**ABSTRACT**

The concepts of evolution and natural selection remain as some of the most challenging topics to teach. The difficulty in teaching these topics arises from the fact that evolution is difficult to observe, and computer simulations do not always result in a clear understanding of evolutionary principles. Recently, the Avida-ED software has been developed to simulate evolution in a laboratory setting. Unlike other simulations, Avida-ED allows students to manipulate the environment, change the genetics of the virtual organisms, and track offspring in real time. We have demonstrated, by using pretest and posttest questionnaires, that students gained a deeper understanding of evolutionary concepts by using this software. In particular, students showed the greatest increase in their ability to explain evolutionary concepts in answers to open-ended questions. Our results show that Avida-ED could be a useful tool in helping students understand and combat preconceived notions about evolution.

**Key Words:** Avida-ED; evolution; natural selection; digital evolution.

**Introduction**

The concept of evolution is one of the core and unifying principles of biology. In the words of the great evolutionary biologist Theodosius Dobzhansky, “nothing in biology makes sense except in the light of evolution” (Dobzhansky, 1973). Additionally, the principles underlying evolution have been validated by scientists and science education organizations, both in the United States and around the world (Plutzer & Berkman, 2010; Brams, 2013). Yet evolution is considered one of the most controversial and difficult topics for instructors of biology to teach in the United States, where the theory still faces a wide range of scrutiny and lack of acceptance among much of the general public. In fact, polling data collected since 1984 indicate that 40–47% of U.S. adults consistently state that they believe humans were created in their present form within the past 10,000 years (Pobiner, 2016). This is radically different from the views held by the majority of adults in European countries and Japan, where approximately 60–80% of adults accept the idea of evolution (Miller et al., 2006). Hence, there is a great need for in-depth and engaging evolutionary instructional tools for the enhancement of evolutionary understanding in U.S. populations.

Furthermore, teaching the ideas of complex evolutionary theory can be a challenge for instructors. For instance, direct observations and the quantification of evolution are difficult in any setting, and can be especially difficult in the college classroom and/or laboratory setting. Additionally, given the integrative nature of evolution in the field of biology (with overlapping subfields including molecular biology, cellular biology, and microbiology, among others), the facilitation of a deeper understanding of evolutionary ideas is even more difficult (Nehm et al., 2009). Many resources, including in-depth case studies (White et al., 2013) and interactive laboratory simulations (Abraham et al., 2009), are available for instructors to use in the dissemination of evolutionary concepts to students. However, these resources are not adequate for helping undergraduate students master evolutionary principles, because of various factors—such as cost-prohibitive equipment/software for analyzing evolution and the inability to directly observe and experimentally manipulate various aspects of evolutionary theory in a research setting.

More recently, an instructional method that uses a digital organismal model has been utilized to help overcome the difficulties of teaching evolution. Specifically, the research platform known as Avida-ED, which was first developed at Cal-Tech in
the late 1990s and has been further developed as an educational tool at Michigan State University (Ofria & Wilke, 2004; Pennock, 2007), allows students to directly observe self-replicating virtual organisms (Avida) that behave in ways similar to bacteria. Additionally, this simulation program allows students to witness evolutionary principles in a real-time setting, which is of tremendous benefit to students. A major advantage of the Avida-ED program that distinguishes it from other evolutionary simulations is that students can study and analyze evolution in an experimental setting. Users have the ability to manipulate resources in the environment, change mutation rates, and track generations of these organisms to observe how these components can alter natural selection. The use of Avida-ED in both experimental and educational settings has become widespread in recent years and has been associated with enhanced student success in learning evolutionary principles (Lenski et al., 2003; Misevic et al., 2006; Chune et al., 2011; Grabowski et al., 2013; Zaman et al., 2014; Smith et al., 2016; Lark et al., 2018).

Given the reported success of Avida-ED in educational settings, we designed a study to investigate whether the implementation of Avida-ED in a first-semester freshman introductory biology course could result in significant increases in the understanding of evolutionary ideas and principles at a liberal arts college in a rural setting. Previous studies have investigated the link between rurality and acceptance of evolutionary ideas, with data exhibiting varying degrees of significance in regard to whether individuals in rural settings accept evolutionary theory (Mazur, 2004; Baker, 2013). Specifically, we used pretest and posttest questionnaires to examine whether Avida-ED was effective in increasing the knowledge and retention of basic evolutionary principles, such as the roles of random mutations, competition, and natural selection. Overall, by comparing students’ pretest and posttest responses, we found that the use of Avida-ED increased students’ understanding of ideas that are central to the theory of evolution. Our results demonstrate how Avida-ED can be used as a valuable tool in evolution education in small colleges and universities.

**Methods**

**Student Population**

Thiel College is a liberal arts college in Greenville, Pennsylvania, that primarily serves students from Pennsylvania, Ohio, and New York. The student population consists largely of individuals from nearby rural counties in these states, many from low-income backgrounds. The Avida-ED lab was used as a part of the laboratory portion of Foundations of Biology (BIO 145), a one-semester introductory course that is required for all majors in the biology department and is a prerequisite for all other biology courses. Data were gathered from 125 students between fall 2016 and fall 2018 (a total of five semesters and pooled from 10 different sections of the course taught by six different instructors). The students included freshman biology majors as well as nonmajors taking the course to fulfill their science core curriculum requirement. The Avida-ED labs were performed in the laboratory sections prior to any instruction on evolution in the lecture portion of the course.

**Avida-ED Software**

The Avida-ED software used in this study is freely available from the developing team at Michigan State University and can be found at https://avida-ed.msu.edu/avida-ed-application/. Students used either the web version or the downloaded software on their individual computers. There were no differences in experimental setup between the two versions.

**Avida-ED Experiments & Lab Design**

Students followed the Avida-ED curriculum that was generated by the design team at Michigan State University. Specifically, Thiel College faculty members implemented the model lessons found in the Avida-ED lab book and curriculum (https://avida-ed.msu.edu/curriculum). The laboratory instruction was divided into two sections spread over two weeks. Week 1 started with students taking a pretest questionnaire that measured their understanding of evolutionary principles prior to any Avida-ED instruction (or any teaching of evolutionary concepts in lecture). Week 1 also focused on introducing the software to the students so that they could become familiar with the tools and features of the program. Once they had become familiar with the software, and all the software-related questions had been addressed, students ran their first experiment: “Exercise 1: Understanding the Introduction of Genetic Variations by Random Mutation.” This concluded week 1 of Avida-ED instruction. Week 2 resumed with the second and third experiments: “Exercise 2: Exploring Fitness, Functions, and Selection” and “Exercise 3: Exploring Mutations and Selection: Pre-adaptive or Post-adaptive?” Week 2 activities ended with a posttest questionnaire (identical to the pretest questionnaire). This progression is shown in Figure 1.

**Questionnaire**

The (identical) pretest and posttest questionnaires were conducted in the laboratory at the beginning of week 1 and at the end of week 2. The laboratory sessions that focus on evolution occur before that material is discussed in the lecture component of the course. The questionnaire is based on questions from the Conceptual Inventory of Natural Selection (Anderson et al., 2002) and from questions provided by the Active LENS Train-the-Trainers Workshop at Michigan State University (which the
authors attended in 2015). The students had the option of choosing whether to participate in the pretest and posttest questionnaires and had the option of opting out (the study was approved by the Institutional Review Board Committee of Thiel College); however, all students in the course were required to participate in the Avida-ED simulation as part of their lab grade for the two lab periods. Students were not compensated for their time, neither monetarily nor in grade form. Several students declined to participate in the questionnaire. Over a period of five semesters, a total of 10 course sections were involved in the study and a total of 125 pretest and posttest questionnaires were collected. All the questions that were administered are available in the Supplemental Material with the online version of this article.

Questionnaire Analysis, Data Entry & Statistics

Questionnaires were graded by either professors or teaching assistants, strictly following a key of correct responses for multiple-choice (MC) questions and acceptable responses for open-ended (OE) questions. Data were entered into Excel and were graphed and analyzed in Prism 7.04 (GraphPad, San Diego, California).

○ Results

Analysis of students’ responses to MC and OE questions before Avida-ED instruction (pre-Avida) and after Avida-ED instruction (post-Avida) (N = 125 students between 2016 and 2018; see above) demonstrated positive effects. Mean percentages of correct MC responses were significantly different between the pre-Avida and post-Avida response groups; specifically, the mean percentage of correct MC responses increased from 44.31 for the pre-Avida instruction group (95% confidence interval [CI]: 41.68–46.93) to 54.61 for the post-Avida instruction group (95% CI: 51.33–57.88) (P < 0.001) (Figure 2). There was an even more significant difference in the OE responses between the pre-Avida and post-Avida response groups; specifically, the mean percentage of acceptable OE responses increased from 43.6 for the pre-Avida instruction group (95% CI: 41.68–46.93) to 69.68 for the post-Avida instruction group (95% CI: 64.93–74.43) (P < 0.001) (Figure 3).

Figure 2. Students’ (N = 125) average performance on a series of multiple-choice (MC) questions that tested their understanding of evolutionary principles. The mean percentage of correct responses before Avida-ED instruction (pre-Avida) was 44.31%. The mean percentage of correct responses after Avida-ED instruction (post-Avida) was 54.61%. The data are statistically significant (****P < 0.0001). The tables show minimum and maximum values, 25% and 75% percentiles, median, mean, standard deviation, standard error, lower and upper 95% confidence limits, and the results of statistical analysis.

Mean fold changes from pre-Avida to post-Avida instruction for both the MC and OE questions were also analyzed. There was a mean fold change of 1.331 (95% CI: 1.224–1.438) in the MC responses (Figure 4) and a mean fold change of 2.096 (95% CI: 1.799–2.393) in the OE responses (Figure 5).

Finally, to determine how much Avida-ED helped (or potentially hurt) students’ understanding of natural selection, we compared the number of answers from the pretest to posttest questionnaire that went from wrong to right (W to R) with the number that went from right to wrong (R to W) (Figure 6). We found that there was a significant increase in the “W to R” answers compared to the “R to W” answers when looking at both MC and OE questions.

Figure 3. Students’ (N = 125) average performance on a series of open-ended (OE) questions that tested their ability to describe evolutionary principles. The mean percentage of acceptable responses before Avida-ED instruction (pre-Avida) was 43.60%. The mean percentage of acceptable responses after Avida-ED instruction (post-Avida) was 69.68%. The data are statistically significant (****P < 0.0001). The tables show minimum and maximum values, 25% and 75% percentiles, median, mean, standard deviation, standard error, lower and upper 95% confidence limits, and the results of statistical analysis.
and enhance introductory biology students manipulate organisms in an experimental setting, would increase evolution simulation program Avida-ED, which allows students to (2003). We examined whether the implementation of the innovative theory; and a greater degree of acceptance sometimes does not result in increased understanding, and vice versa (Sinatra et al., difficulty with both the understanding and the acceptance of evolutionary concepts. The graph shows the fold change in percentage of correct responses calculated following Avida-ED instruction (post-Avida). The mean fold change is 1.331. The table shows minimum and maximum values, 25% and 75% percentiles, median, mean, standard deviation, standard error, and lower and upper 95% confidence limits.

![Figure 5. Students’ (N = 125) fold change in performance between pretest and posttest questionnaires on a series of open-ended (OE) questions that tested their understanding of evolutionary principles. The graph shows the fold change in percentage of acceptable responses calculated following Avida-ED instruction (post-Avida). The mean fold change is 2.096. The table shows minimum and maximum values, 25% and 75% percentiles, median, mean, standard deviation, standard error, and lower and upper 95% confidence limits.](image)

**Discussion**

Evolution is one of the most difficult and controversial topics to teach in the biological sciences. Based on previous research concerning evolutionary instruction, it is thought that students have difficulty with both the understanding and the acceptance of evolutionary theory; and a greater degree of acceptance sometimes does not result in increased understanding, and vice versa (Sinatra et al., 2003). We examined whether the implementation of the innovative evolution simulation program Avida-ED, which allows students to manipulate organisms in an experimental setting, would increase and enhance introductory biology students’ understanding and retention of evolutionary concepts. Our results demonstrate significant increases in the ability of these students to recall and retain important evolutionary concepts.

These results are consistent with previously reported data on the efficacy of the Avida-ED activities in both a high school and a university/college setting (Lenski et al., 2003; Misevic et al., 2006; Clune et al., 2011; Grabowski et al., 2013; Zaman et al., 2014; Smith et al., 2016; Lark et al., 2018). While there were significant increases in the percentage of correct responses from pretest to posttest for both MC and OE questions, the most significant effect we observed was the increase in correct OE responses (26.08% improvement, compared to 10.30% improvement on MC questions).

The ten OE questions asked students to explain concepts rather than simply identify correct answers. The greater increase in acceptable OE answers may indicate that Avida-ED helps students gain a better ability to describe evolutionary processes. While Avida-ED also helped with the declarative knowledge tested by the MC questions, an integrated active-learning tool like Avida-ED may not be as effective in increasing comprehension at this lower level of thinking. Similar results have been found in other areas of biology education research, where active-learning tools such as flipped classrooms have increased deep learning but not surface learning (Lax et al., 2016). Future studies will have to investigate whether or not active-learning laboratory activities like Avida-ED help with long-term retention and understanding of the material over time.

The Conceptual Inventory of Natural Selection from which our pretest and posttest questionnaire is derived (Anderson et al., 2002) was written as a way to test many of the principles central to understanding the theory of evolution – specifically, how natural selection works. The concepts include biotic potential, carrying capacity, limited resources, limited survival, genetic variation, origin of variation, heritability of variation, differential survival, change in population, and origin of species. The questions are also written in such a way that the students do not have to be familiar with the particular jargon associated with the topic to successfully understand the content of the question. This means that students with no background could potentially score well on the pretest and posttest. However, one aspect of our questionnaires that was not investigated was how well students improved in their understanding of these different aspects of evolution by natural selection. Future studies will look into how, if at all, Avida-ED helps students improve in these different areas.

While our results demonstrate that students can benefit significantly from the use of Avida-ED, we acknowledge that our study has some limitations. One limitation is evident in the structure and makeup of our assessment tests. Both the MC and OE portions of the pretest and posttest questionnaire focused on basic, foundational principles of evolution such as which organisms are the most “fit,” how natural selection involves differences in traits, and how genetics/mutations may affect evolution. In order to more accurately measure the performance and understanding of our students,
investigate the semester-long impact of Avida-ED in bolstering evolution concepts. In this way, a future study could incorporate the performance of Avida-ED instruction at Thiel College, we are convinced that this two-week Avida-ED module produced significant increases in student understanding (the "practice" effect). However, given that this was the first time that these students had been exposed to college-level evolutionary instruction at Thiel College, we are convinced that this two-week Avida-ED module produced significant increases in student understanding.

In order to further elucidate the effects of Avida-ED instruction and validate these significant results, future studies should include a control/comparison group of students who do not receive the Avida-ED instruction. Furthermore, with the baseline results presented in this experiment, an opportunity arises to incorporate the performance of more advanced Avida-ED experimental modules in the curriculum. Specifically, this software could be used in a semester-long evolution course to allow students to comprehend more advanced, complex evolution concepts. In this way, a future study could investigate the semester-long impact of Avida-ED in bolstering evolutionary understanding and comprehension.

One other major limitation to this study is the fact that the pretest and posttest questionnaires do not address the question of acceptance of evolutionary theory. The rejection of evolutionary theory by a large proportion of U.S. adults is thought to involve factors that include a literal interpretation of the Bible, political views, and education status (Miller et al., 2006; Hokayem & Boujaoude, 2008; Plutzer & Berkman, 2008; Nelson, 2012; Newport, 2012; Pobiner, 2016). The immediate rejection of evolution by some students and their misconceptions about evolution present a problem to instructors who wish to accurately teach the concepts of evolution to undergraduate students. Some studies have observed a positive relationship between evolutionary understanding and acceptance (Rice et al., 2011; Akyol et al., 2012), while others have not observed the same positive association (Nehm & Schonfeld, 2007). Regardless, the goal of teaching evolution to undergraduate students is not simply to ensure that they understand this concept; we also hope they will discover for themselves that the evidence-based theory of evolution is the most plausible explanation that scientists have for describing the unity and diversity of life on Earth. Allowing students to see that there is scientific consensus and evidence behind seemingly controversial topics could lead to greater acceptance of other controversial ideas such as climate change and vaccines (Kudrna et al., 2013; Walker et al., 2017). In the future, specific assessment strategies and questions can be adapted from other studies that have investigated evolutionary acceptance (Lark et al., 2018), which will allow us to determine if there is a positive association between students understanding evolutionary theory and accepting it, following the use of Avida-ED.

Finally, it is easy for instructors to incorporate Avida-ED into any biology course, large or small. It has a simple, easy-to-use interface, a relatively straightforward learning curve, and, most importantly, it costs nothing. Additionally, after learning how to use Avida-ED in an introductory lab setting, students may then be able to use the program for more complicated lab assignments or for independent study projects in specific lab courses. Given that evolution is considered a significantly difficult concept to teach, the documented ability of Avida-ED to promote and enhance student understanding is a significant benefit for instructors and students alike. Based on our significant results obtained from the analysis of various introductory biology cohorts, it is logical to assume that a similar benefit can be obtained from the implementation of this simulation program in other upper-level evolution courses, including courses on human evolution and population genetics, or in an undergraduate independent research course for senior students. Furthermore, the accessibility and proven benefits of this software would make it ideal for high school instruction of evolution as well.
References

Abraham, J.K., Meir, E., Perry, J., Herron, J.C., Maruca, S. & Stal, D. (2009). Addressing undergraduate student misconceptions about natural selection with an interactive simulated laboratory. Evolution: Education and Outreach, 2, 393–404.

Akyol, G., Tekkaya, C., Sungur, S. & Tracey, A. (2012). Modeling the interrelationships among pre-service science teachers’ understanding and acceptance of evolution, their views on nature of science and self-efficacy beliefs regarding teaching evolution. Journal of Science Teacher Education, 23, 937–957.

Anderson, D.L., Fisher, K.M. & Norman, G.J. (2002). Development and evaluation of the conceptual inventory of natural selection. Journal of Research in Science Teaching, 39, 952–978.

Baker, J.O. (2013). Acceptance of evolution and support for teaching creationism in public schools: the conditional impact of educational attainment. Journal for the Scientific Study of Religion, 52, 216–228.

Bramsreiber, T.L. (2013). Evolving minds: helping students with cognitive dissonance. PhD dissertation, University of Denver.

Clune, J., Stanley, K.O., Pennock, R.T. & Ofria, C. (2011). On the performance of indirect encoding across the continuum of regularity. IEEE Transactions on Evolutionary Computation, 15, 396–367.

Dobzhansky, T. (1973). Nothing in biology makes sense except in the light of evolution. American Biology Teacher, 35, 125–129.

Grabowski, L.M., Bryson, D.M., Dyer, F.C., Pennock, R.T. & Ofria, C. (2013). A case study of the de novo evolution of a complex odometric behavior in digital organisms. PLoS ONE, 8, e60966.

Hokayem, H. & Boulouade, S. (2008). College students’ perceptions of the theory of evolution. Journal of Research in Science Teaching, 45, 395–419.

Kudrna, J., Shore, M. & Wassenberg, D. (2015). Considering the role of “need for cognition” in students’ acceptance of climate change & evolution. American Biology Teacher, 77, 250–257.

Lark, A., Richmond, G., Mead, L.S., Smith, J.J. & Pennock, R.T. (2018). Exploring the relationship between experiences with digital evolution and students’ scientific understanding and acceptance of evolution. American Biology Teacher, 80, 74–86.

Lax, N., Morris, J. & Kolber, B.J. (2016). A partial flip classroom exercise in a large introductory general biology course improves performance at multiple levels. Journal of Biological Education, 51, 412–426.

Lenski, R.E., Ofria, C., Pennock, R.T. & Adami, C. (2003). The evolutionary origin of complex features. Nature, 423, 139–144.

Mazur, A. (2009). Believers and disbelievers in evolution. Politics and the Life Sciences, 23, 55–61.

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

Misevic, D., Ofria, C. & Lenski, R.E. (2006). Sexual reproduction reshapes the genetic architecture of digital organisms. Proceedings of the Royal Society B, 273, 457–466.

Nehm, R.H., Poole, T.M., Lyford, M.E., Hoskins, S.G., Cannuth, L., Ewers, B.E. & Colberg, P.J.S. (2009). Does the segregation of evolution in biology textbooks and introductory courses reinforce students’ faulty mental models of biology and evolution? Evolution: Education and Outreach, 2, 527–532.

Nehm, R.H. & Schonfeld, I.S. (2007). Does increasing biology teacher knowledge of evolution and the nature of science lead to greater preference for the teaching of evolution in schools? Journal of Science Teacher Education, 18, 699–723.

Nelson, C.E. (2012). Why don’t undergraduates really “get” evolution? What can faculty do? In K.S. Rosengren, S.K. Brem, M.E. Evans & G.M. Sinatra (Eds.), Evolution Challenges: Integrating Research and Practice in Teaching and Learning about Evolution (pp. 311–397). New York, NY: Oxford University Press.

Newport, F. (2012). In U.S., 46% hold creationist view of human origins. Gallup Poll, June 1, 2012. https://news.gallup.com/poll/155003/Hold-Creationist-ViewHuman-Origins.aspx.

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

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

Plutzer, E. & Berkman, M. (2008). Trends: evolution, creationism, and the teaching of human origins in schools. Public Opinion Quarterly, 72, 590–553.

Pobiner, B. (2016). Accepting, understanding, teaching, and learning (human) evolution: obstacles and opportunities. American Journal of Physical Anthropology, 159, 232–274.

Rice, J.W., Olson, J.K. & Colbert, J.T. (2011). University evolution education: the effect of evolution instruction on biology majors’ content knowledge, attitude toward evolution, and theistic position. Evolution: Education and Outreach, 4, 137–144.

Sinatra, G.M., Southeterland, 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, 510–528.

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, article 9.

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, 415.

Walker, J.D., Wassenberg, D., Franta, G. & Colmer, S. (2017). What determines student acceptance of politically controversial scientific conclusions? Journal of College Science Teaching, 47, 46–56.

White, P.J.T., Heidemann, M., Loh, M. & Smith, J.J. (2013). Integrative cases for teaching evolution. Evolution: Education and Outreach, 6, 17.

Wiles, J. (2010). Overwhelming scientific confidence in evolution and its centrality in science education – and the public disconnect. Science Education Review, 9, 18–27.

Zaman, L., Meyer, J.R., Devangam, S., Bryson, D.M., Lenski, R.E. & Ofria, C. (2019). Coevolution drives the emergence of complex traits and promotes evolvability. PLoS Biology, 12, e1002023.

DELBERT S. ABI ABDALLAH (dabisabdallah@lecom.edu) is an Assistant Professor of Microbiology and Immunology and CHRISTOPHER W. FONNER (cfonner@lecom.edu) is an Assistant Professor of Physiology at Lake Erie College of Osteopathic Medicine, Erie, PA 16509. NEIL C. LAX (nlax@thiel.edu) is an Assistant Professor of Neuroscience, MATTHEW R. BABELL is a former undergraduate student in the Environmental Science Department, and FATIMATA A. PALÉ (Ipalet@thiel.edu) is a Professor of Biology at Thiel College, Greenville, PA 16125.