Evolution in Action in the Classroom: Engaging Students in Science Practices to Investigate and Explain Evolution by Natural Selection

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Abstract

Current reform efforts at all levels of biology education advocate for the integration of science content and practices and emphasize the importance of phenomena-driven inquiry. We describe an instructional sequence for teaching evolution by natural selection that addresses these goals by engaging students in parallel selection experiments with biological and digital model organisms. These activities address multiple learning objectives in the AP Biology Curriculum Framework and the Next Generation Science Standards while engaging students in authentic science practices to learn about natural selection. We also report results from pre and post assessments in an AP Biology class which demonstrate students' learning gains and increased acceptance of evolution.

Key Words: *evolution; science practices; natural selection; nature of science.*

○ Introduction

Recent reform efforts in biology education at all levels affirm evolutionary theory as one of the key concepts of the discipline and emphasize the importance of engaging students in the practices of science to develop scientific literacy (Brewer & Smith, 2011; College Board, 2012; National Research Council, 2012; NGSS Lead States, 2013). While all of these efforts advocate integrating science practices with content knowledge and engaging students in authentic inquiry to explain phenomena, this can be especially challenging when teaching about evolution. The AP Biology Curriculum Framework asserts, "The science practices enable students to establish lines of evidence and use them to develop and refine testable explanations and predictions of natural phenomena" (College Board, 2012, p. 5). However, educators face many challenges to engaging students in designing and carrying out investigations and analyzing data

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about evolutionary processes in the classroom. These challenges include the long timescales for evolution to occur in most species, technically demanding and cost-prohibitive materials, and the fact that observing changes in populations doesn't necessarily help students to understand the mechanisms of evolution. Here we describe a set of learning activities that we developed to address these challenges and allow students to design and carry out investigations about the process of natural selection. We also report the results of a pre- and post-assessment and pre- and post-survey conducted in one AP Biology classroom (19 students) as preliminary evidence of the efficacy of our approach.

Despite its keystone position in the biological sciences, evolutionary theory is poorly understood by students and the general public, and one in three adults in the United States rejects the idea that humans have evolved (Miller et al., 2006). Although understanding evolution and accepting it as fact are two different issues, we think that biology educators should pay attention to both. Obviously, we want students to understand how evolution works and how it explains the unity and diversity of life on Earth, but students' acceptance of evolution also gives us important information about their understanding of the nature of science. Although many scientific concepts present challenges for learners, evolutionary theory seems to be particularly difficult to understand and is more likely to be rejected for ideological reasons than other scientific theories (Bishop & Anderson, 1990; Gregory, 2009; Meadows et al., 2000). Many science educators believe that understanding evolutionary theory is deeply connected to understanding the nature of science. A few studies have shown a positive relationship between student understanding of the nature of science and their understanding of evolutionary theory (reviewed in Lombrozo et al., 2008; Sinatra et al., 2003).

The nature of science comprises the complex social influences and interdisciplinary nature of the enterprise, as well as the "scientific habits of mind" that include the values and attitudes about knowledge that scientists share (Rutherford & Ahlgren, 1991). These are also closely associated with, though distinct from, the practices of science, or the work that scientists do. Johnson (2016) has argued previously in this journal that "effective science education should engage students in the practices of science while also reflecting on the values, commitments, and habits of mind that have led to the practices of modern science and that give them meaning" (p. 370). However, it has proved very challenging to develop activities that can be carried out in high school classrooms and engage students simultaneously in authentic scientific inquiry, deep reflection on the commitments and habits of mind of scientists, and the mechanisms of evolution.

Guided by the research on positive relationships between student understanding of the nature of science and their understanding of evolutionary theory, we developed a set of learning activities that addresses the following AP Biology Learning Objectives (College Board, 2012):

- LO 1.2: The student is able to evaluate evidence provided by data to qualitatively and quantitatively investigate the role of natural selection in evolution.
- LO 1.4: The student is able to evaluate data-based evidence that describes evolutionary changes in the genetic makeup of a population over time.
- LO 1.5: The student is able to connect evolutionary changes in a population over time to a change in the environment.
- LO 1.13: The student is able to construct and/or justify mathematical models, diagrams or simulations that represent processes of biological evolution.
- LO 1.25: The student is able to describe a model that represents evolution within a population.
- LO1.26: The student is able to evaluate given data sets that illustrate evolution as an ongoing process.

The activities invite students to participate in explicit discussions about the nature of scientific knowledge and to engage in science practices to design an investigation about the mechanism of natural selection in populations of biological and digital organisms. Our approach emphasizes the historical development of Darwin's ideas, integrates evolution concepts throughout the school year, and includes laboratory and computational activities that allow students to observe evolution in action as they develop and test hypotheses about the process of natural selection. We studied the outcomes of the learning activities with 11th grade students who were enrolled in the first author's AP Biology course.

Although this article focuses on how the activities are aligned to the AP Biology Curriculum Framework (College Board, 2012), they can be adapted to different levels of biology courses. For example, the wet-lab and computational activities served as the foundation for the development of curriculum that was implemented in an undergraduate introductory Cell and Molecular Biology course (Smith et al., 2016). The activities we describe below have also been implemented in the first author's general biology course for 11th grade students. The learning activities address the following performance expectations from the Next Generation Science Standards (NGSS Lead States, 2013):

- HS-LS4-2: Construct an explanation based on evidence that the process of evolution primarily results from four factors: (1) the potential for a species to increase in number, (2) the heritable genetic variation of individuals in a species due to mutation and sexual reproduction, (3) competition for limited resources, and (4) the proliferation of those organisms that are better able to survive and reproduce in the environment.
- HS-LS4-3: Apply concepts of statistics and probability to support explanations that organisms with an advantageous heritable trait tend to increase in proportion to organisms lacking this trait.
- HS-LS4-4: Construct an explanation based on evidence for how natural selection leads to adaptation of populations.
- HS-LS4-5: Evaluate the evidence supporting claims that changes in environmental conditions may result in: (1) increases in the number of individuals of some species, (2) the emergence of new species over time, and (3) the extinction of other species.

Overview of the Learning Activities

Although evolution was a recurring theme in every unit of the AP Biology course (for a more detailed rationale, see Olson & Labov, 2012), three main learning activities (Table 1) were developed to address the goals and learning objectives described above. The activities engaged students in many different science practices, but evaluating and using evidence was a central feature of all three. Although the activities could be implemented independently or in a different sequence, we suspect that the combination of three activities in this order is a more powerful approach because of how the activities build on one another.

Since our goal in this instructional sequence was to promote conceptual change, selection and sequencing of the activities was crucial because:

> Moving students to a desired understanding of a phenomenon is not merely a matter of presenting the correct explanation for an encounter, nor simply having direct experiences, but rather creating contexts where teachers explicitly help students scaffold between direct experiences and more accurate interpretations of those experiences so that students begin to question the supporting pillars for their ideas. (Clough, 2006)

The first of these activities includes reading and discussions of works by Paley, Lamarck, and Darwin to highlight the role of empirical evidence in science, scientific versus nonscientific explanations of phenomena, and the observations and questions that Darwin's theory addresses. These discussions were followed by a bacterial selection experiment inspired by Richard Lenski's long-term *E. coli* evolution experiment (Lenski, 2011) that allows students to use artificial selection to model the evolution of antibiotic resistance. Subsequent activities utilized Avida-ED digital evolution software, which provides an instance of evolution that allows students to observe evolution in digital organisms and to develop and test hypotheses about the underlying mechanisms (Pennock, 2007). Because the user interface of Avida-ED represents digital

Table 1. Summary of instructional activities.

Activity	Description	Implementation (Duration, timing)
Historical readings and discussion	Students read and discuss excerpts from *Natural Theology* (1802) by William Paley, *Zoological Philosophy* (1809) by Jean Baptiste Lamarck, and *On the Origin of Species* (1859) by Charles Darwin. Activities adapted from *Modeling for Understanding in Science Education* (MUSE) project (2002).	One week, early in the first semester
Bacterial selection experiment	Students perform an artificial selection experiment adapted from the Kirby-Bauer Disk Diffusion Susceptibility Test (Hudzicki, 2009). *E. coli* K-12 is exposed to antibiotic disks and the most resistant colonies are isolated and re-cultured for multiple rounds of selection. In some trials the *E. coli* become significantly less sensitive to the antibiotic within a few rounds of selection and students observe differences in resistance to different antibiotics and in different trials.	Carried out over six weeks early in the first semester
Avida-ED digital evolution software	Students use the Avida-ED software to work through a set of activities that were designed to highlight the concepts of random mutation, inheritance, and differential reproductive success in different environments. The activities are titled "Studying Evolution with Digital Organisms" and "Evolving TCE Biodegraders," and are available online at www.teachengineering.org.	Two weeks during the second semester (after classical and molecular genetics)

organisms as analogous to a colony of bacteria on a Petri dish, comparisons can be made directly between the bacterial selection experiment and digital evolution. The combination of the bacterial selection experiment followed by the use of the software may prove to be more beneficial than either experience alone since it allows students to "place the often-sterile simulation environment in perspective" (Windschitl, 2000), while providing multiple contexts for learning.

Below we describe each of the three learning activities in turn and report the results from a student assessment and survey.

Historical Readings and Discussion

Early the in the first semester of the AP Biology course, students engaged in a set of activities adapted from the Natural Selection unit developed by the *Modeling for Understanding in Science Education* (MUSE) project of the National Center for Mathematics and Science at the University of Wisconsin–Madison (MUSE, 2002). These activities consisted of historical readings in the development of evolutionary theory and class discussions of the main arguments and evidence included in each. We used activities from "Section Two: Comparing Explanatory Models" of the MUSE Natural Selection Unit. (Activities are available online at http://ncisla.wceruw. org/muse/naturalselection/.)

Our main goal in using the historical readings was to develop a shared understanding of scientific theories as natural explanations for phenomena in the material world. We also wanted students to have a basic understanding of Darwin's theory of natural selection and the importance of empirical evidence in supporting scientific claims before beginning laboratory work. Students read edited excerpts from *Natural Theology* (1802) by William Paley, *Zoological Philosophy* (1809) by Jean Baptiste Lamarck, and *On the Origin of Species* (1859) by Charles Darwin. After each reading, the class discussed the phenomena that the author was trying to explain, the evidence they used to support their arguments, and the inferences they made.

After reading and discussing each piece separately, students were asked to compare the conceptual models of Paley, Lamarck, and Darwin. They recognized that each author accounted differently for the adaptation of organisms to their environment. Paley's argument stood apart from Lamarck's and Darwin's in that it invoked a supernatural force as the mechanism for adaptation. Class discussion highlighted the fact that whereas Lamarck's and Darwin's explanations were empirically testable (and thus "scientific" as we currently characterize the natural sciences), Paley's explanation was not. Based on the class discussion, it appeared that students grasped the fundamental difference between scientific and nonscientific explanations, although they often needed guidance in thinking about how Lamarck's and Darwin's ideas could be tested.

In comparing the models of Lamarck and Darwin, it became evident to the students that Darwin's explanation focused more on the changes occurring at the population level than on individual organisms, and that his mechanism was better supported by their own understanding of genetic inheritance. As they considered Lamarck's explanation of acquired characteristics, they recognized that most modifications made to an organism during its lifetime would not be passed on to offspring. The students came up with examples such as elite athletes that build large muscles and quickly dismissed the notion that their offspring would be born with larger muscles. Overall, students seemed to recognize the power of Darwin's model, although they could not yet clearly articulate it.

The acronym VIST was introduced to elucidate the four key components of Darwin's model: Variation, Inheritance, Selection, and Time (Understanding Evolution, 2017). The acronym aided students in thinking about how the four elements interact to change a population over many generations. After the reading and discussion activities, it was clear that many students did not fully comprehend Darwin's theory of natural selection, but they were convinced that it was testable. This link between the nature of scientific inquiry and evolutionary theory segued nicely into the bacterial selection experiment that followed.

⃝ Bacterial Artificial Selection Experiment

Following the historical readings and discussion, a bacterial selection experiment was introduced to the students as a way to test Darwin's ideas. The similarities between the bacterial selection experiment and Darwin's artificial selection experiments were discussed, as well as the relationship between the experiment and the evolution of antibiotic resistance in natural populations of bacteria. Rather than giving students a set of instructions, the instructor (WJ) demonstrated the techniques and engaged students in planning and carrying out the investigation. The basis of the experiment was the Kirby-Bauer Disk Diffusion Susceptibility Test (adapted from Hudzicki, 2009). The assay involves inoculating an agar plate with bacteria and placing disks that are infused with an antibiotic on top of the agar. The antibiotic diffuses into the agar, creating a ring around the disk in which the concentration of the antibiotic decreases with increasing distance from the disk; this ring is known as the *zone of inhibition*. The assay is a common method for determining the sensitivity of particular strains of bacteria to specific antibiotics.

For the initial demonstration of the protocol, students streaked a nutrient agar plate with a non-pathogenic strain of *E. coli* (*E.coli* K-12), and placed three equally spaced antibiotic disks on top of the agar. The plates were incubated at 37°C for 48 hours and then observed. Students measured the zone of inhibition by determining the diameter of the region around the disk where no bacterial growth occurred. The assay was performed with multiple antibiotics including penicillin, kanamycin, neomycin, novobiocin, streptomycin, erythromycin, chloramphenicol, and tetracycline. Students observed different-sized zones of inhibition in the different antibiotic treatments, and developed an argument from evidence that the size of the zone of inhibition is an indicator of the sensitivity of the *E. coli* to the particular concentration of the specific antibiotic tested.

The instructor led a discussion about how to identify and isolate the most resistant bacterial colonies (those growing in and around the zone of inhibition). Students isolated and re-cultured the most resistant colonies in a liquid medium incubated overnight, and then repeated the assay for five or six rounds of selection. Students shared their results with the class and wrote a formal report about the bacterial selection experiment in which they analyzed the data (size of the zone of inhibition in each round of selection), explained the results, and hypothesized about the phenotypic changes in the bacteria that could have led to decreased sensitivity to the antibiotics.

In addition to allowing students to observe evolutionary changes in a population, the laboratory experience demonstrated many important principles of natural selection. For example, three different groups performed the experiment using kanamycin, and each group observed different changes in the population over the six-week experiment. One group of students observed no change in the size of the zone of inhibition over the course of the experiment, while another observed decreased zone sizes, and the third group observed the development of complete resistance (no zone of inhibition after six rounds of selection). This highlighted the fact that beneficial traits due to random mutations are not induced by the environment, and that selection can only act on variation that is already present in a population.

In addition to relating the bacterial selection experiment to the process of natural selection, there were many opportunities during this experiment for students to reflect on science practices and to discuss the role of evidence in science. For example, discussions about the effect of the depth of the nutrient agar on the diffusion of the antibiotics led the class to develop a procedure to ensure that each group used agar plates of consistent depth. Students decided to determine the mass of the nutrient agar plates that the teacher had poured, and assigned each group of students to use plates of similar mass (±2 g) in each trial to ensure more consistent results between transfers. In addition, the concept of a control treatment was elucidated during the last round. Students recognized that the best way to compare changes in antibiotic resistance was to plate a sample of the original population at the same time that they plated their selected bacteria in order to ensure that the plates were exposed to the same conditions (freshness and depth of the nutrient agar on the plate, temperature of the incubator, their own techniques, etc.). Comparing the evolved and ancestral populations side by side made the changes in the zone of inhibition much more apparent.

Notes for teachers: All lab materials can be purchased from Carolina Biological Supply (Table 2). In addition to the supplies below, you will need basic microbiology equipment, including: inoculating loops and spreaders, an incubator, alcohol lamps (or some other flame sterilizer), and sterile pipets. Students should wear personal protection including goggles, closed-toe shoes, gloves, and laboratory coats or aprons. All contaminated items should be autoclaved before disposal. Please consult the American Society for Microbiology's (2012) *Guidelines for Biosafety in Teaching Laboratories* for a full discussion of safety considerations.

Selection Experiment with Digital Organisms

During the second semester, students engaged in a set of activities using Avida-ED digital evolution software for education (http://avida-ed.msu.edu). Avida is a research platform used by biologists and engineers to study the mechanisms of evolution in order to better understand biological evolution and to apply those mechanisms to solve engineering design problems (Ofria & Wilke, 2004; Zimmer, 2005). Avida-ED is an educational version of Avida designed for teaching and learning about evolution and the nature of science in biology courses (Pennock, 2007). Avida-ED is made freely available to the public online by Michigan State University and has been used in a number of different undergraduate biology courses (Lark, 2015; Smith et al., 2016; Speth et al., 2009).

All of the elements necessary for evolution by natural selection are represented in the digital system: replication of digital organisms (short computer programs), variation due to random replication errors, and selection due to differences in reproductive success (the rate at which the computer programs replicate) in particular environments. Because digital organisms replicate much more rapidly than any biological organisms, the timescales for evolution are amenable to classroom study. The software offers students the opportunity to observe evolutionary changes in a population in real-time and to propose and test hypotheses about the process of natural selection. One of the main benefits of the software is that it allows students to visualize both variation in individuals and evolutionary changes in populations.

We developed guided inquiry lessons that engaged students in collecting and analyzing data in Avida-ED and developing

Table 2. List of lab materials for bacterial selection experiment.

Carolina Biological Item number	Name	Use
741248	Polystyrene Petri dishes	Used to prepare the nutrient agar plates.
776374	Nutrient Agar	Pre-sterilized nutrient agar. Melt and pour approximately 20 mL into each Petri dish.
216655	Luria Broth	Nutrient broth for liquid cultures. Transfer approximately 5 mL into sterile tubes. Inoculate the liquid broth then incubate at 37° C for 24 hours.
805081	Antibiotic Sensitivity Disk Set	Using sterile forceps, remove a disk and place on top of an agar plate that has been inoculated with *E. coli*. Three disks can be evenly spaced in one Petri dish.
155068	*Escherichia coli*, Living, K-12 Strain, Tube	Inoculate nutrient agar plates with 0.1 mL of the *E.coli* culture and spread onto the surface. Periodically re-culture the original stock by placing 0.5 mL into a fresh tube of nutrient broth.

arguments from evidence to explain the results. The purpose of the Avida-ED lessons was to allow students to observe evolutionary changes in a population and to connect those changes to selective pressures acting on variation among individuals. Instructions, hand-outs, and teaching materials are published at www.teachengineering.org. The activities used in this course are titled "Studying Evolution with Digital Organisms" and "Evolving TCE Biodegraders."

Assessment of Student Learning and Acceptance of Evolution

Many formative assessment tasks were embedded in the activities; these, as well as regular classroom assessments and observations, inform our conclusions about student outcomes (for a fuller description, see Johnson, 2012). Two different instruments were used to collect quantitative data about students' understanding and acceptance of evolution by natural selection. The Measure of Acceptance of the Theory of Evolution (MATE; Rutledge & Warden, 1999) instrument was administered before the start of the course and at the end of the school year. The MATE instrument was designed to assess teacher acceptance of the theory of evolution and has since been shown to be a reliable indicator of undergraduate acceptance of evolutionary theory (Rutledge & Warden, 1999; Rutledge & Sadler, 2007). Although others have criticized the MATE for conflating knowledge with acceptance (Smith, 2010) or for unreliability with certain populations (Wagler & Wagler, 2013), we decided that it was useful for us to have some measure of students' acceptance of evolutionary theory before and after the course, despite the limitations of the instrument.

We also developed an assessment with seven constructed response questions to assess students' understanding of natural selection (Appendix Table 3). Students in the AP Biology course completed the assessment immediately before and after working with Avida-ED. Although the bacterial selection experiment was not specifically addressed during the Avida-ED lessons, it served as the context for six of the assessment questions. This was important because we wanted to understand how student thinking about biological evolution changed after their experience with the digital system. The final question of the assessment was intentionally broad and did not apply to any particular system as it was aimed at uncovering student conceptions about the general mechanism of natural selection.

Students in the AP Biology course showed significant gains from pre- to post-assessment after using Avida-ED ($p < 0.001$; 1-tailed paired t-test; $N = 16$). The increase in the pre- to post-assessment scores is attributable to the increased scores on four of the items (1, 3, 6, and 7); scores on the other three items did not show significant changes from pre- to post-test. In addition, scores on the MATE instrument increased significantly ($p < 0.001$; 1-tailed paired t-test; $N = 19$) from pre- to post-test. Out of 100 possible points, the average pre-test MATE score was 75.6, and the post-test MATE average was 91.7.

○ Discussion

The learning activities implemented in this study explicitly addressed the nature of scientific knowledge and allowed students to observe and develop arguments from evidence about the process of evolution by natural selection. The combination of the bacterial selection experiment followed by the use of Avida-ED software was predicted to foster conceptual understanding of the process of evolution by natural selection because it actively engaged students in the practices of science as they learned the content. The assessment results documented significant learning gains and increased acceptance of the theory of evolution, which indicates that this sequence of activities has potential for increasing student understanding of natural selection and the nature of science.

After using Avida-ED, students were much more likely to discuss the random nature of mutations, including the likelihood that certain mutations would occur. Their explanations also focused more on how the frequency of beneficial mutations increases in a population over time. The largest gains were seen on the final question that asked students to reason about a new context, which provides evidence that students were able to apply what they learned using Avida-ED develop a more generalized explanation of natural selection.

We believe that the sequence of curricular activities was particularly important for student learning. None of the assessment questions were directly discussed between the pre- and post-tests. The fact that students were able to offer more complete explanations about natural

selection in biological systems after using Avida-ED suggests that they were able to generalize from the digital system and transfer their understanding to the biological context. Their ability to transfer knowledge suggests that conceptual change occurred in many students. It seems that the use of Avida-ED in tandem with the bacterial model of artificial selection may have been especially powerful for promoting conceptual change in student understanding of natural selection; the two systems share many similarities and may be mutually reinforcing.

In contrast with the pre- and post-tests addressing the process of natural selection, the MATE instrument more broadly assessed students' acceptance of the theory of evolution and particularly their acceptance of the methods of scientific inquiry that allow for the study of the history of life on earth. This instrument measures students' conceptions about both evolution and the nature of scientific knowledge and therefore is well matched to the goals of this study. Our results show that students' acceptance of the theory of evolution increased significantly as measured by the MATE. Interestingly, the pre-test scores on the MATE were higher than expected considering that the students had no prior high school biology. The developers of the MATE consider scores of 65–76 to indicate moderate acceptance, whereas scores of 77–88 indicate high acceptance. The AP Biology students in this study started in the moderate acceptance range and moved into the very high acceptance range (89–100) over the course of the school year.

Observations throughout the school year also support our conclusion that students' understanding and acceptance of the scientific basis for the study of evolution increased. For example, AP Biology students scored higher on questions referring to natural selection and the overall process of evolution on unit exams than students did in previous years. Although the same topics were covered in previous years, the introduction of the bacterial selection experiment and Avida-ED activities, combined with the increased emphasis on the nature and practices of science, likely contributed to increased student learning. Additionally, based on classroom observations and student responses, it was clear that students were very excited about carrying out authentic experiments whose outcomes could not be easily predicted, and the fact that Avida-ED was adapted from an actual research tool made their work seem more relevant and meaningful. Future research is needed to distinguish the effectiveness of the bacterial selection experiment from the Avida-ED activities and to determine which aspects of the nature of science are most important for understanding evolution.

Conclusion

We developed this sequence of activities to address a set of AP Biology learning objectives in order to engage students in the practices of science to explain the process of natural selection. Assessment data collected from AP Biology students indicate that students were able to translate the principles they learned working with the digital system to better explain the process of natural selection in biological systems. In addition to increased understanding, data from the MATE instrument indicate significant gains in students' acceptance of the scientific validity and explanatory power of evolution by natural selection. These findings suggest that the sequence of activities implemented in this study promote conceptual change about the process of evolution by natural selection and the nature of scientific knowledge.

References

American Society for Microbiology, ASM Task Committee on Laboratory Biosafety. (2012). *Guidelines for biosafety teaching in laboratories*. Retrieved from https://www.asm.org/images/asm_biosafety_guidelines-FINAL.pdf

Bishop, B. A., & Anderson, C. W. (1990). Student conceptions of natural selection and its role in evolution. *Journal of Research in Science Teaching, 27*(5), 415–427.

Brewer, C. A., & Smith, D. (2011). Vision and change in undergraduate biology education: A call to action. Washington, D.C.: American Association for the Advancement of Science.

Clough, M. P. (2006). Learners' Responses to the Demands of Conceptual Change: Considerations for Effective Nature of Science Instruction. *Science Education, 15*, 463–494. doi:10.1007/s11191-005-4846-7

College Board. (2012). *AP Biology course and exam description*. www.collegeboard.org.

Gregory, T. R. (2009). Understanding natural selection: Essential concepts and common misconceptions. *Evolution: Education and Outreach, 2*(2), 156–175.

Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. *American Society for Microbiology*. http://www.asmscience.org/content/education/protocol/protocol.3189

Johnson, W. R. (2012). *Evolution in action in the classroom: Engaging students in scientific practices to develop a conceptual understanding of natural selection* (Master's thesis). Available from ProQuest Dissertations and Theses database (UMI No. 1517061).

Johnson, W. R. (2016). Why engaging in the practices of science is not enough to achieve scientific literacy. *American Biology Teacher, 78*(5), 370–375.

Lark, A. (2015). *Teaching and learning with digital evolution: Factors influencing implementation and student outcomes* (Doctoral dissertation). Available from ProQuest Dissertations and Theses database.

Lenski, R. E. (2011). Evolution in Action: A 50,000-Generation Salute to Charles Darwin. *Microbe, 6*(1), 30–33.

Lombrozo, T., Thanukos, A., & Weisberg, M. (2008). The importance of understanding the nature of science for accepting evolution. *Evolution: Education and Outreach, 1*(3), 290–298.

Meadows, L., Doster, E., & Jackson, D. F. (2000). Managing the conflict between evolution & religion. *American Biology Teacher, 62*(2), 102–107.

Miller, J. D., Scott, E. C., & Okamoto, S. (2006). Public acceptance of evolution. *Science, 313*(5788), 765–766.

Modeling for Understanding in Science Education (MUSE) Project. (2002). National Center for Mathematics and Science, Wisconsin Center for Education Research at the University of Wisconsin–Madison. http://ncisla.wceruw.org/muse/.

National Research Council. (2012). *A framework for K-12 science education: Practices, crosscutting concepts, and core ideas*. Washington, D.C.: National Academies Press.

NGSS Lead States. (2013). *Next Generation Science Standards: For States, By States*. Washington, D.C.: National Academies Press.

Ofria, C., & Wilke, C. O. (2004). Avida: A software platform for research in computational evolutionary biology. *Artificial Life*, 10(2), 191–229.

Olson, S., & Labov, J. B. (Eds.). (2012). Thinking evolutionarily: Evolution education across the life sciences: Summary of a convocation. Washington, D.C.: National Academies Press.

Pennock, R. T. (2007). Learning evolution and the nature of science using evolutionary computing and artificial life. *McGill Journal of Education*, 42(2), 211–224.

Rutherford, F. J., & Ahlgren, A. (1991). *Science for all Americans*. New York: Oxford University Press.

Rutledge, M. L., & Sadler, K. C. (2007). Reliability of the Measure of Acceptance of the Theory of Evolution (MATE) instrument with university students. *American Biology Teacher*, 69(6), 332–335.

Rutledge, M. L., & Warden, M. A. (1999). The development and validation of the measure of acceptance of the theory of evolution instrument. *School Science and Mathematics*, 99(1), 13–18.

Sinatra, G. M., Southerland, S. A., McConaughy, F., & Demastes, J. W. (2003). Intentions and beliefs in students' understanding and acceptance of biological evolution. *Journal of Research in Science Teaching*, 40(5), 510–528.

Smith, M. U. (2010). Current status of research in teaching and learning evolution: I. Philosophical/epistemological issues. *Science & Education*, 19(6–8), 523–538.

Smith, J. J., Johnson, W. R., Lark, A. M., Mead, L. S., Wiser, M. J., & Pennock, R. T. (2016). An Avida-ED digital evolution curriculum for undergraduate biology. *Evolution: Education and Outreach*, 9(ar9).

Speth, E. B., Long, T. M., Pennock, R. T., & Ebert-May, D. (2009). Using Avida-ED for teaching and learning about evolution in undergraduate introductory biology courses. *Evolution: Education and Outreach*, 2(3), 415–428.

Understanding Evolution. (2017). *University of California Museum of Paleontology*. http://evolution.berkeley.edu/.

Wagler, A., & Wagler, R. (2013). Addressing the lack of measurement invariance for the Measure of Acceptance of the Theory of Evolution. *International Journal of Science Education*, 35(13), 2278–2298.

Windschitl, M. (2000). Supporting the development of science inquiry skills with special classes of software. *Educational Technology, Research, and Development*, 48(2), 81–95.

Zimmer, C. (2005). Testing Darwin. *Discover*, 26(2), 28–34.

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Appendix Table 3. Student responses were scored by comparing them to an ideal response that included specific elements. For a full description of the scoring procedures, see Johnson (2012).

Item	Question	Ideal Response
1	What happened in the bacteria that allowed them to grow closer to the antibiotic disk? What caused this change?	A random mutation occurred in the DNA and resulted in a protein that made the bacteria more resistant to the antibiotic.
2	Did the change first occur in one or many *E. coli* cells? Explain your reasoning.	The zone around the disk was initially clear, indicating that very few (if any) cells were initially resistant to the antibiotic. The mutation that caused antibiotic resistance likely first occurred in one cell. This cell and its descendants were selected when we swabbed the zone of inhibition, and therefore the antibiotic resistance trait was more prevalent in subsequent rounds of the experiment.
3	Explain how this change in the *E. coli* led to the results that were observed (i.e., many colonies growing much closer to the antibiotic disk).	The bacteria with the mutation had an advantage over the others because they could grow in the presence of the antibiotic. Since the resistant colonies were specifically recultured, the frequency of the trait in the population on the dish increased over many generations.
4	Did the bacteria in this experiment evolve? Explain your answer using your observations from the lab and the fundamental principles of evolution.	Yes. The bacteria evolved because the frequency of the beneficial mutation that conferred antibiotic resistance increased over many generations. The decreased size of the zone of inhibition was a measure of the bacteria's sensitivity to the antibiotic and is evidence of a change in gene frequency in the population.

Appendix Table 3. Continued

Item	Question	Ideal Response
5	In what ways was this experiment similar to natural selection in the wild? In what ways did it differ?	The mechanisms of both artificial and natural selection are nonrandom and cause the resistant bacteria to leave more offspring. In artificial selection humans specifically choose organisms to reproduce, which "speeds up" evolutionary change in the population.
6	If you repeated the experiment many times using the same procedure and same antibiotic, would you expect the exact same results or would there be variability? Explain your answer.	Resistance would develop each time, although there may be variation in the number of generations and the actual sizes of the zone of inhibition. The development of resistance depends on the probability of a mutation that confers resistance and multiple different mutations may be possible.
7	Imagine that a new life form was just discovered on another planet. It is not made up of cells nor does it contain DNA. What characteristics of this life form and the environment would be necessary in order for natural selection to act on it? Explain.	The life form must include genetic information that can be copied and passed to offspring. The genetic information must be able to mutate to introduce variation, and those variations must lead to differential reproductive success within their environment.