ablates the ATG start codon required for mRNA translation (Geyer et al., 1990). Thus there is a direct causal relationship between the y^1 allele and the yellow-body phenotype via lack of yellow protein function.

Herein, we describe a guided-inquiry-based lab series that recapitulates the molecular characterization of a mutant allele that would occur once a genetic locus had been identified for a mutation generated in a forward genetic screen. This series entails generating, analyzing, and interpreting sequences from the 5´ end of the *yellow* gene derived from wild-type vs. yellow-bodied *D. melanogaster*. The entire project is framed around the axiom that if the *yellow* gene controls body color, then it should be possible to identify causal sequence variation between wild-type and yellow-bodied fruit flies in the *yellow* gene. This lab series can be conducted over five to seven weeks and exposes students to several foundational molecular biology techniques including DNA isolation, UV spectrophotometry, agarose gel electrophoresis, PCR, PCR purification, and Sanger sequencing. Furthermore, during the analysis and interpretation stage of the project, students are exposed to several core skills in bioinformatics and sequence analysis, such as working with biological databases, editing chromatograms, mapping reads to a reference sequence, and multiple sequence alignment. Lastly, in addition to considering causal sequence variation, students also consider eukaryotic gene structure and the consequences of point mutations in coding regions on gene expression.

○ Concept-Based Learning Objectives

- Define/explain what is meant by the phrase *causal sequence variant*.
- Define/explain the basic structure of eukaryotic genes.
- Explain how point mutations in coding sequence relate to the genetic code and gene expression.
- Explain how changes in gene expression associated with mutation can cause phenotypic changes at the organismal level.

○ Techniques & Methods Introduced

- DNA isolation
- Use of UV spectrophotometry to quantify and quality DNA isolates
- Use of gel electrophoresis to size separate DNA fragments
- Polymerase chain reaction (PCR)
- Use of spin column technology for purification of PCR products
- Sanger (dideoxy) sequencing
- Use of databases to obtain biological sequences and information about biological sequences
- Use of bioinformatic tools to process and analyze DNA sequences
  - Editing chromatograms
  - Mapping reads to a reference sequence
  - Using multiple sequence alignment to inspect nucleotide variation

○ Lab Series Design

Overview

This inquiry-based exercise is designed to be completed in five to seven weeks and is presented in six modules, beginning with DNA isolation and ending with sequence analysis, that mimic workflows routinely carried out in research labs (Table 1). It does not presume anything about students' molecular biology skills beyond reasonable competency with micropipettes. We have found that the exercise presented by Boker (2012) is useful in helping students develop accuracy with and confidence in their pipetting.

Table 1. Suggested schedule and supplemental materials.

Module	Length	Supplemental Materials
Phenotyping	One 1 hr. session	• Protocol 1
DNA isolation	One 2–3 hr. session	• Protocol 2
Isolate quality control	One 2–3 hr. session	• Protocol 3
PCR	One 1–3 hr. session	• Protocol 4 • PCR presentation slides
PCR purification	One 2–3 hr. session	• Protocol 5
Sequence analysis	One to three 2–3 hr. sessions (depending on endpoint)	• Protocol 6 • Sanger sequencing presentation slides • *yellow* sequences zip archive

Module 1: Exploring the *yellow* Phenotype

Linking phenotype and genotype is the key aim of this series of modules, so the first task is to explore the phenotypic differences between wild-type and yellow-bodied animals. The two objectives of this module are to familiarize students with the use of a stereo dissecting microscope and to use this microscope to compare the body color differences between wild-type and yellow-bodied animals.

Module 2: DNA Isolation

The primary goals of this module are twofold. First, as can be seen in Supplemental Protocol 2, students are made aware of the fact that there are several ways to perform DNA isolations but that all of these approaches consist of a series of physiochemical steps that facilitate specific occurrences (e.g., release of DNA from cells and removal of protein). Second, students are provided with a protocol that will enable them to isolate DNA from whole fruit flies. The protocol that we have provided is for use with Qiagen's DNeasy Blood and Tissue Kit (product 69504), as we have found that this kit's streamlined approach maximizes the likelihood of students generating isolates of sufficient quality for downstream analyses. However, minimally toxic protocols based on ammonium acetate precipitation (e.g., Fetzner, 1999, rescaled for cell-rich tissues from small arthropods) are less expensive and potentially just as effective.

Module 3: Isolate Qualification and Quantification

As can be seen in Supplemental Protocol 3, the third module in this lab series introduces students to UV spectrophotometry as a means of quantifying the yield obtained via DNA isolation and the purity of the isolates. As such, this lab introduces students to the basic relationship between absorbance and concentration via the Beer-Lambert Law. The procedure that we provide in Protocol 3 is for use with a microvolume spectrophotometer, such as a NanoDrop (Thermo Scientific); however, in principle, any instrument that measures absorbance at wavelengths between 200 and 300 nm would be sufficient. In addition, this lab teaches students how to use agarose gel electrophoresis to assess the degree to which their DNA isolates have been degraded. Lastly, students make dilutions suitable for loading template DNA into PCRs using their spectrophotometer results and the equation $V_1C_1 = V_2C_2$.

Module 4: Polymerase Chain Reaction

In this module, students are introduced to PCR and its many applications. In addition to providing students with the background material in Supplemental Protocol 4, we give a 20–30 minute presentation on the mechanics of PCR, including how real-time PCR can be used for viral and mRNA quantification. Students then work together to produce a master mix that is aliquoted into 0.2 ml tubes along with 2 µl of the 10 ng / µl dilution of template DNA that they will have made at the end of the previous session. The PCR recipe and thermal cycler conditions that we provide in Protocol 4 have been thoroughly tested using the Promega GoTaq Flexi Polymerase kit (product M8295) and dNTP mix (product U1515), as well as the T100 thermal cycler that is available from BioRad. However, use of other reagents and equipment will likely only require minor, if any, adjustments. We designed the primers described in Protocol 4 to target a region of *yellow* that includes a portion of the 5´ UTR, all of the first exon, and a portion of the first intron, which is appropriate given that the mutation we are assessing is located in the gene's start codon. We typically use 10 micromolar as our working primer concentration, and the primer volumes in Protocol 4 assume that the primers are at this concentration.

Module 5: PCR Verification & Cleanup

The primary objectives of this module are to (1) determine which PCRs from the previous session were successful, (2) remove impurities from the PCR products to make them suitable for Sanger sequencing, and (3) determine the concentration of the purified PCR product via UV spectrophotometry. In the interest of time, we typically have students set up their gels first and move on to the PCR purification protocol while their gels are running. The procedure we have given in Supplemental Protocol 5 is for use with the Wizard SV Gel and PCR Clean-Up System available from Promega (product A9281); however, in principle, any PCR cleanup procedure could be used in this step.

Module 6: Sanger Sequencing & Sequence Analysis

Between this module and the previous module, we typically submit students' purified PCR products to a genomics facility for Sanger sequencing. However, after doing this over the course of several semesters we have amassed several high-quality wild-type and mutant sequences that are suitable for students to work with, and these are included in the supplemental materials. As such, submission of student PCRs for sequencing is optional. Irrespective of whether student PCRs are sequenced or analyses are based on the. AB1 files we provide, it is advisable that students be introduced to the mechanics of Sanger sequencing, which we typically do via a 20–30 minute presentation. However, exercises such as the one offered by Conley et al. (2016) may be used to further emphasize key features of dideoxy sequencing.

Once the students have an appreciation for cycle sequencing reactions, capillary electrophoresis, and electropherograms, they use FlyBase, the NCBI website, and the freely available bioinformatics suite UGENE (Okonechnikov et al., 2012), to explore and analyze their sequences. Guidance for students on how to use these tools is given in Supplemental Protocol 6. Ultimately, the work students perform when completing this project can have one of two endpoints. The first possible endpoint is answering the question set provided in Protocol 6. The second possible endpoint is production of a scientific manuscript (i.e., introduction, methods, results, and discussion) based on this lab series.

○ Discussion

Our objectives with this series of modules are to teach the link between phenotype and molecular genetics and to introduce students to the skills they need to succeed in upper division classes and the workplace. We chose *D. melanogaster* as our model because it is easy to obtain and maintain, has a large number of well characterized visible mutant phenotypes, has a wealth of genetic information available, and is a model organism used by research laboratories to study a wide range of biological questions. We chose the *yellow* gene in large part because of how well the genetic basis for this phenotype, an A to C transversion in the start codon (Geyer et al., 1990), ties into the larger concept of gene expression, which underpins a large part of modern genetics. The modules form a series of guided inquiries utilizing the same approach that geneticists use to determine the molecular basis of mutant alleles recovered from a forward genetic screen. Each module is part of a whole but also stands alone as a functioning protocol.

Because misconceptions about genetics are common among college students and the general public (Stern & Kampourakis, 2017), it is crucial for instructors to be sensitive to the possibility that students will draw conclusions or make comparisons that were not intended. Given that this lab series examines a body color phenotype that results from a loss of function mutation in a gene that acts pleiotropically to affect behavior, these exercises should be conducted within the context of a course that actively seeks to disabuse students of any essentialist notions that they may have. Given that biological essentialism can be reinforced by examples that strongly link race to the alleles present at a single locus (e.g., sickle cell anemia in African Americans) and is associated with misunderstandings of intraspecific diversity (Donovan, 2015), it is important to convey to students that most traits in natural populations exhibit complex modes of inheritance and are not under single gene control. In addition to introducing students to foundational concepts from quantitative genetics (e.g., polygenes, additivity, and genotype-by-environment interaction), it should be emphasized that while molecular mechanisms are frequently evolutionarily conserved, the genetic and physiological circuitries of humans and other animals differ (Bolker, 2019; Greek & Rice, 2012).

The lab series we describe has worked well and is presented here in its final form after three years of iterative development and testing. Student feedback about the lab series tends to reflect their engagement with a longer duration project that deals with a "big picture idea" rather than a series of unlinked lab exercises or specific modules such as gel electrophoresis or Sanger sequencing. We feel that this lab series would be an excellent model organism module to run in series with others, such as *Caenorhabditis elegans* RNAi (Sengupta, 2013) or *Arabdopisis thaliana* breeding modules (Price et al., 2018). An alternative would be to pair this lab series with other *D. melanogaster* experiments, such as studying the X-linked inheritance of the *yellow* and *white* genes (Lobo & Shaw, 2008) or using a reverse genetics approach to conditionally knock down *yellow* in a wild-type background (Dietzl et al., 2007). In addition, a simple assay in which yellow-bodied vs. wild-type males compete for mates would give students the opportunity to study the influence of *yellow* on a complex trait—namely male courtship / copulatory success (Massey et al., 2019).

○ Supplemental Materials

The protocols for Modules 1–6—containing background and procedures, PowerPoint slides describing PCR and Sanger sequencing, and *yellow*.AB1 sequencing files—are available at https://jaguar-my.sharepoint.com/:f:/g/personal/rpage_tamusa_edu/EvIZrsiSoX1EurucWDeziQYBExfbZ9SJ6sfCHwwk7XidhQ?e=uASYSL.

○ Acknowledgments

We thank T. Marie Tipps for assistance during the early phases of development of this lab series.

References

American Association for the Advancement of Science. (2011). *Vision and Change in Undergraduate Biology Education*. Washington DC: AAAS.

Boker, J. (2012). You sank my … bacteriophage? *American Biology Teacher, 74*, 422–23.

Bolker, J.A. (2019). Selection of models: evolution and choice of species for translational research. *Brain, Behavior and Evolution, 93*, 82–91.

Botstein, D. & Risch, N. (2003). Discovering genotypes underlying human phenotypes: past successes for Mendelian disease, future approaches for complex disease. *Nature Genetics Supplement, 33*, 228–37.

Conley, J.E., Meisel, A.J. & Smith, J.J. (2016). Using M&Ms to model Sanger's dideoxy DNA sequencing method. *American Biology Teacher, 78*, 516–22.

Copeland, N.G., Jenkins, N.A. & Court, D.L. (2001). Recombineering: a powerful new tool for mouse functional genomics. *Nature Reviews Genetics, 2*, 769–79.

Dietzl, G., Chen, D., Schnorrer, F., Su, K.C., Barinova, Y., Fellner, M., Gasser, B., Kinsey, K., Oppel, S., Scheiblauer, S., Couto, A., Marra, V., Keleman, K., Dickson, B.J. (2007) A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*. *Nature*, *448*, 151–56.

Donovan, B.M. (2015). Reclaiming race as a topic of the U.S. biology textbook curriculum. *Science Education, 99,* 1092–117.

Doudna, J.A. & Charpentier, E. (2014). Genome editing. The new frontier of genome engineering with CRISPR-Cas9. *Science, 346*(6213), 1258096.

Fetzner, J.W. (1999). Extracting high-quality DNA from shed reptile skins: a simplified method. *Biotechniques, 26,* 1052–54.

Futuyma, D.J. (1998). *Evolutionary Biology, 3rd Edition*. Sunderland, MA: Sinauer Associates.

Geyer, P.K., Green, M.M. & Corces, V.G. (1990). Tissue-specific transcriptional enhancers may act in *trans* on the gene located in the homologous chromosome: the molecular basis of transvection in *Drosophila*. *EMBO Journal, 9*, 2247–56.

Greek, R. & Rice, M.J. (2012). Animal models and conserved processes. *Theoretical Biology and Medical Modelling, 9*, 40.

Heinze, S.D., Kohlbrenner, T., Ippolito, D., Meccariello, A., Burger, A., Mosimann, C., Saccone, G. and Bopp, D. (2017). CRISPR-Cas9 targeted disruption of the *yellow* ortholog in the housefly identifies the *brown body* locus. *Scientific Reports, 7,* 4582.

Hoatling, S., Slabach, B.L. & Weisrock, D.W. (2018). Next-generation teaching: a template for bringing genomic and bioinformatic tools into the classroom. *Journal of Biological Education, 52*, 301–13.

Kaufman, T.C., Lewis, R. & Wakimoto, B. (1980). Cytogenetic analysis of chromosome 3 in *Drosophila melanogaster*: the homeotic gene complex in chromosome interval 84A-B. *Genetics, 94,* 115–33.

Lobo, I. & Shaw, K. (2008). Thomas Hunt Morgan, genetic recombination, and gene mapping. *Nature Education, 1*(1), 205

Massey, J.H., Chung, D., Siwanowicz, I., Stern, D.L. & Wittkopp, P.J. (2019). The *yellow* gene influences *Drosophila* male mating success through sex comb melanization. *eLife*, *8*, e49388.

Meyer, J.H.F. & Land, R. (2003). Threshold concepts and troublesome knowledge: linkages to ways of thinking and practicing within the disciplines. In International Improving Student Learning Symposium, Rust, C. & Oxford Centre for Staff Development. *Improving student learning theory and practice - 10 years on: Proceedings of the 2002 10th International Symposium Improving Student Learning*. Oxford: Oxford Centre for Staff & Learning Development.

Morgan, T.H. (1911). Random segregation versus coupling in Mendelian inheritance. *Science, 34*, 384.

Morgan, T.H. & Bridges, C.B. (1916). *Sex-linked Inheritance in* Drosophila. Washington DC: Carnegie Institution.

National Research Council. (2009). *A New Biology for the 21st Century*. Washington DC: National Academies Press.

Nüsslein-Volhard, C. & Wieschaus, E. (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature, 287,* 795–801.

Okonechnikov, K., Golosova, O., Fursov, M. & the UGENE team. (2012). Unipro UGENE: a unified bioinformatics toolkit. *Bioinformatics, 28,* 1166–67.

Page, R.B., Espinosa, J., Mares, C.A., Del Pilar, J. & Shelton, G.R. (2018). The curvy road to student success in underserved populations. *Journal of College Science Teaching, 47*, *5*, 6–7.

Price, C.G., Knee, E. M., Miller, J.A., Shin, D., Mann, J., Crist, D.K., Grotewold, E. & Brkljacic, J. (2018). Following phenotypes: an exploration of Mendelian genetics using *Arabidopsis* plants. *American Biology Teacher*, *80*(4), 291–300.

Ross, P.M., Taylor, C.E., Hughes, C., Whitaker N., Lutze-Mann, L., Kofod, M. & Tzioumis, V. (2010). Threshold concepts in learning biology and evolution. *Biology International, 47,* 47–52.

Santoro, M.L., Moretti, P.N., Pellegrino R., Gadelha, A., Abilio V.C., Hayashi, M.A.F., Belangero, S.I. & Hakonarson, H. (2016). A current snapshot of common genomic variants contribution in psychiatric disorders. *American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics, 171*, 997–1005.

Sengupta, S. (2013). Bringing RNA Interference (RNAi) into the high school classroom. *American Biology Teacher*, *75*(9), 698–703.

Stern, F. & Kampourakis, K. (2017). Teaching for genetics literacy in the post-genomic era. *Studies in Science Education, 53,* 193–225.

Walck-Shannon, E., Batzli, J., Pultorak, J. & Boehmer, H. (2019). Biological variation as a threshold concept: can we measure threshold crossing? *CBE—Life Sciences Education, 18,* ar36.

Walsh, B. & Lynch, M. (1998). *Genetics and Analysis of Quantitative Traits.* Sunderland, MA: Sinauer Associates.

Wilson, R., Burnet, B., Eastwood, L. & Connolly, K. (1976). Behavioural pleiotropy of the *yellow* gene in *Drosophila melanogaster*. *Genetics Research, 28,* 75–88.

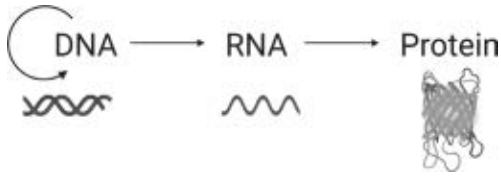
Wittkopp, P.J., True, J.R. & Carroll, S.B. (2002). Reciprocal functions of the *Drosophila* yellow and ebony proteins in the development and evolution of pigment patterns. *Development*, *129*(8), 1849–58.

Xu, X., Oliveira, F., Chang, B.W., Collin, N., Gomes, R., Teixeira, C., Reynoso, D., My Pham, V., Elnaiem, D.E., Kamhawi, S., Ribeiro, J.M., Valenzuela, J.G. & Andersen, J.F. (2011). Structure and function of a "yellow" protein from saliva of the sand fly *Lutzomyia longipalpis* that confers protective immunity against *Leishmania major* infection. *Journal of Biological Chemistry, 286*(37), 32383–93.

ROBERT B. PAGE, PhD, is an associate professor of biology, and MATT CROOK, PhD, is an assistant professor of biology, both in the Department of Life Sciences at Texas A&M University—San Antonio, San Antonio, TX; e-mails: Robert.Page@tamusa.edu and Matthew.Crook@tamusa.edu.

INQUIRY & INVESTIGATION

Teaching the Central Dogma through an Inquiry-Based Project Using GFP



Cynthia Bujanda, Nadja Anderson

Abstract

The Central Dogma is a crucial concept needed to understand biotechnology and molecular biology. High school students often struggle with a meaningful understanding of this abstract concept. This paper presents an inquiry-based approach to increase critical thinking and understanding of the Central Dogma. Commonly used in high school classrooms is Bio-Rad pGLO plasmid containing green fluorescent protein (GFP), because of its accessibility and the fluorescence it emits when exposed to ultraviolet light. We use the expression of GFP in a high school hands-on class project so that students can visualize and understand the abstract concepts of the Central Dogma. Students will also explore protein structure and its importance for a functional protein. During the entire project, students will be guided by the instructor to build hypotheses and design experiments to test those hypotheses, exercising the scientific method.

Key Words: *central dogma; GFP; inquiry; transformation.*

○ Introduction

Green fluorescent protein (GFP), a protein isolated from a bioluminescent jellyfish, *Aequorea victoria*, is of interest to researchers because of its bright fluorescence when exposed to ultraviolet (UV) light (Prasher et al., 1992). Historically, GFP has been used in high school classrooms since the mid-1990s; this was made possible after GFP was cloned and expressed in *E. coli* in 1994 (Ward et al., 2000), making it accessible for biotechnology education. Commonly used in the classroom is the transformation of *E. coli* using the Bio-Rad pGLO plasmid, which contains GFP as well as a gene for ampicillin resistance (bla) and a regulatory protein that binds to the promoter side pBAD (AraC) (Crameri et al., 1995; Deutch, 2019). In the presence of arabinose, the bound regulator on the promoter (AraC) changes shape, allowing RNA polymerase to bind to the promoter. This facilitates the gene expression of GFP by the production of mRNA, followed by the translation of mRNA into protein.

We can use the expression of GFP to explain the Central Dogma, which was first described by Francis Crick in 1970 to explain the transfer of information within cells (Figure 1). The Central Dogma states that the information in DNA is transferred to RNA and that in turn is transferred to a specific sequence of amino acids to produce a polypeptide. This concept is crucial in the understanding of biotechnology and molecular biology. However, biology education research has shown that, when taught under conventional modes of instruction, students have a difficult time understanding the abstract concepts of gene expression and protein translation that are core to the Central Dogma (Lewis & Kattmann, 2004; Newman et al., 2016; Reinagel & Bray Speth, 2016).

Figure 1. Central Dogma overview.

Part of the teachers' challenge to maximize students' learning of abstract concepts is that students are being asked to understand concepts, objects, and processes that are not visible (Hinze et al., 2013). Additionally, using technical vocabulary to convey genetic concepts proves burdensome for students (Reinagel & Bray Speth, 2016). Student understanding of abstract concepts is significantly increased with hands-on, kinesthetic learning (Fyfe et al., 2014; Hayes & Kraemer, 2017); this is even more apparent for ESL students (Llosa et al., 2016; National Academies of Sciences, Engineering, and Medicine, 2018). By broadening students' knowledge on genetic concepts and molecular biology, we educate students in scientific literacy. In today's society this becomes increasingly important because of the relevance of DNA techniques used in medicine, laboratory diagnostics, and criminology (Duncan & Reiser, 2007).

To efficiently teach the Central Dogma, it is important that we present lessons that mirror scientific research by inquiry-based exploration and meaningful reflection on results (Burnette &

The American Biology Teacher, Vol. 84, No. 1, pp. 33–37, ISSN 0002-7685, electronic ISSN 1938-4211. DOI: https://doi.org/10.1525/abt.2022.84.1.33.

Wessler, 2013; Zacharia et al., 2015). We report a lesson in which students will develop their own hypothesis and conduct experiments with the guidance of the instructor in order to reach conclusions and maximize their learning. The main objective of this lesson is to teach the abstract concepts of one of the most important lessons in molecular biology, the Central Dogma, targeting transcription and translation in a way that students experience the concepts rather than merely reading and hearing them.

We further expand our lesson by exploring protein structure and examining how the information flow from DNA will eventually be correctly expressed in proteins. Proteins are complex molecules, their structure being crucial to their function and regulation of cells, and are essential in all biological processes. Their function is dependent on their properly folded three-dimensional structure, which is dictated by the translated sequence of amino acids in the protein. The importance of protein structure can be seen by comparing denatured proteins and native proteins, which is accomplished in this lesson as a secondary objective.

These activities are accessible to schools with polymerase chain reaction (PCR) and electrophoresis materials and flexible to adjust to high school schedules. Teachers can use the pBADgfpuv plasmid, which is readily available through Bio-Rad Laboratories (as pGLO).

○ Objective

Engage students in an inquiry-based hands-on project that will broaden their knowledge of the Central Dogma in molecular biology.

○ Secondary Objectives

1. Students will hypothesize and experimentally test their hypotheses.
2. Students will be introduced to transformation, sterile technique, DNA extraction, PCR, electrophoresis, DNA sequencing, BLAST, and SDS-PAGE protein gels.
3. Students will observe the functional differences between denatured and native GFP.

○ Methods, Materials & Results

This set of lab activities takes approximately 10 days of class time. It can be broken up into different segments to fit the class schedule and curriculum. The details of the protocols and student guides are available on the BIOTECH Project website: http://biotech.bio5.org/publications. Overview of the entire class project can be seen in Figure 2.

○ Transformation of pBADgfpuv Plasmid

Teachers will provide students with an agar plate previously transformed with pBADgfpuv. Students begin the transformation process by extracting the plasmid DNA from glowing *E.coli* (Figure 3A). A single colony of glowing *E.coli* is grown in a liquid LB-plus-ampicillin culture to grow enough cells to extract plasmid DNA. Any plasmid DNA extraction kit can be used; we used

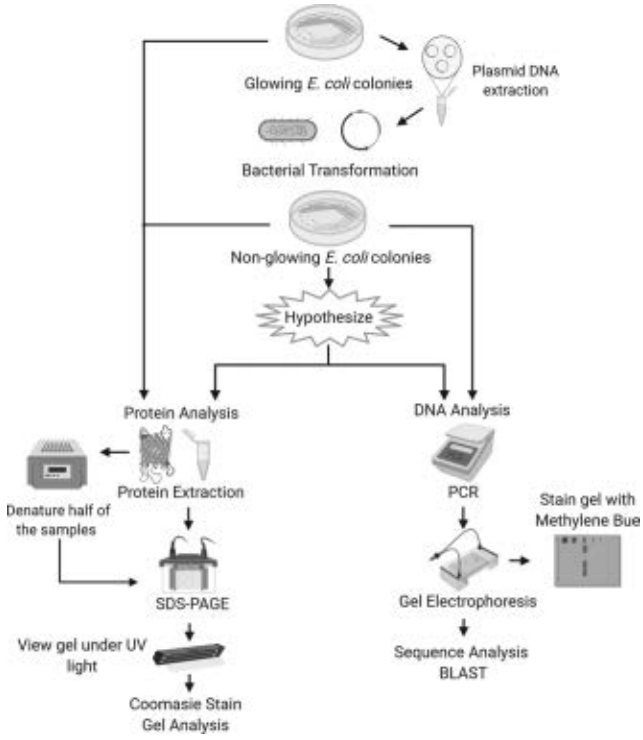


Figure 2. Overview of the entire class project with methodologies. Figure created with BioRender.com and exported under a paid subscription.

Omega BIO-TEK EZDNA Plasmid DNA Mini Kit and followed the manufacturer's instructions. The students can visualize the characteristics of the gene that is being expressed in the initial colonies and show that DNA is the genetic material involved in the transformation process. They should be able to hypothesis that if DNA is the genetic material seen in the transformation, the glowing characteristic will be transferred to the transformed *E. coli*. As an extension, students can further confirm that DNA is the genetic material by adding protease or DNAase to their transformation, much as Oswald Avery did to confirm Fred Griffith's results (Avery et al., 1944; Griffith, 1928). Between 20 and 50 ng of the extracted plasmid DNA can be used for bacterial transformation. Most plasmid DNA extraction kits will yield between 50 and 100 ng/µl.

Students introduce the transformed *E. coli* onto LB and LB-plus-ampicillin plates using a standard chemically competent cells transformation protocol (Green & Rogers, 2013). The following day, students analyze the results and observe transformed colonies (based on ampicillin selection, ampicillin resistance being another gene on the plasmid). At this point, students should wonder why their colonies are not glowing (Figure 3B), since they used plates without arabinose. This being the big question, students will need to find the answer by testing different hypotheses.

The instructor should remind students that their hypothesis of DNA being the genetic material seen in transformation was supported due to the selection of colonies on ampicillin. They should encourage the students to hypothesize as to why their colonies are not glowing, as was seen in the original colonies they used to extract DNA (Figure 3A), and provide guidance as to how they can test each hypothesis. This is a crucial step

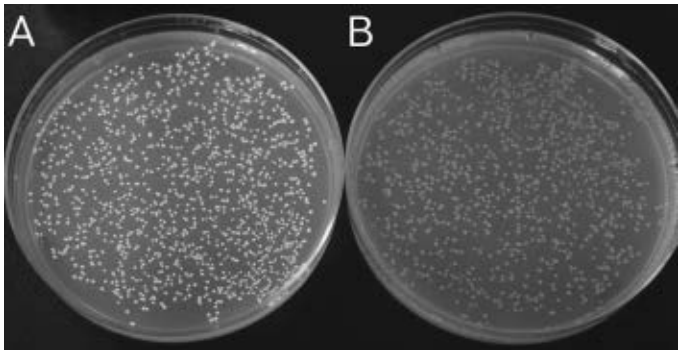


Figure 3. *E. coli* transformed colonies with pGLO. (**A**) Initial agar plate with glowing *E. coli* used for DNA extraction. (**B**) Students transformed nonglowing *E. coli*.

that builds the next set of experiments conducted by the students. Table 1 lists potential hypotheses students may propose. Hypothesis 5 requires students to have more background information, but the others are generally proposed by students (Table 1). It is essential that the instructor guide the students into formulating their thoughts into hypotheses.

Table 1. Student hypothesis—five common hypotheses proposed by students.

Hypothesis	How to Test Hypothesis (Teacher Guided)
GFP gene is mutated	PCR/DNA sequence
GFP gene is not in plasmid	PCR/gel electrophoresis
Plasmid/DNA is not present	Can be eliminated—ampicillin selection was seen
GFP gene is not expressed	Look for presence of mRNA (northern blot, Q-PCR)
Protein is not produced or is improperly folded	Protein extraction/SDS-PAGE

○ DNA Analysis: PCR & Gel Electrophoresis

To test Hypotheses 1 and 2 in Table 1, the instructor guides students to utilize PCR to look for the presence of GFP. The PCR product can then be sequenced and analyzed for mutations. If the students do not have any previous PCR experience, the instructor should dedicate time to give an overview of the technique. There are many animations to help students understand the process, including the DNA Learning Center PCR Animation on YouTube (https://www.youtube.com/watch? v=JRAA4C2OPwg).

To set up the PCR reactions, students can use any scientific supply company's master mix; we used Promega GoTaq, which contains the Taq polymerase, dNTPs, Mg^{2+}, and buffers to simulate the cellular conditions. Each reaction will need forward and reverse primers (GFP Forward: 5´CTCCATACCCGTTTTTTTGC3´ and GFP Reverse: 5´CTGTTTTATCAGACCGCTTC3´) with nuclease-free water making up the remaining volume. A small dab of an *E. coli* colony will be used for the students' template DNA. PCR cycles for amplification of GFP are as follows:

One cycle of:

- 94°C for 5 minutes (initial denaturation and *E. coli* cell disruption)

30 cycles of:

- 94°C for 30 seconds (denaturation of DNA)
- 55°C for 45 seconds (primer annealing)
- 72°C for 1.5 minutes (DNA extension)

One cycle of:

- 72°C for 7 minutes (final extension)

Students will also need to run positive and negative controls to compare their PCR product. A small volume (0.5 µl) of plasmid DNA will be added for the positive control, and either 0.5 µl of water or nothing will be added as the negative control. We have found that a typical class time best allows student groups to set up two reactions; all the groups run a PCR of their nonglowing colony, one group can amplify a positive control, another can amplify the negative control, and two groups can view their results together on an electrophoresis gel. Alternatively, with longer class periods, teachers can choose to have each group run both positive and negative controls. An example of PCR results can be seen in Figure 4.

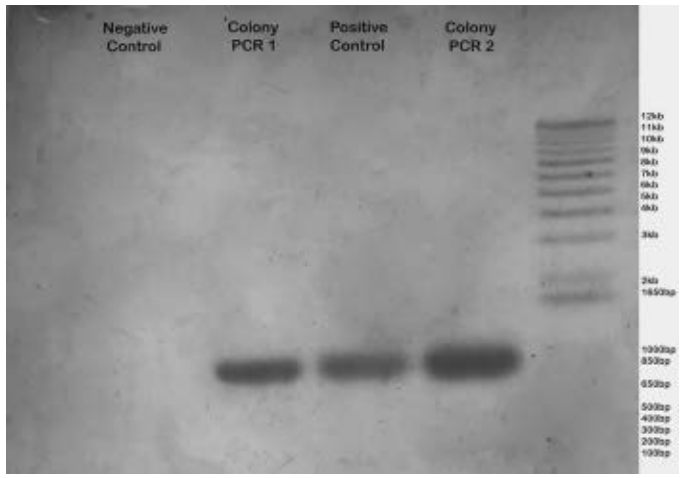


Figure 4. Gel electrophoresis of PCR of GFP, 0.8% agarose in TAE, stained with methylene blue.

PCR products are analyzed by gel electrophoresis and compared to a molecular weight (MW) marker (Invitrogen 1 Kb Plus). If there is a band corresponding to the MW of GFP (~800 bp), then GFP was amplified and can be sequenced (Figure 4). At the end of the DNA analysis, students will have experience in molecular techniques, pipetting, DNA extraction, transformation, PCR, and gel electrophoresis. While students are waiting for the sequencing results, another hypothesis can be investigated. Most high schools do not have the ability to conduct a northern blot or have access to a Q-PCR, potentially making it difficult to test Hypothesis 4, though gene expression will be revisited at the end of the lesson. As an alternative, students can focus on testing for the presence and structure of the protein.

○ Protein Analysis: Protein Extraction, Bradford Assay, & SDS-PAGE

To analyze GFP, students can extract cellular proteins from both the original glowing and nonglowing *E. coli* (Figure 1). We will refer to the glowing sample as *green* and the nonglowing as *white*. Students should collect half the colonies on each plate with a 10µl loop and resuspend the bacteria colonies in a microcentrifuge tube containing 500µl of LB broth by twirling the loop in the broth. Centrifuge the samples, discard the supernatant, and resuspend the bacteria pellet in 400µl of Camiolo buffer. Aliquot half of each sample to a new tube and heat at 95°C for 5 minutes to ensure complete denaturation of the proteins. At this point, each group should have four samples, green heated (G+), green unheated (G-), white heated (W+), and white unheated (W-). An introduction to protein folding and protein structure can be used to illustrate the denaturation process expected with high temperatures (https://youtu.be/8k6D8ajTRlc). Using a Bradford assay, students will determine the protein concentration of each sample. They will make the appropriate dilutions with Laemmli buffer for a final concentration of 0.5 mg/ml of protein in a final volume of 0.1 ml.

Protein analysis is conducted with SDS-PAGE, which separates proteins by size; this is based on an adaptation of "Biotechnology Explorer Protein Electrophoresis of GFP" from Bio-Rad Laboratories. Once the samples have migrated into the gel and the dye front is at the bottom, observation of the gel cassette with UV light will allow students to see one green, structurally functional GFP band glowing at a size of approximately 50 kD (Figure 5B). We recommend using a fluorescent marker, such as BioRad's Precision Plus Protein Kaleidoscope (product 1610375), to assess the size of the glowing band. Students should see the difference between green heated and unheated samples. The instructor can help students interpret that in order to have the functional protein it needs to be folded properly. Students will also see that properly folded, functional, glowing GFP proteins are not visible in the white colony samples.

In order to address whether nonfunctional GFP is present in the students' white colonies, stain the gel with Coomassie blue and visualize the protein profiles of these samples. An example of the Coomassie stained gel can be seen in Figure 5A. Comparing G+ and G-, we can see the contrast in that G+ has abundant protein at ~27 kD (marked with a white arrow), which is interpreted to be the denatured variant of GFP. Folded and denatured proteins can migrate at vastly different "sizes" in the gel, hence the size difference. The predicted size of GFP monomer is 27 kD. Additionally, the functional glowing protein may be migrating as a dimer. The denatured variant of GFP is absent in the white colony samples (W+ and W-). The G-lane shows abundant protein (marked with the red arrow) at the same size as the fluorescent band, which is also absent in the W- or W+. Students may notice that other proteins are expressed in both white colony samples that are not seen in the GFP expressing samples. This is not uncommon, since the expression of GFP is taking up much of the cells resources and therefore they are not able to express their normal abundance of other proteins. These, however, are seen in the white colonies where resources are not being taxed by the overexpression of a nonessential protein. After finishing the protein analysis, students should conclude that the GFP wasn't being produced by their nonglowing, transformed colonies.

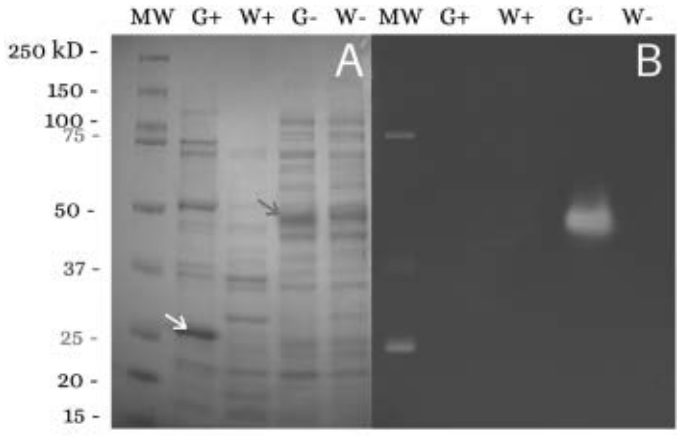


Figure 5. 12% SDS-PAGE. A) Coomassie stained gel after destaining. B) Gel under UV light, prestaining.

○ Sequence Analysis of GFP: NCBI/ BLAST

Instructors can find the document with DNA sequence information on the BIOTECH Project website. We recommend doing the protein analysis before the sequence analysis. Returning to the hypothesis of whether the GFP gene was mutated, students will analyze the sequence of the PCR product. Students are introduced to the NCBI website, specifically the BLAST tool. In this program, the sequence query (PCR product) will be compared to sequences in the database to find similarities. In the BLAST results, an option of "cloning vector pBAD-GFPuv, complete sequence" will display. The query alignment to the subject (pBAD-GFPuv Accession U62637) will show no apparent mutations, thus refuting the first hypothesis (Table 1). Looking at the sequence entry of the entire plasmid sequence, three genes are features in this plasmid: araC, gfpuv, and bla. Further investigation of the genes allows students to determine that bla provides ampicillin resistance and that araC encodes for the araC protein. At this point, students should question the purpose of araC. Students can Google *araC*, leading them to the AraC Wikipedia page (https://en.wikipedia.org/wiki/AraC). The information on this page indicates that araC is a component of the L-arabinose operon in *E. coli*. Further investigation of the L-arabinose link will allow the students to elucidate that arabinose is necessary to activate the genes of the L-arabinose operon. The instructor helps the students identify that AraC acts as a repressor on the promoter of these genes by blocking the DNA binding site for RNA polymerase, thus blocking the expression of the BAD genes (genes on the L-arabinose operon). In the presence of arabinose, AraC protein is altered, allowing the binding of RNA polymerase and expression of the BAD genes. AP biology classes will be able to relate this to Lac-operon gene expression.

The students should hypothesize that pBAD-GFPuv is using this regulated promoter for GFP expression and, if so, that the addition of arabinose to these cells will allow GFP to be expressed and the colonies to glow. By discovering the function of AraC on BAD promoter expression, they are tying together the concepts of the Central Dogma. The instructor provides arabinose to be added to their transformed nonglowing plates (which have been stored at 4°C to avoid overgrowth of colonies). After incubation at 37°C overnight, students can see glowing colonies. This reinforces that DNA is the

instruction manual that has the information. To produce a protein, DNA must transcribe its information in RNA so that the translation to amino acids (the language of proteins) can occur.

○ Conclusion

This lab project aims to explain gene expression and how information transfers within cells. The most important takeaway from this project is the Central Dogma of molecular biology, in a way that students can experience through an inquiry-based project rather than lecture-based instruction. Additionally, protein folding/denaturation and protein function were addressed. Using heat to alter the shape of the protein, rendering it nonfunctional, students can learn how important protein structure is for proper function.

The role of the instructor is to ignite curiosity and engagement in the students, to guide in students' development of hypotheses and apply the scientific method. This inquiry-based project will promote critical thinking to an abstract concept—the Central Dogma.

References

Avery, O.T., Macleod, C.M. & Mc Carty, M. (1944). Studies on the chemical nature of the substance inducing transformation of pneumococcal types: Induction or transformation A Deoxyribonucleic acid fraction isolated from pneumococcus type III. *Journal of Experimental Medicine*, *179*(2), 379–84. https://doi.org/10.1084/jem.179.2.379.

Bio-Rad laboratories. (2017). *Bio-Rad Explorer Protein Electrophoresis of GFP: A pGLOTM Bacterial Transformation Kit Extension*. https://www.bio-rad.com/webroot/web/pdf/lse/literature/M1660023.pdf.

Burnette, J.M. & Wessler, S.R. (2013). Transposing from the laboratory to the classroom to generate authentic research experiences for undergraduates. *Genetics*, *193*(2), 367–75. https://doi.org/10.1534/genetics.112.147355.

Crameri, A., Whitehorn, E.A., Tate, E. & Stemmer, W.P.C. (1995). *Improved Green Fluorescent Protein DNA Shuffling*. 315–19. https://doi.org/10.1038/nbt0396-315.

Deutch, C.E. (2019). Transformation of *Escherichia coli* with the pGLO plasmid : going beyond the kit. *American Biology Teacher*, *81*(1), 52–55. https://doi.org/10.1525/abt.2019.81.1.52.

Duncan, R.G. & Reiser, B.J. (2007). Reasoning across ontologically distinct levels: Students' understandings of molecular genetics. *Journal of Research in Science Teaching*, *44*(7), 938–59. https://doi.org/10.1002/tea.20186.

Fyfe, E.R., McNeil, N.M., Son, J.Y. & Goldstone, R.L. (2014). Concreteness fading in mathematics and science instruction: a systematic review. *Educational Psychology Review*, *26*, 9–25. https://doi.org/10.1007/s10648-014-9249-3.

Green, R. & Rogers, E.J. (2013). Chapter twenty eight - transformation of chemically competent *E. coli*. *Methods in Enzymology*, *529*, 329–36. https://doi.org/10.1016/B978-0-12-418687-3.00028-8.

Griffith, F. (1928). The significance of pneumococcal types. *Journal of Hygiene*. *27*(2), 113–59. https://doi.org/10.1017/S0022172400031879.

Hayes, J.C. & Kraemer, D.J.M. (2017). Grounded understanding of abstract concepts: the case of STEM learning. *Cognitive Research: Principles and Implications*, *2*(1). https://doi.org/10.1186/s41235-016-0046-z.

Hinze, S.R., Rapp, D. N., Williamson, V.M., Shultz, M.J., Deslongchamps, G. & Williamson, K.C. (2013). Beyond ball-and-stick: Students' processing of novel STEM visualizations. *Learning and Instruction*, *26*, 12–21. https://doi.org/10.1016/j.learninstruc.2012.12.002.

Lewis, J. & Kattmann, U. (2004). Traits, genes, particles and information: revisiting students' understandings of genetics. *International Journal of Science Education*, *26*(2), 195–206. https://doi.org/10.1080/09500690320000072782.

Llosa, L., Lee, O., Jiang, F., Haas, A., O'Connor, C., Van Booven, C.D. & Kieffer, M.J. (2016). Impact of a large-scale science intervention focused on English language learners. *American Educational Research Journal*, *53*(2), 395–424. https://doi.org/10.3102/0002831216637348.

National Academies of Sciences, Engineering, and Medicine. (2018). *English Learners in STEM Subjects: Transforming Classrooms, Schools, and Lives*. Washington, DC: National Academies Press. https://doi.org/10.17226/25182.

Newman, D.L., Snyder, C.W., Fisk, J.N. & Wright, L.K. (2016). Development of the central dogma concept inventory (CDCI) assessment tool. *CBE Life Sciences Education*, *15*(2). https://doi.org/10.1187/cbe.15-06-0124.

Prasher, D.C., Eckenrode, V.K., Ward, W.W., Prendergast, F.G. & Cormier, M.J. (1992). GFP cloned and sequenced. *Gene*, *111*, 229–33. https://doi.org/10.1016/0378-1119(92)90691-h.

Reinagel, A. & Bray Speth, E. (2016). Beyond the central dogma: model-based learning of how genes determine phenotypes. *CBE—Life Sciences Education*, *15*(1), ar4. https://doi.org/10.1187/cbe.15-04-0105.

Ward, W.W., Swiatek, G.C. & Gonzalez, D.G. (2000). *Green Fluorescent Protein in Biotechnology Education*, 305, 672–80.

Zacharia, Z.C., Manoli, C., Xenofontos, N., de Jong, T., Pedaste, M., van Riesen, S.A.N., Kamp, E.T., Mäeots, M., Siiman, L. & Tsourlidaki, E. (2015). Identifying potential types of guidance for supporting student inquiry when using virtual and remote labs in science: a literature review. *Educational Technology Research and Development*, *63*(2), 257–302. https://doi.org/10.1007/s11423-015-9370-0.

CYNTHIA BUJANDA is a designated campus colleague in the Department of Molecular and Cellular Biology at the University of Arizona and a high school teacher at Sunnyside High School; e-mail: cindybjd@email.arizona.edu. NADJA ANDERSON is the director of the Biotech Project in the Department of Molecular and Cellular Biology at the University of Arizona; e-mail: nadja@bio5.org.

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 practitioners at every level, with a special focus on high school and post-secondary biology instruction.

Revised September 2021

Article Categories

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

Feature Article (up to 4,500 words) includes topics of general interest to readers of *ABT*. Consider the following examples of content that would be suitable for 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 and life science education standards
- Social and ethical implications of biology and how to teach such issues as genetic modification, energy production, agriculture, climate change, health care, nutrition, and cultural responsiveness
- Reviews and updates of recent advances in the life sciences in the form of an "Instant Update" that brings readers up-to-date in a specific area
- Imaginative views of the future of biology education and suggestions for adjusting to changes in schools, classrooms, and student populations
- Other timely, relevant, and interesting content such as discussions of the role of the Next Generation Science Standards in biology teaching, considerations of the nature of science with implications for the classroom, considerations of the continuum of biology instruction from K–12 to post-secondary teaching environments, or contributions that consider the likely/ideal future of science and biology instruction

Research on Learning (up to 4,500 words) includes reports of original research on innovative teaching strategies, learning methods, or curriculum comparisons. Studies should be based on sound research questions, hypotheses, discussion of appropriate design and procedures, data and analysis, discussion on study limitations, and recommendations for improved learning outcomes.

Inquiry and Investigations (up to 3,500 words) is the section of *ABT* that features discussion of innovative laboratory and field-based strategies. Strategies in this section should be original, engaging, practical, and related to either a particular program such as AP and/or linked to standards such as NGSS. Submissions should also be focused at a particular grade/age level of student and must include all necessary instructions, materials list, worksheets, and assessment tools. Other appropriate contributions in this category are laboratory experiences that engage students in inquiry.

Tips, Tricks and Techniques (up to 1,500 words but may be much shorter) features a range of suggestions useful for teachers including laboratory, field, and classroom activities; motivational strategies to assist students in learning specific concepts; modifications of traditional activities; new ways to prepare some aspect of laboratory instruction; etc.

Writing & Style Guidelines

The *Chicago Manual of Style, 17th Edition* is the guide for questions of punctuation, abbreviation, and style. List all references in alphabetical order on a separate page at the end of the manuscript. Please review a past issue for examples. Use first person and a friendly tone whenever appropriate. Use concise words to emphasize your point rather than capitalization, underlining, italics, or boldface. Use the SI (metric) system for all weights and measures.

While calls for specific themed issues of *ABT* are infrequent, February and April are traditionally themed editions on Evolution and the Environment, respectively.

Preparing Tables, Figures, and Photographs

General Requirements

- When your article is accepted, we will require that figures be submitted as individual figure files in higher resolution format. See below for file format and resolution requirements.
- Authors should be aware that color is limited within the journal. All artwork, figures, tables, etc. must be legible in black and white. If color is important to understanding your figures, please consider alternative ways of conveying the information.

Article Photographs

Digital files must meet the following guidelines:

- Minimum resolution of 300 DPI, 600 DPI is preferred
- Acceptable file formats are TIFF and JPEG
- Set to one-column (3.5" wide) or two-column size (7" wide)
- If figure originates from a website, please include the URL in the figure caption. Please note that screen captures of figures from a website are normally too low in resolution for use.

Tables and Figures

- Minimum resolution of 600 DPI, 1200 DPI is preferred
- Acceptable file formats are TIFF, BMP, and EPS
- Set to one-column (3.5" wide) or two-column size (7" wide)

If you have any questions, contact Valerie Haff at managingeditor@nabt.org.

continued

Submission Guidelines

NOTE: All authors must be current members of NABT or a charge of $100 per page is due before publication.

All manuscripts must be submitted online at http://mc.manuscriptcentral.com/ucpress-abt

- Authors will be asked to register the first time they enter the site. Upon receiving a password, authors can proceed to upload their manuscripts through a step-by-step process. Assistance is available in the "Author Help" link found in the menu on the left side of the page. Additional assistance is available from the Managing Editor (managingeditor@nabt.org).
- Manuscripts must be submitted as Word or WordPerfect files.
- Format manuscripts for 8.5 × 11-inch paper, 12-point font, double-spaced throughout, including tables, figure legends, and references.
- Please place figures (including photos) and tables where they are first cited in the text along with appropriate labels. Make sure to include figure and table citations in the text, as it is not always obvious where they should be placed. At the time of initial submission, figures, tables and images should be low resolution so that the final file size remains manageable.
- If your article is accepted, the editors will require that figures be submitted as figure files in higher resolution form. See section on *Preparing Tables, Figures, and Photographs*.

Supplemental Materials

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

Editorial Procedures

- Communications will be directed only to the first author of multiple-authored articles.
- Typically, three individuals who have expertise in the respective content area will review each article.
- The editors attempt to make decisions on articles as soon as possible after receipt, but the process can take six to eight months, with the actual date of publication to follow. Authors will be emailed editorial decisions as soon as they are available.
- Accepted manuscripts will be forwarded to the Copy Editor for editing. This process may involve making changes in style and content. However, the author is ultimately responsible for scientific and technical accuracy. Page proofs will be sent to authors for final review before publication at which time only minor changes can be made.

Submitting Images

Cover Images

Submissions of cover photographs from NABT members are strongly encouraged. Covers are selected based on the quality of the image, originality, composition, and overall interest to life science educators. *ABT* has high standards for cover image requirements and it is important for potential photographers to understand that the required size of the cover image generally precludes images taken with cell phones, point-and-shoot cameras, and even some older model digital SLR cameras.

Please follow the requirements listed below.

- Email possible cover images to Kathleen Westrich at kmwestrich@yahoo.com.
- *ABT* covers feature an almost-square image with a slight vertical orientation.
- Choose an image with a good story to tell. Do not crop the subject too tightly. It is best to provide an area of background around the subject.
- Include a brief description of the image, details of the shot (i.e., circumstances, time of day, location, type of camera, camera settings, etc.), and your biographical information in an email message.
- Include your name, home and email addresses, and phone numbers.
- Please ensure that the image meets the minimum standards for publication listed below and has not been edited or enhanced in any way. The digital file must meet the minimum resolution of 300 pixels per inch (PPI)—preferred is 400 PPI— and a size of 8.5 x 11.25". We accept TIFF or JPEG images only.

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

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

Valerie Haff, Managing Editor
managingeditor@nabt.org

INQUIRY & INVESTIGATION

Using DNA Barcoding Methods to Identify Wild Huckleberry, *Vaccinium membranaceum*, as a Classroom Project

NABT BioClub RECOMMENDATION

Jeff Dykes, Kristy Kappenman, Emily D. Nissen

Abstract

In 2019 the Biology 100 class of Wenatchee Valley College at Omak (WVCO) worked on a DNA barcoding project with Tabitha Graves's huckleberry research project for bear habitat in Glacier National Park, Montana. Students isolated DNA from huckleberry leaf samples from the National Park. They then ran a PCR with an rbcL primer pair to target the rubisco gene in plant chloroplasts. The PCR product was sequenced by a private company, Genewiz, and the DNA sequence was analyzed through DNA Subway. Twelve student groups, one or two students per group, isolated DNA from huckleberry leaf samples that was sequenced and analyzed. Twelve samples were determined to be of the genus Vaccinium. One of the twelve samples distinguished between the five species of huckleberry, identified the sample as Vaccinium membranaceum, and was published in GenBank. They showed that DNA barcoding can be used successfully to aid in the identification of this species of huckleberry. There were many student outcomes, including hands-on skills with the tools of DNA technology, contributing to a real-world project, and analyzing data for DNA sequence matches. This is a great lab exercise to use for AP biology classes, two-year community college biology classes, and four-year college biology classes at the 100 to 200 level.

Key Words: *Bear; chloroplast DNA; Cold Springs Harbor Laboratory; DNA barcoding; DNA Subway; gel electrophoresis; gene; Glacier National Park; huckleberry; PCR; rbcL; rubisco; taxonomy; USGS Northern Rocky Mountain Science Center; Vaccinium; Wenatchee Valley College at Omak.*

○ Introduction

Since 2014 the science department of Wenatchee Valley College at Omak (WVCO), in North Central Washington, has experimented with methods in DNA barcoding. We have followed the procedure outlined by Cold Springs Harbor Laboratory DNA Learning Center (DNALC, 2018). Our campus has a botanical garden, the WVCO Native Plant Garden, that contains plants native to our geographical area. Students can sample plant leaves and use the DNA barcoding methods to identify the species of numerous plants from the Native Plant Garden. In many cases student results were subsequently published in GenBank, the U.S. Government repository for DNA sequences. To date, our WVCO students have published in GenBank 32 plant DNA sequences and one American black bear DNA sequence.

In brief, the DNA barcoding lab consists of grinding the leaf of a green plant and placing the pulverized tissue in a microfuge tube with silica. The silica selectively binds the chloroplast DNA and separates the chloroplast DNA from other cell debris. Next, the plant DNA sample is combined with primers that select a specific DNA sequence of nucleotides that are multiplied by the polymerase chain reaction (PCR). The product of the PCR amplification is visualized with gel electrophoresis. Correctly sized DNA from PCR is then sent to Genewiz (http://www.genewiz.com/) for DNA sequencing. Finally, sequences are run in the DNA Subway (https://dnasubway.cyverse.org) collection of programs to find a match.

"There were many student outcomes, including hands-on skills with the tools of DNA technology, contributing to a real-world project, and analyzing data for DNA sequence matches."

In 2018 WVCO's science department began a collaborative project with Tabitha Graves, research ecologist, USGS Northern Rocky Mountain Science Center in Glacier National Park, Montana. Her work was to "understand variables influencing huckleberries to identify management options to improve resilience of bear food system" (Graves, n.d.). The value of our collaboration was to add a method for identifying species of huckleberry using DNA barcoding. Samples from Graves were identified in the field by workers who used traditional methods of taxonomic keys. Indeed, some of the samples did not come with the species identified. The goal of our work was to determine if DNA barcoding

The American Biology Teacher, Vol. 84, No. 1, pp. 40–44, ISSN 0002-7685, electronic ISSN 1938-4211. DOI: https://doi.org/10.1525/abt.2022.84.1.40.

could be a reliable means for identifying species of huckleberries. Our students were to determine the DNA barcode for five known species of huckleberries found in Glacier National Park, Montana. The possible species were *Vaccinium membranaceum*, *V. myrtilloides*, *V. caespitosum*, *V. myrtillus*, and *V. scoparium* (Graves, n.d.).

The overall goal of DNA barcoding is to establish a database that allows the identification of an unknown organism by matching a specific sequence of its DNA to a known sequence for the corresponding known organism. This method takes the emphasis away from the taxonomic keys that often require extensive familiarity with the specific characteristics of the organism to be identified. DNA barcoding uses DNA primers to select and amplify an appropriate region of DNA using PCR. With PCR, primers are recommended for the type of organism, fish, plant, fungus, animal, etc. (DNALC, 2018). When mixed with DNA (template), isolated from the sample, PCR will generate millions of copies of the DNA sequence from the template. These copies of the DNA are then sequenced, and their order of DNA letters is determined. Isolated DNA samples are easily and cheaply sequenced by commercial companies, such as Genewiz. The DNA sequence is entered into the web application DNA Subway to determine the identity of the species of organism. DNA Subway is a collection of several applications combined in one system. DNA Subway takes the entered sequence and compares it with known sequences of plants by doing a BLASTN search of GenBank. The compared matches are ranked according to the number of nucleotides that are not matching (DNALC and CyVerse, n.d.). In this project we looked for a 100% match to determine the species level of identity.

As a result of this work, one student was successful in determining the DNA barcode for a species of huckleberry named *Vaccinium membranaceum* (sample 114). We present this paper with the hope that other students will become involved with DNA barcoding in the science classroom.

○ Literature Review

In 2003 Paul D. N. Hebert and colleagues proposed using a version of the universal price code (UPC), or barcode, to represent a sequence of DNA for a given species of organism. He coined the term *DNA barcode*. His choice to identify species of insects was the 658 bp fragment of the mitochondrial cytochrome c oxidase I (COI) gene. The COI gene has a faster rate of evolution than the 12 S and 16 S ribosomal genes. Thus, there is more of a chance to see variations among species that would act as unique markers to identify the species. The primer pair of LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) was used in the PCR reaction that generated the 658 bp COI gene fragment (Hebert et al., 2003). His work showed that the COI gene sequences could distinguish between different species.

For fungi, Bellemain and colleagues (2010) determined that from nuclear DNA the internal transcribed spacer (ITS) region of DNA worked well for identifying fungi using the DNA barcode technique. Various primers were selected for sequencing the ITS regions. It was found that some of the primers worked better with basidiomycetes (ITS_1-F, ITS_1, and ITS_5) while others primers (ITS_2, ITS_3, and ITS_4) favored ascomycetes.

For land plants, the Consortium for Barcode of Life Working Group (CBOL), found that a combination of primers, called a 2-locus barcode, for more than one gene worked very well. These primers were the rbcL and matK primers (Hollingsworth et al., 2009). The primer rbcL targets the region of the ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) gene, and the matK primer targets the maturase K gene. Both genes are found in the chloroplast of plants. The rubisco gene codes for the enzyme that fixes (incorporates) carbon dioxide into sugars in the Calvin cycle of photosynthesis. The maturase K gene creates a product that is involved with editing of RNA in the chloroplasts. RbcL was selected for our work as the best primer for use with land-based plants, according to DNALC (2018).

A DNA barcode is a sequence of DNA letters or nucleotides (ATCG), with each letter represented by a different color and formatted like a grocery store barcode with vertical lines. In this way any gene can be represented as a barcode. Figure 1 shows the DNA barcode, as generated by Bio-Rad's DNA Barcode Generator (http://biorad-ads.com/DNABarcodeWeb) for the plant species of thin leaf huckleberry.

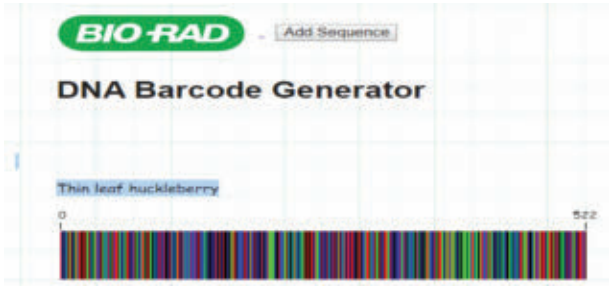


Figure 1. A DNA barcode for thin leaf huckleberry. In this case it indicates the DNA letter sequence (ATCGs) for 522 letters or base pairs. This was created by inputting the DNA sequence into Bio-Rad's DNA Barcode Generator.

Once the DNA gene region(s) have been sequenced, the sequence is compared to published sequences using the DNA Subway website. DNA Subway is a collection of programs and databases created by Cold Springs Harbor Laboratory and Cyverse at the University of Arizona (n.d.). DNA Subway is used to identify the organism associated with the DNA barcode (DNA sequence) of a sample.

○ Materials & Methods

DNA was isolated from huckleberry plants received from Graves's huckleberry research project in Glacier National Park, Montana. The species of huckleberry plant and the location where collected (latitude and longitude) and included with the samples. Protocols from DNALC (2018) were followed with slight modifications. For example, we paid specific attention to cleaning the mortars and pestles prior to each use to prevent contamination. Each student individual or group was given a specific huckleberry leaf sample from Graves's Glacier National Park samples. Students selected from these samples (Graves, n.d.).

Isolation of DNA from Huckleberry Leaf

A 1 cm^2 sample of huckleberry leaf was immersed in liquid nitrogen and, with a mortar and pestle, the leaf was pulverized to a fine powder. The 600 ul of **lysis buffer** (***6 M Guanidine***

hydrochloride MW 95.53) was added to the pulverized sample and transferred to a 1.5 ml microfuge tube. Following a 10-minute incubation at 65°C, the microfuge tube was centrifuged (14,000 rpm) for 1 minute to pellet the leaf debris. The supernatant was transferred to a fresh tube, and 3 ul of a **silica resin** (***50% w/v silicon dioxide MW 60.08***) was added. The supernatant and silica resin were mixed for 5 seconds on a vortex machine, followed by incubation for 5 minutes at 57°C. The DNA was selectively bound to the silica resin. The DNA/silica was pelleted by a 30-second spin in the centrifuge (14,000 rpm). The supernatant was removed, and 500 µL of iced **wash buffer** (***0.02 M Tris, 0.05 M NaCl, 0.001 M EDTA, 50% ETOH***) was added to the tube. The tube was vortexed for 5 seconds, then the DNA/silica was again centrifuged for 30 seconds and the supernatant wash buffer was discarded. This was repeated one more time. After removal of the wash buffer, the remaining DNA/silica pellet was air-dried to remove all the buffer. Then 100 µL of **deionized water** was added to the DNA/silica pellet to selectively suspend the DNA in water. Water detaches the DNA from the silica. This was incubated for 5 minutes at 57°C followed by a 30-second spin in the centrifuge (14,000 rpm). Finally, 90 µL of the DNA containing supernatant was transferred to a fresh microfuge tube and stored at -20°C until ready for the PCR step.

PCR of the Isolated DNA

In a clean 0.2 ml PCR tube, 23 µL of primer/loading dye mix (640 µL distilled water, 460 µL Cresol red loading dye, 20 µL of 15 pmol/µL 5' primer, and 20 µL of 15 pmol/µL 3' primer) was added. Primers were rbcLaF and rbcLa rev. Next, one illustra PuReTaq PCR Ready-to-Go PCR Bead (VWR 89497-132); per 25 µL: 2.5 units Taq DNA polymerase, 10 mM Tris-HCl [pH 9.0], 50 mM KCl, 1.5 mM $MgCl_2$, and 200 µm of each dNTP) was added to the tube followed by 2 µL of sample huckleberry DNA. This was run in a thermocycler with the following settings.

- Initial step: 95°C for 1 minute
- Denaturing step: 35 cycles of 94°C for 15 seconds
- Annealing step: 54°C for 15 seconds
- Extending step: 72°C for 30 seconds, and then held at 4°C or stored at –20°C until the gel electrophoresis step

Gel Electrophoresis

After the PCR step, 5 µL of huckleberry sample DNA was loaded onto a 2% agarose gel in 1× TBE (Tris-boric acid-EDTA) buffer and run for 30 minutes at 130 V. The DNA was visualized using GelGreen nucleic acid gel stain, 10,000× (Minipcr.com, RG-1550-01) added directly to the molten agarose gel, and a UV transilluminator. A DNA ladder was added to the outside lanes to confirm a DNA band size of between 550 and 600 nucleotides for the target DNA. Samples that had the appropriate size DNA band were chosen for the sequencing step.

DNA Sequencing

The 20 µL of selected huckleberry PCR samples were sent to Genewiz (2021) for sequencing.

DNA Subway

The sequenced files for the samples that were successfully sequenced by Genewiz (2021) were uploaded to DNA Subway (https://dnasubway.cyverse.org) and compared to potential matches to establish the identity (genus, species) of the student samples. When the DNA sequence was a 100% match between the DNA Subway database and our huckleberry samples, we adopted the name of the plant for the student sample and submitted the sample to GenBank (through DNA Subway) for publication of the DNA sequence.

○ Results

There were 14 students in the class, and 12 samples had sufficient DNA to run a sequence and determine if the sample was from *Vaccinium*. One student had a successful DNA sequence match (sample 114) of their huckleberry DNA to a known sequence for *Vaccinium membranaceum*. Figure 2 shows the DNA gel of several huckleberry samples. Twelve samples (not all are shown) were sent to Genewiz for DNA sequencing. Figure 3 shows the BLASTN search from DNA Subway results for sample 114 between the published BLASTN *Vaccinium membranaceum* and sample 4-M13F_DO1.ab1and sample 4-M13R_F02.ab1(our sample 114). Figure 4 shows the DNA Subway alignment viewer for sample 114 and one of the results from BLASTN (Ascension MG219766.1).

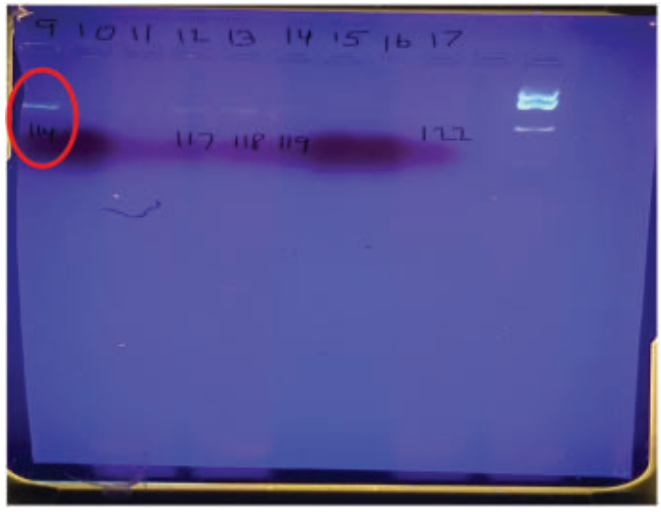


Figure 2. DNA gel showing several of the student samples. Lane 9, sample 114, is the sample that had a successful match with *Vaccinium membranaceum*. Eleven other samples were sequenced and were successfully identified as the genus *Vaccinium*. However, the species was not able to be determined.

Figure 3. DNA Subway's BLASTN results showing zero DNA base pair mismatches between the student sample and *Vaccinium membranaceum*. Zero mismatches indicates the student sample was *Vaccinium membranaceum*.

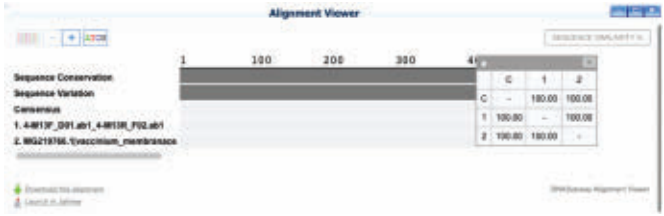


Figure 4. DNA Subway's Alignment Viewer indicating a 100% sequence match between the student sample (4-M13F…) and the BLASTN database sample (MG219766.1) of *Vaccinium membranaceum*. That the sequences match indicates they are the same species.

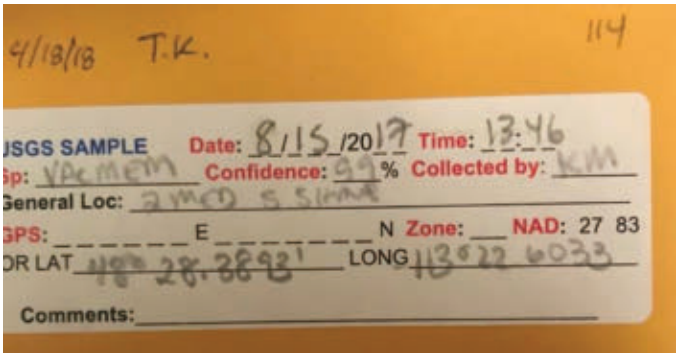
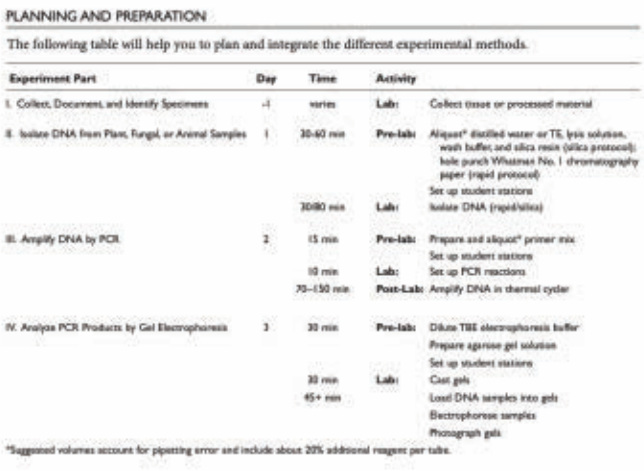


Figure 5. Huckleberry leaf samples from Graves's huckleberry project showing the date and location of sample collection.



PLANNING AND PREPARATION

The following table will help you to plan and integrate the different experimental methods.

Experiment Part	Day	Time	Activity	
I. Collect, Document, and Identify Specimens	-1	varies	Lab:	Collect tissue or processed material
II. Isolate DNA from Plant, Fungal, or Animal Samples	1	30-60 min	Pre-lab:	Aliquot* distilled water or TE, lysis solution, wash buffer, and silica resin (silica protocol); hole punch Whatman No. 1 chromatography paper (rapid protocol)
				Set up student stations
		30/80 min	Lab:	Isolate DNA (rapid/silica)
III. Amplify DNA by PCR	2	15 min	Pre-lab:	Prepare and aliquot* primer mix
				Set up student stations
		10 min	Lab:	Set up PCR reactions
		70–150 min	Post-Lab:	Amplify DNA in thermal cycler
IV. Analyze PCR Products by Gel Electrophoresis	3	30 min	Pre-lab:	Dilute TBE electrophoresis buffer
				Prepare agarose gel solution
				Set up student stations
		30 min	Lab:	Cast gels
		45+ min		Load DNA samples into gels
				Electrophorese samples
				Photograph gels

*Suggested volumes account for pipetting error and include about 20% additional reagent per tube.

Figure 6. A time table from Cold Spring Harbor Laboratory DNA Learning Center indicating the estimated time for completion of the in-lab sessions.

○ Discussion of Results

Fourteen students isolated DNA that was sequenced, but not all students were able to distinguish between the five species of huckleberry. Eleven samples were determined to be in the genus *Vaccinium*. One student sample (sample 114) of huckleberry leaf matched

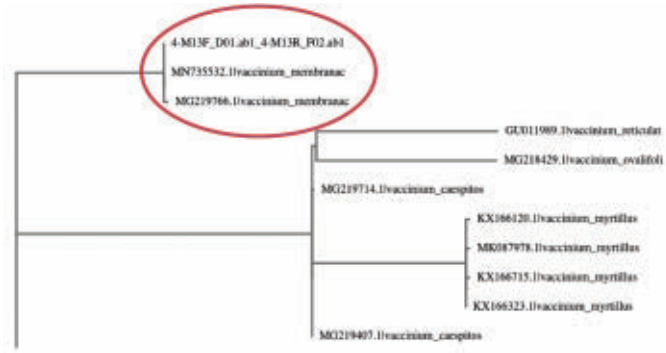


Figure 7. PHYLIP ML chart from DNA Subway for sample 114. It shows the relationship between the species. The red circle indicates that the sample 114 (4-M13F_D01.ab1_4-M13R_F02.ab1) and MN735532.1 and MG219766 are closely related.

100% for the sequence of the huckleberry species *Vaccinium membranaceum* and was published in GenBank (National Center for Biotechnology Information, 2019).

For the DNA published in GenBank, the DNA Subway results confirmed that the student sample 114 (from latitude 48°28′3893″ N, longitude 113°22′6033″ W from Graves's huckleberry project. Figure 5 came from the huckleberry plant *Vaccinium membranaceum*. This was determined by comparing the student's huckleberry sample DNA sequence to that of DNA Subway's BLASTN search (Figure 3). The 507 bp final sequence (it was longer but the DNA Subway programs trim nucleotides that do not correspond to both inputted sequences) from the sample matched 100% with two candidates, accession MN 735532.1 and MG 219766.1. Both were *Vaccinium membranaceum*. This was further verified by using DNA Subway's alignment view (Figure 4) to show that BLASTN search accession MG 219766.1 was a 100% match with the student sample 114. Additionally, DNA Subway's PHYLIPP MS Chart (Figure 7) indicates that the 114 sample is *Vaccinium membranaceum*.

We conclude that DNA barcoding is able to resolve, to the genus and species level, the huckleberry plant *Vaccinium membranaceum* using the primer pair rbcLaF and rbcLaRev. Students were unable to resolve other species of huckleberry plants, *V. myrtilloides, V. caespitosum, V. myrtillus,* and *V. scoparium* (Graves, n.d.) with this primer pair. Unofficially, in later experiments we were successful using 2-locus barcode methods. A 2-locus barcode method simply means that two different primer pairs are used. For example, instead of using only the rbcL primer pair, one might use the MatK primers and rbcL primers. This gives more specificity in identifying the species.

The fact that 12 groups isolated DNA from huckleberry plants indicates that the experiment was successful. These groups were able to determine the genus was *Vaccinium* but were unable to get a resolution of the five species.

From working with students doing the DNA barcode methods, some things can be attributed to errors in the recovery of DNA. Early on we found that we were getting results that were inconsistent with what we were analyzing. We concluded that this was due to a residue left on the mortar and pestle. To combat this we had students thoroughly wash the mortar and pestle with soap and water prior to use.

The DNA barcoding was carried out over two to four weeks but could be consolidated to perhaps four lab sessions where session 1 is the DNA isolation, session 2 is the PCR step, session 3 is gel electrophoresis and mailing the PCR sample to Genewiz for sequencing, and session 4 is analysis of the sequences on the DNA Subway applications. (See figure 6 for a time frame).

Occasionally, if students had no match in their DNA BLASTN search, we would go to another website, BOLD Systems (http://www.boldsystems.org), and download the sequence of DNA that could be compared to the species of plant their sample came from. They would download the rbcL-determined sequences and enter this into the DNA Subway alignment tool to determine if the sequence was a match. This was done to give something to base the identity of the student sample on when the DNA Subway BLASTN search did not provide any suitable matches. Students would then compare the BOLD systems sequences to their sample and determine the plant identity.

To complete this lab as a classroom exercise with 24 students, the instructor would need to assemble basic DNA equipment. This would include two water-baths, micropipettes (2–20 µL and 100–1000 µL), microfuge, thermocycler, gel electrophoresis equipment, and UV transilluminator. Consumables would be the Ready-to-Go PCR Beads, agarose, TBE buffer, and reagents for the silica DNA isolation method (see Materials & Methods section).

Prior to introducing the DNA barcoding methods, it is recommended that students have a basic understanding of DNA, genes, and PCR. Once students have that knowledge base, then they can perform the experiment with an understanding of the process of DNA barcoding. All in all, the DNA barcoding / huckleberry project gave our students the opportunity to be involved in a real-life research project and to have the possibility that their work could be published in GenBank.

Our GenBank-published DNA sequence may be found at http://www.ncbi.nlm.nih.gov/nuccore/MN735532.1.

○ Acknowledgments

Special thanks to Dr. Tabitha Graves at the U.S. Geological Survey, Northern Rocky Mountain Science Center. For more information on Dr. Graves' huckleberry research, see USGS "Predicting Changes in Bear Foods," https://www.usgs.gov/centers/norock/science/climate-change-impacts-bear-foods? qt-science_center_objects=0#qt-science_center_objects and her USGS profile, https://www.usgs.gov/staff-profiles/tabitha-graves.

Special thanks to my biology class students at Wenatchee Valley College at Omak for helping with this project, namely Araseli R. Almeida, Isabella M. Dinsmore, Samantha N. Eiffert, Kody J. Erickson, Morgan L. George, Shelby L. Gorr, Krista G. Marchand, Chase W. McDaniel, Maria D.C. Merida, Hunter A. Munts, Emily D. Nissen, Malia J. Whitmore, Samanta A. Yanez, and Sage Young.

References

Bellemain, E., Carlsen, T., Brochmann, C., et al. (2010). ITS as an environmental DNA barcode for fungi: an *in silico* approach reveals potential PCR biases. *BMC Microbiology*, *10*, 189. https://doi.org/10.1186/1471-2180-10-189.

Bio-Rad. (n.d.). DNA Barcode Generator. http://biorad-ads.com/DNABarcodeWeb.

BOLD Systems. (2014). Barcode of Life Data System. http://www.boldsystems.org.

Cold Spring Harbor Laboratory DNA Learning Center. (2011). History of DNA barcoding [video]. DNALC. https://dnalc.cshl.edu/resources/dnatoday/111103-history-dna-barcoding.html.

———. (2018). *Using DNA Barcodes to Identify and Classify Living Things*. DNALC. https://dnabarcoding101.org/files/using-dna-barcodes.pdf.

Cold Spring Harbor Laboratory DNA Learning Center and CyVerse. (n.d.). DNA Subway. https://dnasubway.cyverse.org.

Genewiz. Sanger Sequencing. (2021). https://www.genewiz.com/en/Public/Services/Sanger-Sequencing.

Graves, T. *Huckleberry Ecology: Finding Berry Treasure*. (n.d.). USGS Northern Rocky Mountain Science Center.

Hebert, P.D.N., Cywinska, A., Ball, S. L. & deWaard, J.R. (2003). *Biological Identifications through DNA Barcodes*. Royal Society. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1691236/pdf/12614582.pdf.

Hollingsworth, P.M., Forrest, L.L., Spouge, J.L., et al. (2009). *A DNA Barcode for Land Plants*. Proceedings of the National Academy of Sciences, *106*(31), 12794–97. https://doi.org/10.1073/pnas.0905845106.

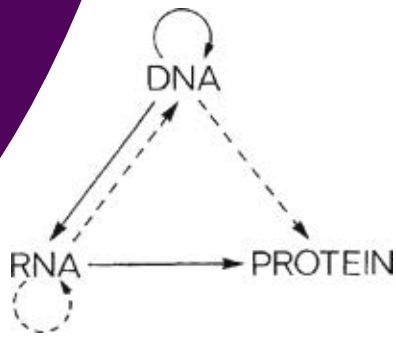
National Center for Biotechnology Information. (2019). Vaccinium membranaceum isolate DNAS-Emily Nissan 114 5.8 S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence. U.S. National Library of Medicine. GenBank. MN735532.1. https://www.ncbi.nlm.nih.gov/nuccore/MN735532.1.

JEFF DYKES is an adjunct faculty member at Wenatchee Valley College in Omak, WA; e-mail: jdykes@wvc.edu. KRISTY KAPPENMAN is a former faculty member at Wenatchee Valley College in Omak, WA; e-mail: kkappphd@gmail.com. EMILY D. NISSEN is a former student at Wenatchee Valley College in Omak, WA; e-mail: Nissenemily37@gmail.com.

TIPS, TRICKS & TECHNIQUES

Teaching Molecular Biology with the Historical Accounts in *The Eighth Day of Creation*

Elizabeth A. Morton, M. Bryce Taylor



Central Dogma of Molecular Biology, Francis Crick, copyright 1970. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Nature.

Abstract

The Eighth Day of Creation is a narrative history of molecular biology by science journalist Horace Freeland Judson. It uses first-person interviews to tell the story of how scientists in the mid-20th century discovered the basic rules of life that we now call the Central Dogma. The book presents both an in-depth analysis of the foundational research and a look into the lives of the scientists involved. We used this book as the primary text for an advanced undergraduate seminar course at the University of Washington in winter 2020, a class designed to help students critically think about approaches to science, the role of social factors in scientific progress, and the conceptual development of paradigm shifts. In this piece we reflect on our approach designing the course and our experience teaching it and share our syllabus (annotated with some reflections on the course) as inspiration for others

Key Words: *molecular biology; nature of science; philosophy of biology; history of science; history of biology; genetics; heredity; molecular genetics.*

○ Introduction & Motivation for the Course

Molecular biology was presented in our science courses as a set of conclusions (e.g., the Central Dogma is …), without deep exploration of the experiments that allowed scientists, over the course of decades, to come to these conclusions. In our experience, this ahistorical format can lead to the misrepresentation of science as a linear progression of discovery. It omits the technical and conceptual challenges faced by scientists, the misdirection of intellectually attractive but unsupported hypotheses, and the role of social forces and institutions in shaping our views. It also leaves out the human element of being a scientist—the self-doubt and uncertainty that accompany scientific investigation, the excitement of a new discovery, and the many different personal approaches to exploring the mystery of life that suggest there is no single "right" way to do science.

The Eighth Day of Creation is a narrative history of molecular biology chronicled by science journalist Horace Freeland Judson in the sixties and seventies (H. F. Judson, 1979). The book details the *molecular revolution*—the short period during the mid-20th century in which scientists began explaining life in mechanistic terms, what we now call the central dogma. Over the course of a decade (O. Judson, 2013), Judson conducted lengthy interviews with over a hundred scientists (H. F. Judson, 1979), including Francis Crick, Linus Pauling, James Watson, Jacques Monod, Matthew Meselson, and Sydney Brenner. Much of the text is direct quotes, presenting insight into the personalities behind textbook names and allowing these scientists to explain their discoveries and their thought processes at the time (Peifer, 2020). This work has been lauded as one of the first to approach science history from a journalistic perspective (Marchant, 2018; O. Judson, 2013; Pontin, 2011).

After reading the book as postdocs, we both came away wishing we had done so earlier in life. We wondered how it would have impacted our early-life navigation in science. While not universally true, our personal educational experience included only rare instances of historical or personal context for biological discoveries. How did early scientists conceptualize the gene and its function before molecular biology was formalized? How were early results misinterpreted due to preconceptions of the time? How did the personal experiences of scientists contribute to their great discoveries? How did political and social events influence the course of science? These were the details provided in *The Eighth Day of Creation* that captured our attention.

We set out to develop the course we would have liked to take. The experience was gratifying, and we felt like it had a positive impact on our students. To our knowledge this book is rarely if ever used as a course text in modern classes. In conversation with colleagues we (anecdotally) have found that while senior faculty frequently recognized the title immediately, most in our peer group and younger did not. For these reasons we wanted to share our experience with others and promote the book as a useful resource in biology education.

The American Biology Teacher, Vol. 84, No. 1, pp. 45–49, ISSN 0002-7685, electronic ISSN 1938-4211. DOI: https://doi.org/10.1525/abt.2022.84.1.45.

○ Course Structure & Reflection

We structured the course around group discussion. Each week students were assigned 30- to 50-page reading excerpts from the text and a choice of prompting questions to which they had to write a short response (Table 1).

We tried to make these questions open-ended and thought-provoking (with mixed success!). Each week during class, we opened with a paired effort then group effort to generate a timeline of the events covered in the week's reading (Figure 1).

These were frequently followed by diagramming of the more technical aspects of any benchmark experiments up for discussion that week (Figure 2).

Table 1. Course schedule and *The Eighth Day of Creation* reading list.[a]

1	**Topic:** What is molecular biology?
	Assigned pages: NA
	Writing prompt: NA[b]
2	**Topic:** Biology before it went molecular
	Assigned pages: 27–41, 50–61
	Writing prompt: Crick talks about boldness versus caution in terms of scientific approach. What are the benefits of each? Are there types of research or stages in the development of an idea that benefit most from one or the other?
3	**Topic:** Pursuing the molecular basis of the gene
	Assigned pages: 94–104, 108–14, 118–29, 133–38, 141–44
	Writing prompt: On page 94 Chargaff offers the comment "To the scientist nature is as a mirror that breaks every thirty years." What did he mean by that? Would you agree? If so, would you consider that a feature or a bug of the scientific process?
4	**Topic:** The structure of DNA
	Assigned pages: 147–86, 196–98
	Writing prompt: There were a number of wrong turns and incorrect models put forward before Watson and Crick proposed the double helix structure. What do you think the multiple incorrect models proposed reflect? • The hurried nature of the research (the perception of a competition) • The difficulty of the problem • The nature of all science (faulty models don't get as much press) • Other aspects of the story
5	**Topic:** Difficulties posed by RNA
	Assigned pages: 186–95, 225–28, 233–34, 248–82
	Writing prompt: Pages 193–194 includes discussion about competition: whom Watson saw himself as competing with, opinions on whom he was really competing with, and Watson's assertion that competition is "the dominant motive" in science. Do you agree with Watson's statement? What role do you think competition plays in the advancement of science, and is this to science's benefit or detriment?
6	**Topic:** The Central Dogma
	Assigned pages: 333–38, 344, 346–47, 348–84
	Writing prompt: Had you heard of Lysenko and the movement of Soviet Science during this time period? What surprised you in the segments that covered his work and influence?
7	**Topic:** Gene regulation and the *lac* operon
	Assigned pages: 384–424
	Writing prompt: One of the prevailing ideas at the time was that rRNA was the messenger and ribosomes (as made of protein + rRNA) were specific for specific gene products. Does the eventual overturning of this idea with the discovery of mRNA count as a revolution? Do any of the revelations in this section (the idea of regulation of expression, the PaJaMo experiment, etc.) count as revolutions in biology? What makes a discovery revolutionary?

8	**Topic:** Solving the code
	Assigned pages: 424–46, 460–61, 463–69, 470–82, 483–89
	Writing prompt: Barbara McClintock (a) seemed to have similar ideas about gene regulation as Jacob and Monod, earlier and in a more complex system, and (b) was working under conditions almost certainly biased against her (sometimes she worked without pay). Comment on this.
9	**Topic:** Protein structure-function
	Assigned pages: 70–93, 568–93
	Writing prompt: On page 70 Linus Pauling is described as exemplifying the Medawar quote "Humility is not a state of mind conducive to the advancement of learning." What do you think about the meaning and accuracy of this statement in general? What do you think about it as applied to Pauling?
10	**Topic:** What comes/came next?
	Assigned pages[a]**:** 201–22
	Writing prompt: NA[b]

[a] Page numbers are from the 1st edition (H. F. Judson, 1979). More detailed descriptions of assigned sections as well as page numbers for the 2013 commemorative edition can be found at https://depts.washington.edu/genomicssalon/the-eighth-day-of-creation-reading-guide.

[b] We did not have an assignment before the first class period. We did not have a writing assignment before the last class period to give students time to focus on their final essays.

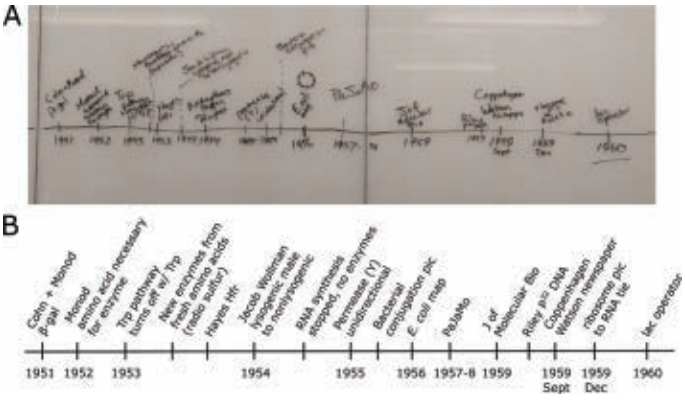


Figure 1. (**A**) Photo of the Week 7 timeline generated by the students of the winter 2020 quarter, covering events surrounding the discovery of the lac operon (starting with the observation that bacteria can only synthesize new enzymes [β-galactosidase] if all amino acids are available and ending with the publication of mutations demonstrating the existence of the lac operator). (**B**) Schematized version of A. The timeline includes a mix of key experimental and theoretical advances, as well as notes on character development. This week was more heavily biased toward experimental details.

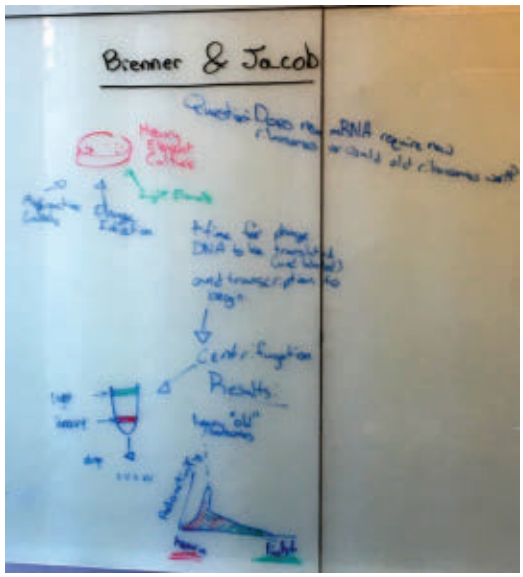


Figure 2. Week 8 student-produced diagram of the 1960 experiment by François Jacob and Sydney Brenner. "Question: Does new mRNA require new ribosomes or could old ribosomes work?" Experiment described on pages 436–41 (H. F. Judson, 1979).

The bulk of class time was then devoted to open-ended discussion, paired or whole-group, of more personal reactions to aspects of the text. The majority of a student's grade was determined by discussion participation. Each class ended with a brief, informal student presentation reviewing a biological concept that might help them better understand the next week's reading (e.g., "what is lysogeny?" before we dove into Lwoff and Jacob).

Our course was scheduled to meet 2 hours per week for 10 weeks. To fit this limited timeframe and credit load, we cut the 618-page book approximately in half, assigning excerpts instead of full chapters (Table 1). We worried about this abridgement but feel we were able to hit the highlights and give students a condensed but useful experience. Each week we provided feedback on writing assignments in the form of our responses both to the students' thoughts as well as their writing styles, in the hope of helping them strengthen their writing skills. Students were then encouraged to reuse segments from their (revised) weekly writing assignments in their final essay.

Over the course of the quarter, we shifted much of the explanatory load onto the students. For example, in later class periods we had the students diagram important experiments on a whiteboard

(Figure 2), a task that one of us had performed in early classes. Similarly, in early class periods, discussions often resembled Q&A sessions with us as panelists. By the end, students were breaking into side conversations and carrying out lively debate. Along the way we attempted to model a comfort with uncertainty as we all worked through tricky subject matter together, which the scientists themselves demonstrated as they struggled with complex data and incorrect hypotheses.

One interesting observation from our discussions was the attention students paid to individuals with outsized or undersized influence on the story. Two characters that seemed to particularly capture our students' interest were Oswald Avery and Barbara McClintock. Avery was a meticulous worker who revealed that DNA held the secret to heritability, with relatively little fanfare. McClintock was a reclusive figure whose groundbreaking work on gene regulation (and many other phenomena) went largely unnoticed or misunderstood for decades. Though given short treatment in the text (McClintock criminally so in our opinion), both featured prominently in our students' discussions.

In contrast, James Watson, Francis Crick, and Linus Pauling were leaders in the theoretical development of molecular biology and occupy a large portion of the text. All three advocated a style of science that used a minimal amount of data when forming theories, before testing those theories against larger datasets. Our students worried this was an example of cherry picking that, at best, left to others the burden of actually collecting substantiating data and at worst misled the field with incorrect conclusions. This point led to lively discussion and many evolving opinions throughout the remainder of the course. It was wonderful to see the students thinking through scientific approaches and their ramifications at this level. The background and context offered in the text presented many such opportunities to consider the ethics and merit of different scientific approaches.

Due to the pandemic, our last class period was cancelled and we were unable to conclude with a discussion of the book in a modern context. We planned to spend this period discussing the perspectives on molecular biology in Chapter 4 and the essay on Rosalind Franklin at the end of the commemorative edition (O. Judson, 2013). The first is an interlude on the future directions and implications of molecular biology as it stood in the seventies. This section makes an interesting study in light of the actual development of the field and its impacts. For instance, the book spends little time on evolution, yet the rules spelled out for the molecular basis of life not only make evolution possible, they make it inevitable. The latter essay would have allowed more discussion of the social context of the book and the perspective and biases brought by the author. We hoped to take this opportunity to discuss the contributions of the many female scientists (e.g., Barbara McClintock, Dorothy Hodgkin, and Nettie Stevens) who receive little or no coverage in the text and to discuss other ways in which bias manifests in historical records.

In spite of this setback we felt like our course accomplished the goal we had set. Our interactions with our students while teaching this course gave us a deeper appreciation of the remarkable scientific achievements in this period of history as well as Judson's monumental efforts to document them. We encourage anyone with an interest in science history, intellectual revolution, or the roots of molecular biology to read this book. Although our experience was with an upper-division undergraduate seminar course, we feel that historical context and insight into the experience of scientific inquiry are equally valuable in early science education. The historical details of many classic experiments (relevant to foundational biology courses) as well as personal thoughts of scientists are provided with page numbers in Table 2. We particularly hope that trainees like ourselves can experience the same moments of revelation we had on our first read and that fellow biology educators at all levels will consider incorporating this rich resource into their teaching. Our syllabus, condensed reading list, and additional reflections on our teaching strategies can be found at https://depts.washington.edu/genomicssalon/the-eighth-day-of-creation-reading-guide. Please contact us with any questions.

Table 2. List of select landmark discoveries and personal reflections described in *The Eighth Day of Creation*.

Category	Event	Pages[a]	Conclusion or Relevance
Key experiment	Salvador Luria & Max Delbrück perform the fluctuation test (1943).	50–53, 55–57	Mutations are spontaneous.
Key experiment	Alfred Hershey & Martha Chase further demonstrate DNA is the heritable material (1952).	130–31	DNA is the heritable material.
Key experiment	Francis Crick, Rosalind Franklin, James Watson, & Maurice Wilkins show the structure of DNA (1953).	102–4, 108–14, 135–36, 153–54, 156–61, 164–66, 171–75[b]	DNA is a double helix.
Key experiment	Matt Meselson & Franklin Stahl reveal semiconservative DNA replication (1957).[c]	187–92	DNA replication follows a semiconservative model.
Key experiment	Marshall Nirenberg & Johann Matthaei demonstrate a solution to the coding problem (1961).	470–72, 476–78, 480–82	The first specific mRNA codon was identified for an amino acid.
Key theoretical advance	Francis Crick proposes the Central Dogma (1957).	333–38	The central dogma describes the flow of information in a cell.

Science as an experience	Author extracts DNA from bacteria with Sidney Altman.	30–33	What does working at the bench feel like?
Science as an experience & key experiment	Oswald Avery describes his results showing that DNA is the heritable material (1944).	34–41	DNA is the heritable material. What does making a revolutionary discovery feel like?
Science as an experience	François Jacob lobbies to join André Lwoff's lab and work on prophage (1950).	384–85	What does starting from scratch in a lab feel like?

[a] Page numbers are from the 1st edition (H. F. Judson, 1979).
[b] Most of pages 94–198 is devoted to this discovery. The provided page numbers represent an abridged selection of some notable events within this period.
[c] The experiment was performed in 1957 and published in 1958.

○ Acknowledgements

We thank Katherine Xue for introducing us to the text and Charles Laird for encouraging us to design a course around it. We thank Clara Amorosi, Abigail Keller, and Chris Large for comments on our selected reading list. We thank Katherine Xue for helpful comments on an early draft of this piece. Lastly, we would like to thank the students of our winter 2020 class, both for taking a chance on a brand new course and for their engagement and enthusiasm that made teaching it so enjoyable.

References

Crick, F. (1970). Central dogma of molecular biology. *Nature*, *227*, 561–63. https://doi.org/10.1038/227561a0.

Judson, H. F. (1979). *The Eighth Day of Creation*, *1st ed*. New York, NY: Simon and Schuster.

Judson, O. (2013). Growing up with *The Eighth Day*: a reminiscence. In H. F. Judson, *The Eighth Day of Creation*, *commemorative ed*. (pp. xv–xvii). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Marchant, J. (2018). The best biology books recommended by Sean B Carroll. Interview. Five Books. https://fivebooks.com/best-books/biology-sean-carroll.

Peifer, M. (2020). *The Eighth Day of Creation*: looking back across 40 years to the birth of molecular biology and the roots of modern cell biology. *Molecular Biology of the Cell*, *31* (2), 81–86. https://doi.org/10.1091/mbc.E19-11-0619.

Pontin, J. (2011). Horace Freeland Judson Obituary. In H. F. Judson, *The Eighth Day of Creation*, *commemorative ed.* (pp. xiii-xiv), Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

ELIZABETH MORTON is a research scientist in the Department of Genome Sciences at the University of Washington, Seattle; e-mail: emorton@u.washington.edu. BRYCE TAYLOR is an assistant professor in the Department of Biology at Loras College, Dubuque, IA. He was formerly an acting instructor in the Department of Genome Sciences at the University of Washington, Seattle; e-mail: bryce.taylor@loras.edu.


NABT
National Association of Biology Teachers
SESSION PROPOSAL DEADLINE:
MARCH 15, 2022
LEARN MORE
nabtconference.com
NABT INDY
November 10-13, 2022
JW Marriott Indianapolis

Down To Earth with Zac Efron (Netflix Original Series, 1 season, 8 episodes, ~40 minutes each; more information at https://downtoearthzacefron.com)

In this Emmy-winning travel-adventure nature program hosted by Zac Efron, best known for his rise to fame with *High School Musical*, viewers travel around the world investigating a variety of unconventional sustainable solutions to global problems. Each show starts with Efron explaining that the purpose of the series is to find new perspectives on very old problems and healthy, sustainable living solutions. He is accompanied by self-proclaimed wellness expert Darin Olien, who lacks credibility, but adds some entertainment value to the show. The pair travel to eight different locations to examine creative, sustainable solutions addressing the importance of bees, renewable energy, ecovillages, longevity and genetics, biodiversity, medicinal plants, sustainability, and water.

The show is structured to be consumed by a general audience. The language is not science-dense, and the silly, light-hearted interactions between Efron and Olien are central to each episode. Students will appreciate the entertainment and approachability of the material. The episodes are filmed on location in Iceland, France, Costa Rica, Sardinia, Lima, Puerto Rico, London, and Iquitos and expose viewers to a variety of landscapes, cultural norms, and communities. There are multiple claims that lack credibility and validity. Because the content is loosely based on science and subject to debate, this show should only be used by an experienced educator as a springboard for discussions, research, or fishbowl activities with secondary and older students.

Each episode ends with a short debrief of the takeaways, which distill down to the overall message of the series that if everyone makes one small change it can have a large impact. The eight episodes passively expose viewers to diverse ecosystems from deserts to rainforests, behaviors such as volunteering and recycling, and careers like blogging and tourism. Asking students to take note of these would lead to rich classroom discussion and may validate students' career interests.

Every episode highlights the efforts of a scientist who briefly explains their work. The majority of the show is spent on exploring different aspects of sustainability in general. Some notable quotes from the series include

- "You have to start somewhere."
- "When everyone tries to do a little, a lot can change."
- "Work with nature, not against it."
- "The problem might be the solution."

Reinforcing the topics and the impact of alternative solutions from the show with a short research activity followed by a design challenge or debate could make it a valuable resource to add to a teacher's library.

Teachers may want to incorporate these episodes into their media routine because they are not typical documentary style. The series could be used in a number of secondary science courses, including biology, ecology, and environmental science, to fuel debate about reliable and valid claims, discover career options, and to expose students to different cultures, languages, and values but should not be considered a swap for other research-based science content.

Bonnie Nieves
Biology Teacher / Author
Nipmuc Regional High School, Upton, MA
bnieves@mursd.org

The American Biology Teacher, Vol. 84, No. 1, pp. 51, ISSN 0002-7685, electronic ISSN 1938-4211. DOI: https://doi.org/10.1525/abt.2022.84.1.51.

NABT BioClub MEMBERS

Abington Heights High School, Clarks Summit, PA
American International School of Muscat, North Chesterfield, VA
Arcadia High School, Phoenix, AZ
Archbishop Curley High School, Baltimore, MD
Arroyo High School, San Lorenzo, CA
Athens High School, Troy, MI
Ayala High School, Chino Hills, CA
The Barstow School, Kansas City, MO
Bethlehem High School, Bardstown, KY
Bishop Garcia Diego High School, Santa Barbara, CA
Brentwood Academy, Brentwood, TN
Broad River Elementary, Beaufort, SC
Cabarrus Kannapolis Early College High School Concord, NC
Canadian Valley Technical Center, OK
Caney Valley High School, Ramona, OK
Cardinal Gibbons High School, Raleigh, NC
Carrboro High School, Carrboro, NC
Castle Park High School, Chula Vista, CA
Center for Advanced Professional Studies, Overland Park, KS
Central Carolina Technical College, Sumter, SC
Central Falls High School, Central Falls, RI
Central Magnet School, Murfreesboro, TN
Charleston High School, Greenup, IL
Chelan High School, Chelan, WA
Chester High School, Chester, PA
Clayton High School, Clayton, MO
Colonia High School, Colonia, NJ
Coronado High School, Colorado Springs, CO
Cuyohoga Community College, Macedonia, OH
Darnell-Cookman School of the Medical Arts, Jacksonville, FL
DeVry Advantage Academy, Chicago, IL
Dora R-III School, Dora, MO
Dougherty Valley High School, San Ramon, CA
Eastern Mennonite High School, Harrisonburg, VA
El Centro College, Dallas, TX
Emmett High School, Emmett, ID
Fairhaven High School, Fairhaven, MA
Florida SouthWestern State College, Naples, FL
Freedom High School, Freedom, WI
George Washington High, Charleston, WV
Gillette College, Gillette, WY
Grafton High School, Grafton, WI
Grand View University, De Moines, IA
Greater Lowell Technical High School, Tyngsborough, MA
Greater New Bedford Regional Vocational Technical High School, New Bedford, MA
Greensburg Salem High School, Greensburg, PA
Harmony School in Innovation, Katy, TX
Heathwood Hall Episcopal School, Columbia, SC
Hillsboro High School, Hillsboro, OR
Hilltop High School, Chula Vista, CA
Holt High School, Holt, MI
The Independent School, Wichita, KS
Kenmore West High School, Buffalo, NY
Kent County High School, Worton, MD
Kettle Run High School, Nokesville, VA
Lake Metroparks, Concord, OH
Lakeville North High School, Lakeville, MN
Lexington High School, Mansfield, OH
Los Fresnos High School, Los Fresnos, TX
Martin Luther College, New Ulm, MN
Mary Persons High School, Forsyth, GA
Marysville High School, Marysville, KS
Metropolitan Community College, Omaha, NE
Midland Park High School, Midland Park, NJ
Minnetonka High School, Minnetonka, MN
Moscow High School, Moscow, ID
Mount Abraham Union High School, Bristol, VT
Nassau Community College, Garden City, NY
Northampton Area High School, Northampton, PA
Palm Tree School, Fairfax, VA
Panorama High School, Panora, IA
Perkins High School, Sandusky, OH
Pike High School Freshman Center, Indianapolis, IN
Pikeview High School, Princeton, WV
Putnam City High School, Oklahoma City, OK
Riverside City College, Riverside, CA
Salem High School, Salem, IN
Saltsburg High School, Saltsburg, PA
Seabury Hall, Makawao, HI
Seneca East High School, Attica, OH
Sherando High School, Winchester, VA
Skyline High School, Sammamish, WA
Snow College, Ephraim, UT
Southeast Community College, Lincoln, NE
Southern Wells High School, Poneto, IN
St. Andrew's Episcopal School, Potomac, MD
St. Clair High School, St. Clair, MI
State Library of PA, Lykens, PA
Stillwater High School, Stillwater, OK
Stouffville District Secondary School, Whitchurch-Stouffville, ON, Canada
The Summit County Day School, Cincinnati, OH
Sunlake High School, Land O'Lakes, FL
Tiffin Columbian High School, Tiffin, OH
Unionville High School, Kennett Square, PA
University Christian High School, Hickory, NC
Valley View High School, Archbald, PA
Vincennes University, Vincennes, IN
Visitation Academy - Saint Louis, St. Louis, MO
West Mifflin Area High School, West Mifflin, PA
Worthington Christian High School, Worthington, OH
York Community High School, Marion, IL

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

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

THE VACCINE SKEPTICS OF 1721

DOUGLAS ALLCHIN, DEPARTMENT EDITOR

The response to the recent COVID pandemic has been marked by sometimes virulent opposition to vaccination. Many regard the widespread skepticism of an effective medical treatment (and vaccines, in particular) to be not only alarming but also unprecedented—this month's Sacred Bovine.

A brief excursion into the scourge of smallpox in the 18th century, however, may prove informative (Carrell, 2003; Remillard-Hagen, 2012). The episode from history offers some fascinating insights into how many people, even some doctors, have responded in surprisingly cautious ways to potentially life-saving therapies. Their perspectives help illustrate the complex psychology of the public reception to medical claims and the factors that shape how people assess scientific credibility, expertise, and empirical evidence. (For accompanying classroom visuals, see http://shipseducation.net/smallpox.)

○ Smallpox, Complexion & Children

Let us join the story in 1717 in Constantinople (present-day Istanbul, Turkey), the heart of the Ottoman Empire. There, Lady Mary Wortley Montagu, the wife of the newly arrived British ambassador, encounters the local women and greatly admires their pristine complexions. Lady Mary's awareness has been shaped by her own experience. Just over a year earlier, she contracted smallpox, a disease that had swept through England and left one in five infected patients dead. While Lady Mary survived, the disease had left her face pockmarked. For an elite woman in 18th-century England, beauty was an utmost treasure, integral to social stature and regard among men. But uncomfortable with her disfigurement, Lady Mary had become accustomed to masking her face in public.

Yet here in Turkey, the women at the baths seem uniformly unblemished. How had they all apparently escaped the ravaging effects of smallpox, so devastating across the continent? Lady Mary learns that they rely on a medical procedure largely unrecognized in western Europe: ***variolation***. She describes it in a letter to a friend:

> A propos of distempers, I am going to tell you a thing, that will make you wish yourself here. The small-pox, so fatal, and so general amongst us, is here entirely harmless, by the invention of engrafting, which is the term they give it. There is a set of old women, who make it their business to perform the operation, every autumn, in the month of September, when the great heat is abated. People send to one another to know if any of their family has a mind to have the small-pox; they make parties for this purpose, and when they are met (commonly fifteen or sixteen together) the old woman comes with a nut-shell full of the matter of the best sort of small-pox [pus from a patient's pustule], and asks what vein you please to have opened. She immediately rips open that you offer to her, with a large needle (which gives you no more pain than a common scratch) and puts into the vein as much matter as can lie upon the head of her needle, and after that, binds up the little wound with a hollow bit of shell, and in this manner opens four or five veins. . . . The children or young patients play together all the rest of the day, and are in perfect health to the eighth. Then the fever begins to seize them, and they keep their beds two days, very seldom three. They have very rarely above twenty or thirty [pocks] in their faces, which never mark, and in eight days time they are as well as before their illness. Where they are wounded, there remains running sores during the distemper, which I don't doubt is a great relief to it. Every year, thousands undergo this operation, and the French Ambassador says pleasantly, that they take the small-pox here by way of diversion, as they take the waters in other countries. There is no example of any one that has died in it. (Montagu, 1784)

Nowadays, of course, we recognize this process as induced immunization. Namely, variolation was an early form of vaccination using natural sources.

Lady Mary has already lost her brother to smallpox in 1713. She is now concerned about protecting her own son, almost age five. But is the procedure safe and effective? What are the risks? (Here is an excellent opportunity to engage students in inquiry and discussion: "Placed in this position, what will guide your reasoning? How will you assess the credibility of the local Turkish practice? What evidence is sufficient to warrant action?")

Here, one may begin to appreciate the many factors that contribute to assessing the scientific merit of a claim. Empirical evidence is surely important, yes. But what counts as evidence, or sufficient evidence, or adequate documentation of results over many years, or perhaps even generations of collective experience? The Turks report no deaths. But who can be trusted to speak for the evidence? For a Christian (such as Lady Mary), should the testimony of a non-Christian be trusted unquestionably, or possibly even discounted? The engrafting is not even performed by professional physicians.

The American Biology Teacher, Vol. 84, No. 1, pp. 53–54, ISSN 0002-7685, electronic ISSN 1938-4211. DOI: https://doi.org/10.1525/abt.2022.84.1.53.

Who is an expert and why? How does one measure their expertise? How does one establish the relevant trust? What would justify a final decision?

Ultimately, Lady Mary deems the available evidence—primarily the low incidence of smallpox among the Turks—as sufficient. She has her son variolated, although by an English physician who has accompanied them on their journey East.

We know now that the procedure is effective, but one may wonder if perhaps Lady Mary was unduly biased by her own fears at the time. Was it appropriate for her to extend trust to nonphysicians as experts in this case, and to trust anecdotal, rather than systematically collected, quantitative evidence?

○ The Royal Family & the Prisoners of Newgate

Lady Mary returns to England two years later. She tries to share her experience with others. She becomes an advocate for variolation, although publishing under a pseudonym. In 1721, another epidemic of smallpox spreads through London and Lady Mary decides to have her four-year-old daughter inoculated. The physician who had earlier inoculated her son, Charles Maitland, is now reluctant. He fears for his reputation. While the effectiveness of variolation was reported to the premier scientific institution in England, the Royal Society, by foreign correspondents in 1713 and 1716, the procedure has not yet been accepted by the medical establishment. And so the event is witnessed by three other members of the Royal College of Physicians, including its president, Hans Sloane, the very doctor who had tended Lady Mary during her own bout of smallpox in 1716. The variolation procedure proves effective again.

Lady Mary continues her promotion of variolation. She encourages Caroline, the princess of Wales, to have her children inoculated. But here the significance of the decision is greatly amplified. Any decision regarding the royal family needs to be approved by the king! (This occasion offers a second opportunity to invite students to reflect on the case in a historical perspective: As a member of the royal family in 1721, what will you do, and why?)

Some individuals are persuaded by the successful demonstration with Lady Mary's daughter. For example, one of the physician-witnesses soon has his own children inoculated. Others are skeptical. Despite Lady Mary's status in British society, many regard the procedure as foreign, a practice of a less civilized culture, and thus inherently untrustworthy. What was the evidence? (Without reputable medical journals easily accessible via the internet, what would even constitute a reliable report of the relevant evidence?) Is one case performed in England sufficient? Whose testimony, based on what expertise, matters? Despite her enthusiasm, Lady Mary is certainly not a trained physician. Did the physicians who witnessed the one recent case really develop enough experience to vouch for the procedure? In this new context, and under these slightly different circumstances, what are the appropriate criteria for establishing credible claims and for guiding judgments?

Princess Caroline is favorably disposed to the prospect, but the king less so. He is ultimately persuaded, however, to approve a formal experiment. They enlist Hans Sloane (who is also a physician to the royal family) to test prisoners from the notorious Newgate Prison. Six individuals sentenced to execution are offered the prospect of a full pardon if they "volunteer" to participate in a trial inoculation. They are compared with another prisoner who has already survived smallpox. In addition, one prisoner (a female) is exposed to a contagious smallpox patient afterward, to ensure that the procedure is not only safe but also effective. For greater assurance on the safety with young children, Caroline has a handful of orphans from the local parish inoculated (in this case, no consent is sought). Notwithstanding the now unacceptable ethics of testing, the results indicate that, as before, the procedure is safe. And the prisoners are freed. Ultimately, the king grants permission for inoculating Caroline's daughters. But not the sons. Perhaps that is just too risky for the royal lineage?

Controversy continues. Many of society's elite who have lost family members or friends to smallpox enroll their children. Dr. Maitland, who has performed the procedure throughout, publishes a small booklet on the virtues of variolation. Other tracts soon follow, condemning it (and Maitland). For example, William Wagstaffe, a physician at the distinguished St. Bartholomew's Hospital in London declares

> Posterity will scarcely be brought to believe that a method practiced only by a few *Ignorant Women*, amongst an illiterate and unthinking People should on a sudden, and upon slender Experience, so far obtain in one of the most Learned and Polite Nations in the World as to be received into the *Royal Palace*. (quoted in Hopkins, 2002, p. 47)

For some, gender and culture seem to shape the interpretation of the evidence. Others contend that the procedure violates God's intentions: religious perspectives at work. Again, the question arises (for students to address explicitly): What constitutes sufficient evidence, scientifically? Do the results from the Newgate Prison experiment alter the balance? In what ways might a scientific view differ from individual perspectives, based on psychological, social, or cultural factors?

By 1730, fewer than 900 individuals in England have been inoculated. Smallpox epidemics reappear in 1731, 1734, and 1736, and again with exceptional virulence in 1752. Tens of thousands die. The Royal College of Physicians finally endorses inoculation in 1754, but death rates do not decline significantly until well into the next century. Skepticism about inoculation, it seems, has a long and complex (and fascinating) history.

In retrospect, the case of Lady Mary Wortley and smallpox variolation invites us to reflect: What factors should, ideally, guide our judgments about scientific claims, and what factors, by contrast, actually do shape those judgments, for better or worse? How might the story of the skeptics of 1721 inform an understanding of the public reception—and our own views—of vaccines today?

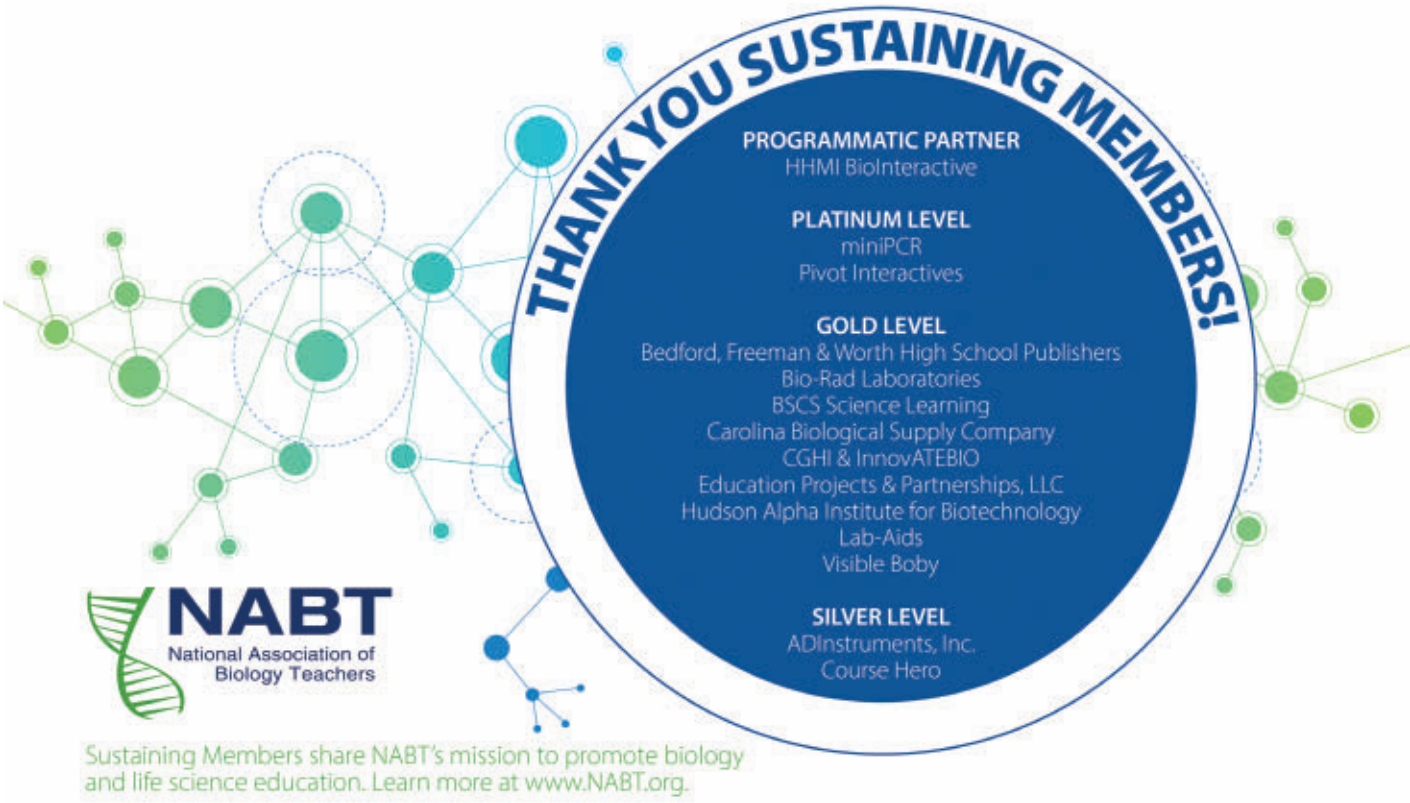
References

Carrell, J.L. (2003). *The Speckled Monster: A Historical Tale of Battling Smallpox*. Dutton/Penguin.

Hopkins, D.R. (2002). *The Greatest Killer: Smallpox in History*. University of Chicago Press.

Montagu, M.W. (1784). Letters of Lady Wortley Montagu. London: Cadell, Evans, Murray and Baldwin.

Remillard-Hagen, E. (2012). Lady Mary Wortley Montagu and Smallpox Inoculation in 18th-century England. St. Paul, MN: SHiPS Resource Center. http://shipseducation.net/modules/biol/smallpox.htm.



STATEMENT OF OWNERSHIP MANAGEMENT AND CIRCULATION, 09/26/21

Title: *American Biology Teacher* Pub. No. 0002-7685. Frequency: Nine issues published annually. Subscription price: $328.00 institutions. Location of office of publication: 155 Grand Avenue, Suite 400, Oakland, CA 94612-3764. Headquarters of publishers: 155 Grand Avenue, Suite 400, Oakland, CA 94612-3764. Publisher: University of California Press, 155 Grand Avenue, Suite 400, Oakland, CA 94612-3764. Editor: William F. McComas, NABT, 12100 Sunset Hills Road, Suite 130, Reston, VA 20190. Owner: National Association of Biology Teachers, 12100 Sunset Hills Road, Suite 130, Reston, VA 20190. The purpose, function, and nonprofit status of this organization and the exempt status for Federal income tax purposes have not changed during the preceding twelve months.

Extent and nature of circulation	Average number copies each issue for preceding twelve months	Actual number copies for single issue nearest to filing date
Total number of copies printed	2,777	2,550
Paid outside county mail subs	2,334	2,155
Paid distribution outside the mails	160	138
Total paid circulation	2,494	2,293
Free distribution outside the mails	3	6
Total free or nominal rate distribution	3	6
Total distribution	2,497	2,299
Copies not distributed	280	251
Total	2,777	2,550
Percent Pd. and/or Requested Circ.	99.88%	99.74%

I certify that the statements made by me above are correct and complete.
Erich van Rijn, Director of Finance

Join NABT!

MEMBERSHIP APPLICATION

☐ **Yes,** I want to join the National Association of Biology Teachers (NABT) and receive all member benefits.

☐ **Renew** my membership in NABT.

- 9 issues of *The American Biology Teacher*
- Special member only opportunities
- Bi-monthly electronic issues of *News & Views*
- Conference and workshop discounts & more!

Dr. _____

Preferred Mailing Address _____

_____

City _____

State _____ Zip _____

Province _____ County _____

E-mail Address _____

Fax _____

Type of Membership

☐ **FULL MEMBER** $79/yr
☐ **FOREIGN MEMBER*** $150/yr (U.S. check or international money order only)
☐ **STUDENT MEMBER** $35/yr (open to regularly matriculated students not in full-time employment) Online (only) subscription to ABT
Signature of faculty member required below:
_____
☐ **EARLY CAREER & ADJUNCT MEMBER** $35/yr (open to early career (<3 years), adjunct and part-time employed educators) Online (only) subscription to ABT
Signature of department chairperson required below:
_____
☐ **RETIRED MEMBER** $60/yr; $35/yr online (only) subscription to ABT
☐ **SPOUSE MEMBER** $35/yr (when accompanied by an active membership)
☐ **SUSTAINING MEMBER** Platinum level: $1750/yr; Gold level: $1250/yr; Silver level: $900/yr; Bronze level: $750/yr
☐ **ORGANIZATIONAL MEMBER** $149/yr

*U.S. rates apply to citizens of Canada and Mexico.

NOTE: $48 of membership dues goes toward a print subscription to *The American Biology Teacher,* for each year joined, except spouse members, in which case only the sponsoring active member receives a subscription to be shared by the two persons.

Payment Method

NABT accepts checks drawn on U.S. banks, international money orders (payable to NABT), major credit cards, purchase orders, or join online at **www.nabt.org.** Membership payments are non-refundable and all sales are final.

☐ Check ☐ Purchase Order # _____ *(please attach)*

☐ MasterCard ☐ VISA If paying by credit card, please complete the information below:

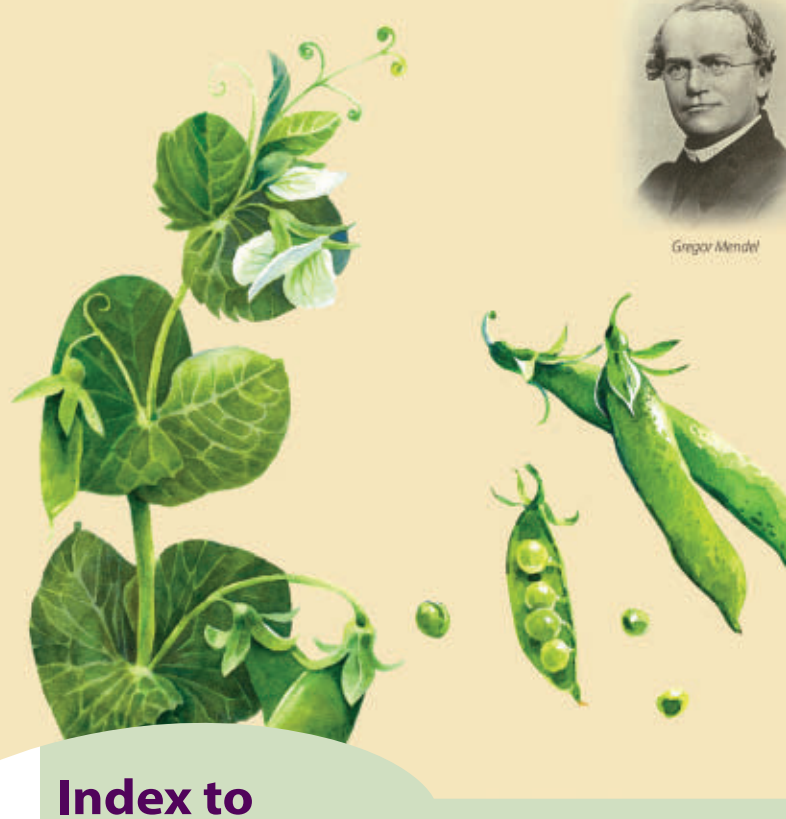
Acct. # _____ CVV Code _____

Expiration date _____

Card holder _____

Signature _____

Complete and return to: NABT • PO Box 3363, Warrenton, VA 20188
FAX (202) 962-3939



Index to Advertisers

NABT empowers the individual educator and fosters a supportive professional environment to create a diverse community who continually improves and enhances biology education.

Help NABT continue to grow.

Donate now at

www.nabtdonations.org

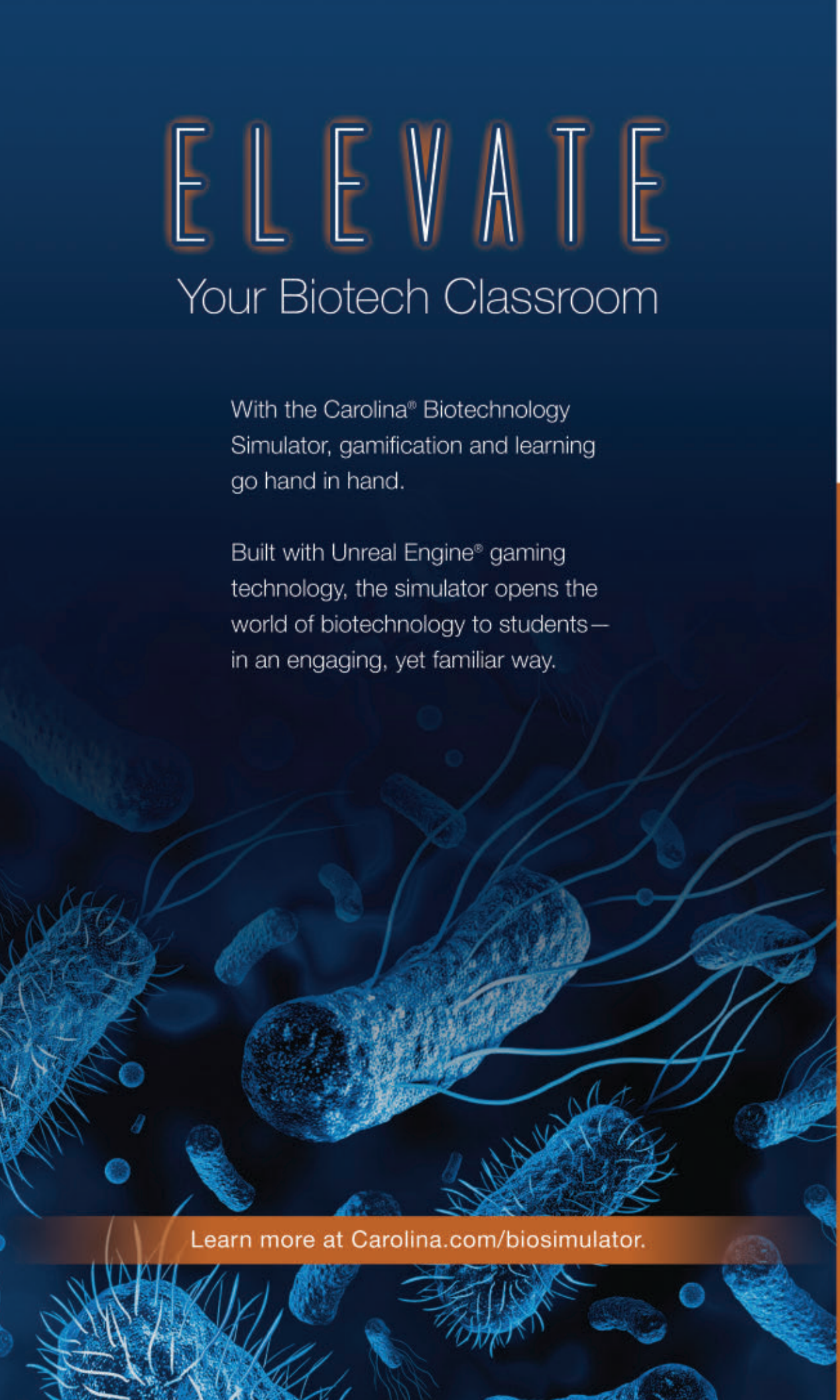
NABT
National Association of Biology Teachers

ELEVATE

Your Biotech Classroom

With the Carolina® Biotechnology Simulator, gamification and learning go hand in hand.

Built with Unreal Engine® gaming technology, the simulator opens the world of biotechnology to students—in an engaging, yet familiar way.



EPIC MegaGrants RECIPIENT

Learn more at Carolina.com/biosimulator.

CAROLINA®