EVOLUTION IN ACTION IN THE CLASSROOM:
ENGAGING STUDENTS IN SCIENTIFIC PRACTICES TO DEVELOP A
CONCEPTUAL UNDERSTANDING OF NATURAL SELECTION

By

Wendy Renae Johnson

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ABSTRACT

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Public understanding and acceptance of the theory of evolution in the United States is not commensurate with its acceptance in the scientific community and its role as the central organizing principle of the biological sciences. There are a multitude of factors that affect student understanding of the theory of evolution documented in the literature including the proposition that understanding of evolution is intimately linked to understanding the nature of science. This study describes the development, implementation, and assessment of learning activities that address the process of natural selection and the scientific methodology that illuminates these mechanisms. While pre and post-test scores were higher for students in an Advanced Placement Biology course than students in a general biology course, similar learning gains were observed in both groups. Learning gains were documented in understanding the random nature of mutations and their importance to the process of natural selection, explaining selection as a competitive advantage of one variation over another type and specifically linking this to reproductive success, and in connecting inheritance, variation, and selection to explain the process of natural selection. Acceptance of the scientific validity of the theory of evolution as measured by the Measure of Acceptance of the Theory of Evolution (MATE) Instrument also increased significantly in both groups over the course of the school year. These findings suggest that the sequence of activities implemented in this study promote conceptual change about the nature of science and the process of evolution by natural selection in students.

DEDICATION

Kevin,
Thank you for making it possible for me to have it all.

ACKNOWLEDGEMENTS

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Chapter I: Introduction and Literature Review

The theory of evolution is the basis for a rich understanding of the unity and diversity of life and raises the study of biology from a collection of interesting facts to a truly amazing intellectual pursuit for uncovering the history of life on earth. Darwin's revolutionary notion of a branching tree of life connecting all species has not only been validated by evidence from every domain of the biological and earth sciences but has proven to be the most important organizing principle in biology. Dobzhansky's (1973) famous declaration that "Nothing in biology makes sense except in the light of evolution" eloquently summarizes the centrality of evolutionary theory in modern biology. 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 theory of evolution (Miller 2006). While barriers to learning exist in all scientific disciplines, evolutionary theory seems to be particularly difficult to comprehend and more likely to be rejected based on philosophical or religious reasons than other scientific theories.

In addition, preconceived ideas and misconceptions about genetics, adaptation, and the tree of life interfere with learning about evolutionary theory. Everyday experiences may lead to the development of misconceptions about science. "A significant disconnect between the nature of the world as reflected in everyday experience and the one revealed by systematic scientific investigation" (Gregory 2009) makes these misconceptions highly resistant to change. In addition to the misconceptions introduced by everyday experiences of the physical world, the nature of science itself is poorly understood. Only 42% of Americans demonstrated an understanding of scientific inquiry in surveys by the National Science Foundation in 2010 (National Science Board 2012). Misconceptions or lack of prior knowledge about the nature of

science and the process of scientific inquiry likely contribute to the concept-specific misconceptions held by students. Thus, an effective method for increasing student understanding of evolution must include learning activities that focus on both content knowledge as well as relevant scientific practices for studying the process of evolution. The hypothesis that student understanding of evolution is intimately connected to their understanding of the nature of science leads to the prediction that deep understanding of evolutionary theory requires competency in scientific thinking. This study describes the development and implementation of such learning activities and their effects on learning outcomes.

Scientists and professional science education associations are in agreement that biological evolution is a major unifying concept that should be required learning for all students. The National Association of Biology Teachers Position Statement on Teaching Evolution asserts that "biological evolution must be presented in the same way that it is understood within the scientific community: as a well-accepted principle that provides the foundation to understanding the natural world" (NABT 2011). The National Science Teachers Association Position Statement on the Teaching of Evolution adds "if evolution is not taught, students will not achieve the level of scientific literacy they need (NSTA 2003). The American Association for the Advancement of Science has also issued a statement on the teaching of evolution that calls evolution "one of the most robust and widely accepted principles in modern science" and asserts that "it is the foundation for research in a wide variety of scientific fields and, accordingly, a core element in science education" (AAAS 2006). The National Academy of Sciences argues that "to teach biology without explaining evolution deprives students of a powerful concept that brings great order and coherence to our understanding of life" (NAS 1998).

In recent years scientists and professional science education associations have worked together to develop curriculum frameworks for biology education at all levels throughout the K-16 continuum. The National Research Council's Next Generation Science Framework lists "Biological Evolution: Unity and Diversity" as one of the four "Core Ideas" of the life sciences that are "essential for a conceptual understanding of the life sciences and will enable students to make sense of emerging research findings" (NRC 2012). Similarly, the Advanced Placement Biology Curriculum Framework is organized around four "Big Ideas," the first of which is evolution (College Board 2011). The AAAS Vision and Change in Undergraduate Biology Initiative recognizes five "Core Concepts" for the discipline of biology with evolution named first (Vision and Change 2009). In addition to including evolution as a key concept, all three documents outline a set of scientific practices in which students should develop competency including asking scientific questions, designing and carrying out investigations, developing and using models, applying mathematics and computational thinking, and working with scientific explanations and theories. All three documents also speak to the importance of integrating scientific practices and content knowledge to foster deep conceptual learning at all levels.

Principles of Evolution

Although scientists are still uncovering all of the mechanisms of evolutionary change, there is consensus among experts that natural selection is the primary driving force of evolution. Thus, the curriculum frameworks for K-12 education all include Darwin's theory of evolution by natural selection as a major learning goal as well as learning goals related to variation, inheritance, and the role of natural selection in adaptation and generating the diversity of living organisms. While Darwin was not the first to propose the general idea of evolution, he was the

first to combine many observations about living organisms, geologic processes, fossils, and artificial selection to propose a workable mechanism for how it occurs. Other naturalists, including Alfred Russel Wallace, also proposed theories of natural selection, but Darwin's theory was developed earlier and was significantly better supported by observations such as artificial selection performed by animal breeders (Bowler 2009). Darwin's powerful image of a tree of life clearly illustrates the idea of common descent that was radical for his time, but his explanation of the process of natural selection responsible for the tree's branches was truly visionary given the intellectual and scientific culture of his period. Darwin explained the process of natural selection in the introduction to *The Origin of Species*:

> As many more individuals of each species are born than can possibly survive; and as, consequently, there is frequently recurring struggle for existence, it follows that any being, if it vary however slightly in any manner profitable to itself, under the complex and sometimes varying conditions of life, will have a better chance of surviving, and thus be naturally selected. From the strong principle of inheritance, any selected variety will tend to propagate its new and modified form (Darwin 1859).

Although Darwin correctly connected the natural variability he observed in populations to the branching tree of life produced by natural selection, he recognized that his lack of understanding of the [genetic] basis of variation and heredity was an important problem. Darwin's theory was not widely accepted until the modern synthesis of the 1940s because the mechanisms of variation and inheritance were not well known and the prevailing essentialist world view which viewed species as immutable (Mayr 2001).

The modern synthesis merged several branches of biology including Mendelian genetic principles and Darwinian evolution to explain how natural selection and genetic drift lead to the unity and diversity of life. One of the key developers of the modern synthesis, Ernst Mayr (2001), organized Darwin's theory of evolution by natural selection into five facts and three inferences, which are summarized in Figure 1.
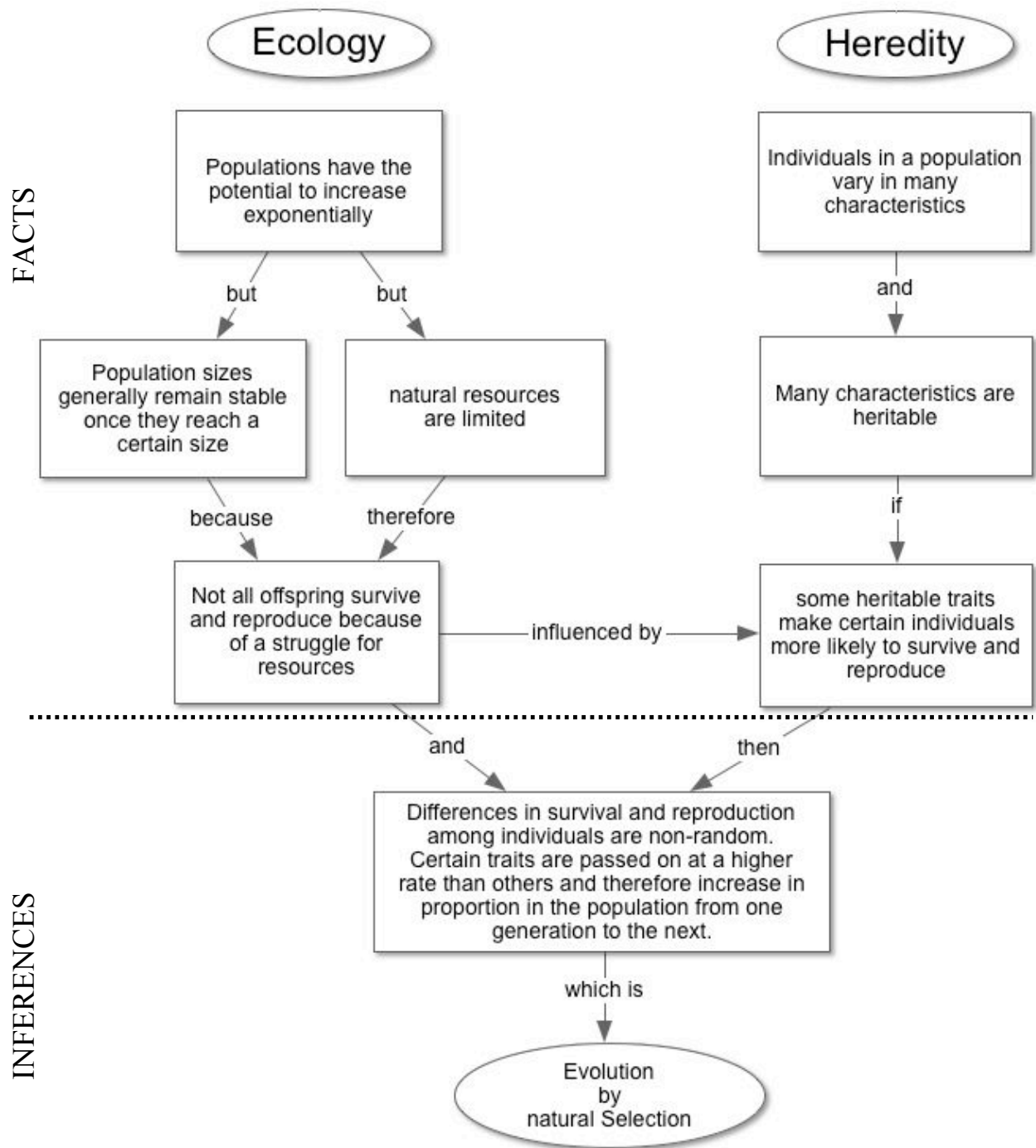
Figure 1. Darwin's facts and inferences based on works by Mayr (2001) and Gregory (2009).

Mayr emphasized populations rather than individuals and described natural selection as "a process of elimination" that concentrates the fittest phenotypes in a population. Additionally he argued that natural selection must be understood as a two-step process that involves the random production of variation through mutation and sexual reproduction and the nonrandom aspects of survival and reproduction.

Learning Challenges and Misconceptions

Darwin's theory, while elegantly simple, is notoriously difficult to fully comprehend. As Mayr's summary (Figure 1) demonstrates, explaining the theory of evolution by natural selection requires simultaneous attention to individual genotypes and phenotypes as well as population-level understanding. Internalizing Mayr's model requires a certain amount of statistical reasoning that can be challenging. Additionally, applying natural selection to speciation requires the integration of geological timescales, which is a barrier for many students. Many specific misconceptions regarding evolution have been described in the literature (e.g., Gregory 2009; Bishop and Anderson 1990), but even when these misconceptions are uncovered and targeted by instruction measured learning gains remain relatively low (e.g., Nehm and Reilly 2007; Bishop and Anderson 1990). These misconceptions often arise through common-sense-thinking and intuitive cognitions at the unconscious level and are thus very resistant to change. In addition, the cognitive biases and personal beliefs of students may also hinder learning about evolution. The Intelligent Design movement has reinvigorated the creation versus evolution debate in the United States, and certainly religious beliefs can sometimes hinder student learning of evolution. Many other factors such as misconceptions about the nature of science, confusing terminology, and students' dispositions also influence learning about evolution. Teachers' acceptance and

understanding of evolutionary theory along with the integration (or lack thereof) of evolutionary concepts within textbooks and curricula undoubtedly also play a role in student learning (e.g., Nehm et al. 2009; Meadows et al. 2000, Rutledge and Warden 2000).

The segregation in biology curricula that treats evolution as a discrete unit of study may hinder deep understanding of evolution as a unifying principle (McVaugh et al. 2010). While this curricular compartmentalization probably diminishes student learning of many biological concepts, its effect is likely heightened for a complex idea like evolution by natural selection. Additionally, since evolutionary theory is the unifying conceptual framework of modern biology, its segregation in the curriculum is probably especially detrimental to student learning. Kumala (2010b) argues that, "We describe and teach the strands of biology as disjointed units when we have a real opportunity to portray it as a cohesive discipline by building around an evolutionary framework." Content analyses of the most common introductory college textbooks have also demonstrated a segregation of evolutionary concepts from most other topics (Nehm et al. 2009), contributing to student misconceptions and leading to poor understanding of the theory and its central organizing role in biology.

In addition to the isolation of evolution concepts in curricula and textbooks, students sometimes feel that their religious beliefs are in conflict with scientific explanations of the origin of species. If students think that evolutionary theory threatens their religious beliefs they may actively chose not to learn about the topic (Meadows et al. 2000). Unfortunately, a vocal minority of the American public believes that science and theology are incompatible. Although much attention is given to religious beliefs in the creation versus evolution debate in the United States, it is also likely that some scientists perpetuate misconception about science and religion. By using evolution "to underpin claims about the nature of the universe, the meaning of it all for

us humans, and the way we should behave" (Ruse 2003), some scientists misrepresent science and confuse the public about its relationship to philosophical beliefs. Science is limited to natural explanations for the physical world. It is important that students understand that, "Science and religion ask fundamentally different questions" and that most scientists and religious leaders do not see any conflict between science and religion (AAAS 2006).

While there is much attention given to the effect of religious beliefs on acceptance and understanding of evolution in the United States, student dispositions such as motivation, emotions, and openness to new ideas are also important factors in their learning. Recent research has suggested that intuitive cognitions that occur on an unconscious level may have a significant impact on the acceptance of evolutionary theory. Intuitive feelings not based on logical reasoning may interact with logical reasoning in complex ways and lead to a "feeling of certainty that plays a moderating role in relationships among evolutionary knowledge and beliefs" (Ha et al. 2012). The work of Ha, Haury, and Nehm (2012) suggested that having an emotional feeling of certainty plays an important role in the acceptance of evolution. Their research supported the use of a theoretical model of factors influencing the acceptance of evolutionary theory that included level of education, knowledge, religion, and feeling of certainty.

In addition to emotional biases, students often hold cognitive biases about nature that make learning about evolution especially challenging. These cognitive biases include essentialist thinking that rigidly categorizes nature, teleological thinking that attributes purpose to nature, and the attribution of intentionality to natural events (Sinatra et al. 2008). Given these challenges it is not surprising that misconceptions about evolution are common among students and are highly resistant to change. "Learning about a complex process like evolution, which likely conflicts with students' prior experiences, background knowledge, and perhaps personal

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beliefs, most probably requires conceptual change. As changing conceptions is always difficult, we should expect that evolution will be difficult for many students to learn" (Sinatra et al. 2008).

Bishop and Anderson (1990) assessed students' conceptions of natural selection in a college nonmajors biology course and compared their responses to accepted biological theory. Although most students in the study believed that they "already had a basic understanding of the process of evolution by natural selection," before instruction, the researchers found that "their ideas about how and why evolution occurred were very different from those accepted by biologists." The work demonstrated that students failed to recognize the two separate aspects of natural selection (the source of variation versus the action of selection) and instead tended to believe that the environment itself causes traits to change over time or to be satisfied with an explanation that invokes a causal link between the need for a certain trait and its emergence within a particular species. Unlike biologists, the students did not employ population-level thinking; they failed to recognize the importance of variation within a population and often attributed evolutionary changes to gradual changes in individuals. Although the course included specific instruction that explicitly addressed these common misconceptions and was designed to help students develop more scientific explanations of natural selection, many students retained their original misconceptions. After instruction the percentage of students in the study who demonstrated a scientific understanding of natural selection increased from less than 25% to over 50%. Interestingly, the number of previous biology courses had very little effect on student understanding of natural selection. Bishop and Anderson concluded that "the concepts of evolution by natural selection are far more difficult for students to grasp than most biologists imagine."

Language appears to present a significant barrier to many students' learning of natural selection. Bishop and Anderson noted that students' conceptual misconceptions were reinforced by their unscientific usage of the terms "adapt" and "fitness." Gregory's (2009) literature review of common misconceptions about evolution also noted the importance of these two terms among others, and advocated a much greater focus on precision and accuracy in scientific language. For example, the term "natural selection" seems to personify nature and may contribute to the misconception that evolution is a goal-oriented process that inevitably leads to "more evolved" organisms like humans. Many words have different colloquial and cultural meanings than they do in science; the terms theory and hypothesis themselves are known to be associated with many misconceptions among students (Schwartz 2007). In evolutionary biology the problem seems to be exacerbated by a large number of specific terms that are used differently in everyday language than by biologists. In addition, experts often employ vague "forces" and "pressures" in their explanations of evolutionary change that students do not relate to natural selection. A study of undergraduate biology students found that references to evolutionary "pressures" were ubiquitous in textbooks, lectures, and student assessment responses, yet these "forces" were never defined. Approximately 20 percent of the test item responses by students included references to forces or pressures that act on organisms to cause adaptations, but when asked to explain their reasoning about evolutionary pressures their answers tended to be scientifically inaccurate or very shallow (Nehm et al. 2010). Shorthand explanations that employ force-talk or colloquial language that is not explicitly defined are commonly used by experts and likely contribute to student misconceptions. When students employ this language it may or may not suggest a misconception, but it is difficult for instructors to assess student understanding.

Clearly there are many factors that contribute to student misconceptions about evolution by natural selection. The low acceptance of the historical fact of evolution and a lack of formal education coupled with inaccuracies propagated by nonscientists are often blamed for low student understanding. However, numerous studies suggest that these reasons cannot fully explain why misconceptions about natural selection are so prevalent or so resistant to change (Gregory 2009). In addition to personal beliefs and experiences of students, teacher acceptance and understanding of evolutionary theory likely play a major role in student learning. One study of 552 Indiana public high school biology teachers (Rutledge and Warden 2000) showed that more than 20 percent of teachers were undecided or did not accept the main tenets of evolutionary theory, particularly its ability to be scientifically tested and the evolution of humans. Additionally the teachers scored an average of only 71 percent on a multiple-choice test about basic evolutionary concepts and demonstrated many misconceptions about the nature of science. Rutledge and Warden concluded that there were significant relationships between teacher acceptance of evolutionary theory and both their understanding of evolution and the nature of science. Their study indicates the need for increased teacher-education efforts in the areas of evolutionary biology and the nature of science.

Nature of Science and Learning Evolution

Although Rutledge and Warden's study confirmed that teachers' acceptance of evolutionary theory was significantly related to their understanding of evolutionary theory, many studies have failed to demonstrate this correlation between belief and understanding in students (Bishop and Anderson 1990, Sinatra et al. 2003, Shtulman 2006, Brem et al. 2003). However, other studies have shown a relationship between student understanding of the nature of science

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and their understanding of evolutionary theory (Lombrozo et al. 2008, Sinatra et al. 2003). The nature of science refers to the scientific practices of experimentation and observation, 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 and Ahlgren 1991). Unfortunately, teachers have often placed more emphasis on memorizing facts and concepts than on the nature of scientific inquiry that leads to that knowledge. This not only promotes misconceptions about the goals and processes of science, but likely hinders content learning, especially of complex concepts such as evolutionary theory that draw on many observations, inferences, and lines of evidence to explain phenomena.

"Science is a process of seeking natural explanations for natural phenomena" (AAAS 2006). While philosophers may still debate the boundaries of science from other types of knowledge, the essential commitment to methodological naturalism is well accepted among scientists (Pennock 2011). Additionally, although a single scientific method as simplistically portrayed by textbooks does not exist, there is a general consensus among scientists on the methods of scientific inquiry and among science educators on a set of learning objectives about the nature of science. These concepts include the durable yet tentative nature of scientific ideas, the limitation of science to studying natural phenomena, the distinction between laws and theories, and the relationships among history, society, technology, and science (McComas 1998). It is important that students develop an understanding of the process of science in order to participate in a society that is heavily reliant on technology and other applications of scientific knowledge. Unfortunately, students harbor many misconceptions about how scientific knowledge is generated and used and often their classroom experiences only serve to support those misconceptions. "Due to years of school science instruction and everyday out-of-school

experiences that have consistently conveyed, both explicitly and implicitly, inaccurate and simplistic portrayals of the nature of science, students carry deeply held misconceptions that rarely respond to implicit instruction that faithfully reflects the nature of science" (Clough 2006). Since learning is dependent on prior knowledge, science curricula must explicitly confront these misconceptions and supplant students' simplistic ideas about the nature of science with more accurate mental models. Engaging students in developing and testing hypotheses, analyzing and communicating data, and refining conclusions, while explicitly highlighting the differences between science and other ways of knowing, helps students to develop a more sophisticated understanding of the process of science.

"The best science teaching reveals not just the science of nature but also the nature of science. It is all very well and good for a student to learn the facts that science has discovered, but to do no more than that is to miss what is most important and distinctive about science, namely, its methods of investigation" (Pennock 2004). The theory of evolution provides an ideal context for teaching students about the nature of science. As students learn about evolution many important concepts about the nature of science can be seamlessly integrated including: the relationships among observation, experimentation, data, and inference, the development and testing of hypotheses, and the human dimension of scientific discoveries and theories. This type of contextualized learning of the nature of science is likely to help students to develop a more accurate understanding of the nature of scientific inquiry, and may also significantly increase their learning of evolutionary concepts. A historical approach that explicitly attends to the nature of science issues that influenced the development of the theory of evolution combined with an application of scientific inquiry that engages students in developing and testing hypotheses about the mechanisms of natural selection should significantly enhance student understanding of both

evolution and the nature of science. Consequently, this study involves the development and implementation of a curriculum that integrates nature of science instruction with the theory of evolution by natural selection and the assessment of subsequent student learning.

Learning Strategies

Biology teachers who expect to guide their students to a better understanding of evolution by natural selection certainly face many challenges given the prevalence of misconceptions and their resistance to change. In addition, students' intuitive biases, the apparent simplicity yet complexity of natural selection, the misleading portrayal of evolution by the media, the language of experts, low acceptance of evolution by the general public and attacks by creationists, and the isolation of evolutionary concepts in textbooks and curriculum all add to the challenge teachers face. Current understanding about how students learn makes it evident that teaching methods that rely heavily on lecture and fail to take into account students' previous knowledge and engage them in building new knowledge do not necessarily lead to deep understanding of complex ideas (Donovan and Bransford 2005). Additionally, there are many perceived barriers to demonstrating natural selection in the classroom due to the timescales and equipment typically required of evolution experiments that utilize living organisms. The goal of this study is to develop, implement, and assess the effectiveness of learning activities that deeply engage students in thinking about the nature of science and allow them to apply science practices to develop a conceptual understanding of evolution by natural selection. Thus careful attention is given to the historical development of Darwin's ideas, integration of evolution concepts throughout the school year, and laboratory activities that allow students to observe evolution in action while they develop and test hypotheses about natural selection.

Many active-learning strategies have been developed for teaching evolution; these include project-based learning (Cook 2009), group discussions and problem solving (Nehm and Reilly 2007), and case-study approaches (Stewart and Rudolph 2001), among others. Research has suggested that novice learning in evolutionary theory tends to be highly contextualized and students have difficulty building a conceptual understanding of evolution that they can apply to different situations (Nehm and Ridgeway 2011). Rather than focus on one particular learning strategy, this study incorporates many types of learning experiences spread throughout the school year to foster critical thinking and continual development of student understanding of the nature of science and evolution by natural selection. The learning activities have been adapted from a number of sources and apply many different pedagogical methods in order to foster conceptual change in student understanding of the nature of science and evolutionary theory. The novelty in the approach lies in the integration of evolutionary concepts throughout the school year as well as the specific laboratory activities, readings, and discussions that guide students in thinking critically about the historical development of evolutionary theory, the mechanisms that lead to evolutionary change, and the scientific practices that allow for these explanations.

Conceptual change is the goal of the learning activities developed in this study. This requires careful selection and sequencing of activities 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 process of science, scientific versus nonscientific explanations of phenomena, and the observations and questions that Darwin's theory addresses. These

discussions are enhanced 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 development of antibiotic resistance. Subsequent activities utilize Avida-ED, digital

evolution software that provides an instance of evolution that is easily studied in the classroom to

observe natural selection in action and develop and test hypotheses about the mechanism

(Pennock 2007). Since the user interface of Avida-ED represents digital organisms as analogous

to a colony of bacteria on a Petri dish, comparisons can be made directly between the bacterial

selection experiment and the digital evolution concepts. 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.

Avida is a research platform used by biologists and engineers to study the mechanisms of

evolution in order to apply those mechanisms to understanding biological evolution and to

engineering design problems (Ofria and Wilke 2004). 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 has previously been used in undergraduate biology courses

(Speth et al. 2009), but its use in high school classrooms has not been studied. The software

allows students to observe digital organisms similar to computer viruses that self-replicate and

are subject to random mutation during replication. The digital organisms are essentially short

computer programs defined by a sequence of fifty commands, which is roughly analogous to the

genome of a biological organism. The programming language includes twenty-six commands,

which are represented by the letters A – Z. The default organism is capable of replication only,

but random errors during replication substitute one command for another and can result in

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offspring with sequences that execute logic functions. The digital environment specifies rewards for certain logic functions, which increase the organism's computing power allowing for faster replication. The differences in the rate of replication among digital organisms leads to competition for limited space on a grid. Thus, all of the elements necessary for evolution by natural selection are represented: replication, variation of the programs due to random replication errors, and selection due to differences in reproductive success. Because digital organisms replicate much more rapidly than any biological organisms, the timescales for evolution are amenable to classroom study. "This is different from traditional evolution simulations used for research or education in that the populations of digital organisms are not constrained in how they adapt to solve problems… Because they are autonomous self-replicators, the digital organisms do not just appear to evolve; they actually do evolve in their digital environment" (Pennock 2007). Students can actively manipulate variables and observe the effects in real-time. Thus the software allows for deep learning of evolutionary concepts as well as nature of science concepts such as experimental design, data analysis, and drawing conclusions.

Chapter II: Methods

The curriculum developed for this study was implemented at Lansing Catholic High School in Lansing, Michigan during the 2011 – 2012 school year. The school is a private high school accredited by the Michigan Association of Nonpublic Schools. The student population is just under 500 students, and the average class size is 24 students. Ninety percent of the student population is white, and a majority of the students are members of the Roman Catholic Church. There is significantly more diversity in terms of socio-economic background and academic ability than racial or cultural diversity. The historical readings, *E. coli* selection experiment, and Avida-ED lessons were implemented in one section of an Advanced Placement (AP) Biology course with 20 students and five sections of a biology course with 113 total students. All students assented to the study and obtained parental permission for participation by signing a consent form (Appendix A). Nearly all of the students were juniors who had completed a physical science course during their freshman year and a chemistry course during their sophomore year. Although the AP Biology course covers many more topics and explores concepts in greater depth than the biology course, the learning activities developed for this study were implemented nearly identically in both courses. The AP Biology course included extension activities and lab reports that were not incorporated in the biology course. Tables 1 – 4 outline the topic sequence and major activities in both courses. The highlighted activities indicate those that specifically address evolutionary theory and the asterisks indicate those that were developed and assessed directly in this study. Evolutionary concepts were purposely integrated throughout the year to highlight the centrality of evolutionary theory in modern biology and to promote conceptual change in student ideas about evolution. Assessments were included at the beginning and end of each course as well as directly before and after the Avida-ED lessons.

Table 1. AP Biology Curriculum First Semester

Dates	Topics	Labs, Activities, Assignments
Aug 23 – 26	The process of science	Animal behavior lab Experimental design
Aug 29 – Sept 1	Biological inquiry & evolutionary theory	**Evolution NYT June 2011 Article** **Paley, Lamarck, & Darwin readings***
Sept 6 – 9	Chemistry of life	Properties of water lab *E. coli* **selection lab***
Sept 12 – 16	Organic compounds	Organic compounds activities *E. coli* **selection lab continued***
Sept 19 – 23	Cell structure	Cells lab; Acids, bases, and buffers lab; *E. coli* **selection lab continued***
Sept 26 – 29	Cell membrane	Diffusion & osmosis lab *E. coli* **selection lab continued***
Oct 3 – 6	Coupled reactions & enzymes	Rates of enzyme catalysis lab *E. coli* **selection lab continued***
Oct 10 & 11	photosynthesis	
Oct 17 – 21	Photosynthesis & respiration	Photosynthesis lab
Oct 24 – 28	respiration	Cell respiration lab *E. coli* **selection lab report**
Oct 31 – Nov 4	Cell cycle	Mitosis lab
Nov 7 – 11	Meiosis & introduction to genetics	Meiosis lab
Nov 14 – 18	Inheritance patterns	Fast Plants genetics lab
Nov 21 & 22 **No classes**	Human genetics	Chapter 12 self study and sex chromosomes online case study
Nov 28 – Dec 2	RNA and DNA structure and function	Bacterial transformation lab
Dec 5 – 9	Transcription & translation	Modeling gene expression
Dec 12 – 14	Nucleic acid technology & applications	Gel electrophoresis lab
Christmas Break	Gene regulation & population genetics	**"Kettlewell & the Peppered Moths"** **"Haldane & Population Genetics"**
Jan 4 – 6	Population genetics	**Population genetics lab**
Jan 9 – 13	Microevolution	**Digital evolution lab**
Jan 16 – 20	Speciation & macroevolution	**TCE Biodegraders Avida-ED activity*** **Video: "What Darwin Never Knew"**

Table 2. AP Biology Curriculum Second Semester

Dates	Topics	Labs, Activities, Assignments
Jan 24 – 27	Evolution and Biodiversity: prokaryotes, protists & fungi	Sordaria genetics lab
Jan 30 – Feb 3	Evolution and Biodiversity: plants & animals	**Phylogenetic trees activities** **Population genetics & evolution test**
Feb 6 – 10	Plant nutrition & transport	Transpiration lab
Feb 13 – 16	Plant reproduction & development	Flower and seed dissections
Feb 21 – 24	Animal tissues & organ systems	Animal tissues lab
Feb 27 – Mar 2	Nervous system	Neuron function activity
Mar 5 & 9	Skeletal and muscular systems	MME and ACT testing Chapter 35 self study
Mar 12 – 16	Endocrine system	
Mar 19 – 23	Circulatory system	Effect of temperature on Daphnia heart rate Heart dissection
Spring Break	Reading Assignment: **"Your Inner Fish" by Neil Shubin**	Make notes of connections to class content and interesting concepts for discussion
Apr 2 – 5	Immune system	**"Your Inner Fish" discussion** Blood types lab
Apr 9 – 13	Reproduction & development	Nova Video: *Life's Greatest Miracle*
Apr 16 – 20	Population Ecology Social Interactions Community Interactions	
Apr 23 – 27	Ecosystems	Aquatic primary productivity lab AP practice exam
Apr 30 – May 4	The biosphere Review for AP Exam	Go over the practice exam
May 7 – 11	Review for AP Exam	**Extra credit reading: *The Neverending Story: The Origin and Diversification of Life* by Marcus Kumala**
May 14	National AP Biology Exam Day	
May 15 – Jun 1	Projects	

Table 3. Biology Curriculum First Semester

Dates	Topics	Labs, Activities, Assignments
Aug 23 – 26	The process of science	Pillbug behavior lab
Aug 29 – Sept 1	Biological inquiry	Observation, data, and inferences readings and activities
Sept 6 – 9	Evolutionary theory	**Paley, Lamarck, & Darwin readings*** *E. coli* **selection lab***
Sept 12 – 16	Evolutionary theory	*E. coli* **selection lab continued*** Test 1: The science of biology
Sept 19 – 23	Biochemistry: chemical bonds	*E. coli* **selection lab continued*** Properties of water lab
Sept 26 – 29	Biochemistry: pH	*E. coli* **selection lab continued*** pH and buffers lab
Oct 3 – 6	Biochemistry: organic compounds	*E. coli* **selection lab continued*** Organic compounds activities
Oct 10 & 11	Biochemistry review & test	Test 2: Biochemistry
Oct 17 – 21	Energy & the environment	Food webs activity
Oct 24 – 28	Energy & chemical reactions	Calorimetry lab Test 3: Energy
Oct 31 – Nov 4	Enzymes & ATP	Enzyme lab Quiz: Enzymes & ATP
Nov 7 – 11	Cell structure	Cell structure microscope lab
Nov 14 – 18	Cell membrane & diffusion	Diffusion lab Test 4: Cells
Nov 28 – Dec 2	Photosynthesis	Light & pigments lab
Dec 5 – 9	Respiration	Quiz: Photosynthesis Floating leaf disk assay lab
Dec 12 – 14	Respiration	Test 5:Photosynthesis & Respiration
Christmas Break		Extra Credit: *The Immortal Life of Henrietta Lacks* by Rebecca Skloot
Jan 4 – 6	Multicellularity& homeostasis	
Jan 9 – 13	Transport systems in plants	Transpiration lab
Jan 16 – 20	Transport systems in animals	Circulation lab Test 6: Multicellularity & Transport

Table 4. Biology Curriculum Second Semester

Dates	Topics	Labs, Activities, Assignments
Jan 23 – 27	DNA structure	DNA extraction lab Video: Hidden Staircase
Jan 30 – Feb 3	The cell cycle & DNA replication	DNA replication lab Mitosis lab
Feb 6 – 10	Genetic coding	Test 1: DNA & the Cell Cycle
Feb 13 – 16	Transcription & translation	Gene expression simulation Test 2: Genetic Coding & Expression
Feb 21 – 24	Meiosis	Meiosis simulation lab
Feb 27 – Mar 2	Animal reproduction	Test 3: Meiosis & Reproduction
Mar 5 & 9	Viruses	MME & ACT Testing
Mar 12 – 16	Animal Growth & Development	Stem cells project
Mar 19 – 23	Human Development	Video: *Life's Greatest Miracle* Test 4: Animal Growth & Development
Spring Break		Extra Credit: *The Language of Life* by Francis Collins
Apr 2 - 5	Evolution of plants	**Video: "How to Grow a Planet" impacts of plant evolution on earth**
Apr 9 – 13	Plant reproduction & development	Flower and seed dissections Test 5: Plant Growth & Development
Apr 16 – 20	Introduction to genetics	Probability and practice problems
Apr 23 – 26	Mendelian Genetics	Sex chromosomes case study
Apr 30 – May 4	Non-Mendelian Genetics	Blood types lab Test 6: Genetics
May 7 – 11	Population genetics	PTC tasting lab
May 14 – 18	Evolution	**Digital evolution lab***
May 21 – 25	Evolution	**Digital evolution lab continued*** Test 7: Population Genetics & Evolution
May 29 – Jun 1	The "Modern Synthesis"	**Video: "What Darwin Never Knew"**

Analysis of Activities

1) Historical Development of the Theory of Evolution

Evolution was introduced early in the year as an explanation for the unity and diversity of living organisms and as a case study for the process of science. A set of activities was adapted from the lessons available online which were 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 class discussions regarding data, inferences, and scientific models including hypotheses and theories. These concepts were then applied as students read 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. Students were asked to note the general phenomena that each author addressed as well as the data and inferences that each employed in their explanations.

After reading and discussing each piece separately students were asked to compare the 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 and Darwin's in that it invoked a supernatural force as the mechanism for adaptation. Paley's work was discussed as an example of a philosophical explanation and contrasted with the testable explanations proposed by Lamarck and Darwin. Based on class discussion is appeared that students grasped the fundamental difference between the types of 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 observations about inheritable traits. As they considered Lamarck's explanation of acquired characteristics they recognized that most modifications made during an organism's lifetime would not be passed on to offspring. The class discussed 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 clearly define it. The acronym VIST, was introduced to elucidate the components of Darwin's model which includes variation, inheritance, selection, and time. 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 mechanism of natural selection, but they seemed mostly 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.

2) Bacterial Selection Experiment

Following the historical readings and discussion of scientific inquiry a bacterial selection experiment was introduced to the students. The similarities between the bacterial selection experiment and Darwin's artificial selection experiments were highlighted, as well as the relationship between the experiment and the evolution of antibiotic resistance in natural populations of bacteria. Students were not given a set of instructions, but rather the techniques were demonstrated and class discussions led the experimental design and data analysis in order to provide students with an authentic research experience. The experimental procedures, experimental results, and the pedagogical framework for the experiment are summarized below.

During the third week of school the disk diffusion antibiotic sensitivity method was introduced. 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 the distance from the disk. 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 worked in groups of four to streak the *E.coli* K-12 on an agar plate and then equally spaced three antibiotic disks on top of the plate using a set of pre-made antibiotic standard disks obtained from Carolina Biological Supply Company. The plates were incubated at 37º 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. Penicillin G (10μg) did not inhibit the growth of the *E. coli* very much, but inhibition of growth was observed with kanamycin (30μg), neomycin (30μg), novobiocin (30μg), streptomycin (10μg), and tetracycline (30μg) disks. The Groups compared their results and concluded that the size of the zone of inhibition is an indicator of the sensitivity of the *E. coli* to the particular concentration of the antibiotic tested.

After the demonstration of the assay, each group chose a particular antibiotic to use over the course of the next month to conduct an artificial selection experiment using the disk diffusion antibiotic sensitivity method. The biology students used the pre-made disks. The AP Biology students chose to use common chemicals in their environment including dilute concentrations of bleach, Dial soap, peroxide, and Penicillin G (2mg/mL). They impregnated sterile filter paper with the solutions by dipping them into a 10μl drop of solution. Students followed the same procedure as the demonstration by inoculating an agar plate with 0.1mL of the *E. coli* K-12 culture and spreading it over the surface of the agar and then equally spacing three disks on top

of the inoculated plate. The plates were incubated at 37ºC for 48 hours and then the zones of

inhibition were measured. The most resistant bacteria were selected for reculturing by swabbing

the clear areas near the disks with a sterile loop and swirling in a vial of nutrient broth. If the

zones were perfectly clear students were instructed to swab the clear area near the disks but to

also select the colony growing nearest to the disk to be sure that they had bacteria in their vial to

culture for the next round. The cultures were incubated at 37ºC for at least 24 hours and then the

assay was repeated using the selected bacteria. The students repeated the procedure three more

times, measuring the change in zone size and degree of growth within the zones for a total of

four rounds. In addition, during the last round students ran a standard by inoculating a plate with

the original *E. coli* culture for comparison.

Unfortunately a few groups did not complete all four rounds of artificial selection due to

contamination or other issues. Over the course of the experiment decreased zones of inhibition

were observed in the Kanamycin, Erythromycin, Penicillin G (2mg/mL solution), peroxide,

Chloramphenicol, bleach, and Dial soap treatments. The size of the zones decreased as well as

the degree of bacterial growth within the zones of inhibition. Students attempted to monitor the

"cloudiness" of the zones, which indicated limited growth of resistant bacteria, and also took

pictures of the plates after each round for comparison. The most dramatic decrease in sensitivity

was seen in the Penicillin G (2mg/mL) treatment as there were no discernable zones of inhibition

after four rounds of selection. Students were intrigued by the different results of each treatment

and enjoyed comparing results among groups.

The bacterial selection experiment was designed to give students an authentic experience

in scientific inquiry. They were excited to learn that the instructor had not tested many of the

antibiotics they were using and thus could not predict the outcome. Class discussions centered

around the fact that they were observing changes in the characteristics of a population over time, just as Darwin had done with his artificial selection experiments. The experiment was compared to the development of antibiotic resistance in natural bacterial populations and the importance of taking antibiotics exactly as prescribed to prevent the development of resistant populations was discussed. Although students enjoyed the experiment, it was unclear how well they understood the connection between the results of the artificial selection they observed and the process of natural selection. The AP Biology students seemed to grasp the conceptual context and had very few technical problems with the procedures. In the biology classes there was enormous variation among the groups in terms of their skill in handling the bacteria and analyzing the results. The biology students made many technical mistakes, which led to contamination and excessive use of materials, and even inadvertently started a small fire on the lab table while sterilizing an inoculating loop.

In addition to relating the bacterial selection experiment to the process of natural selection, many important considerations about the nature of scientific inquiry were directly illuminated in this activity. For example, discussions about the effect of the depth of the nutrient agar of the plates on the diffusion of the antibiotics led to a procedure for weighing the plates and assigning each group a narrow weight range of plates to ensure more consistent results between transfers. Multiple groups observed contamination on their plates, which prompted them to develop better sterile technique. The concept of a control treatment was elucidated during the last round when they recognized that comparing the zone sizes to the data they had collected weeks ago in the first round was not as reliable as inoculating a new plate because the conditions would be more similar (age of the plate, temperature of the incubator, their technique, etc.).

AP Biology students wrote a formal report about the bacterial selection experiment in which they explained their results and hypothesized about the phenotypic changes in the bacteria that could have led to the decreased sensitivity to the chemicals. Biology students did not write a formal lab report. The bacterial selection experiment was designed to engage students in scientific thinking and practices and to give them a shared experience in the scientific investigation of natural selection through the process of artificial selection. The experiment spanned five weeks and ran into the biochemistry unit. After the completion of the experiment, units on cells and genetics were presented and the bacterial selection experiment was not specifically incorporated. The bacterial selection experiment was purposely situated at the beginning of the year to attract student interest, apply science practices, and familiarize students with bacterial growth on a Petri dish because the Avida-ED software likens the growth of a population of digital organisms to bacteria on a Petri dish.

3) Digital Evolution Using Avida-ED (lesson plans Appendix B)

The Avida-ED software was introduced after a unit on population genetics and its relationship to evolution. The goal of the Avida-ED lessons was to link genetics concepts to the process of natural selection to help students better understand the mechanism. The students worked with Avida-ED for five class periods using a set of lessons (Appendix B) that had been designed to highlight the concepts of random mutation, inheritance, and unequal reproductive success in different environments while teaching the students to use the software. The lessons were divided into two parts. The first section explored individual digital organisms in Avida-ED and populations on the virtual Petri dish. The second section explored competition between digital organisms and the effect of the environment on populations.

Part I of the lessons began with students reading a *Discover Magazine* article called "Testing Darwin" which discussed the use of the original Avida software to answer questions about biological evolution (Zimmer 2005). Students were puzzled by the concept of digital evolution, but they were very receptive to using Avida-ED. The lessons were designed to allow students to work at their own pace. All instructions were included in a handout, which also contained discussion questions to answer for each section. In part I students examined the default digital organism's command sequence (a circular "genome" of 50 letters which do not code for any logic functions), watched it replicate and observed random replication errors occur, grew a population of digital organisms from the default organism, and examined the command sequences of evolved organisms in the population. Discussion questions brought attention to the learning objectives and challenged students to think about the processes they observed in Avida-ED. Students wrote their answers to the discussion questions as they worked with the software and then added to them during class discussions.

In part II of the lessons students placed the default organism and one of its evolved descendants from the population they had grown in part I into the same virtual Petri dish. They observed the competition for space between the two populations and the quick extinction of the default organism's descendants. The evolved organism replicated much faster because it was better suited to the default environment (i.e., it performed logic functions that were rewarded with increased processing power) and thus its descendants quickly out-competed the default organism's descendants for space on the dish. Next students explored the role of the environment by placing the same evolved organism that had out-competed the default organism in the default environment into an environment with no rewards (none of the logic functions would be rewarded with increased computing time and thus would provide no advantage). As

the population grew, random mutations knocked out some of the functions that the original organism was able to perform and new functions would appear by random mutation only to disappear later. This highlighted the random nature of mutations and the nonrandom nature of the selection pressures from the environment. The students followed this experiment by another in which only the functions that the evolved organism could not perform were rewarded. Again they observed the loss of functions in the population, but when a new function appeared it quickly increased in frequency in the population due to the reproductive success it conferred. Students collected data in a table for the number of organisms in each population at specific time points and the number of organisms performing each of the logic functions. At the end of part II they constructed a graph in Avida-ED of the population size over time and the average fitness value of the populations grown from the default organism in part I, the evolved organism with no rewards, and the evolved organism with limited rewards. Class discussion focused on the effect of the environment on the fitness (reproductive success) of individuals and how that affected the population over time. Students compared their graphs to those produced by other students and found that while each run was unique, there were discernable trends. For example, the average fitness of the population with no rewards showed little to no increase because there was very little advantage to any of the variation within the population. Additionally, students could directly correlate the spikes in the average fitness of the population subject to limited rewards on their graph to their data tables because they recorded which functions had appeared by random mutation at each point in time.

The AP Biology class was also assigned an extension activity (Appendix C) that asked them to develop and test a hypothesis for evolving a strain of bacteria that could perform a particular biochemical function using Avida-ED as a model. The students did the activity

outside of class and wrote a lab report about their experiment. The reports served as a formative assessment and were not included in the results of this study. Class discussions and writing prompts indicated that students in both courses enjoyed working with Avida-ED. However, there were some technical issues with the software related to saving their work and starting new experiments that frustrated the students, and a few students never seemed to become comfortable with the software. The AP Biology students were definitely more comfortable connecting what they saw in Avida-ED to biological processes, but the class discussions in all classes revealed that students were actively drawing connections between the digital organisms in Avida-ED and biological organisms.

Assessments

Two main pre and post assessments were administered in both AP Biology and in the biology class. One was used to gauge students' overall acceptance of the theory of evolution and its scientific underpinnings at the beginning and end of the course, while the other was a self-produced assessment that was used to assess their understanding of the process of natural selection. The pre and post assessments allowed for quantitative analysis and direct comparison of pre and post tests scores. Formative assessments throughout the historical readings, bacterial selection experiment, and Avida-ED activities consisted of ungraded written responses to questions and participation in class discussions. In addition, qualitative observations were noted throughout the year on unit exams, assignments, and class discussions, as well as on the AP Biology bacterial selection lab reports. These data were not analyzed quantitatively, but inform the conclusions of this study.

The Measure of Acceptance of the Theory of Evolution (MATE) is an instrument that was designed to assess teacher acceptance of the theory of evolution and has since been shown to be a reliable indictor of undergraduate acceptance of evolutionary theory (Rutledge and Warden 1999; Rutledge and Sadler 2007). This instrument was selected to assess student acceptance of evolution at the beginning and the end of the course because it includes concepts related to the nature of science as well as specific to evolutionary theory. The MATE is assumed to be appropriate for this group of students based on their age and reading comprehension levels, which are not far behind typical undergraduates. Because acceptance of the theory of evolution is based on both the facts and inferences that make up the theory and an understanding of the scientific methodology inherent in uncovering them, the MATE instrument is well matched to the goals of this study.

The MATE is a 20-item Likert-scale instrument in which each item requires students to indicate their degree of agreement or disagreement with a statement on a five-point scale. Six concepts are tested in the MATE instrument: the scientific validity of evolutionary theory (six items), the process of evolution (four items), evidence of evolution (four items), evolution of humans (two items), the scientific community's view of evolution (two items), and the age of the earth (two items). Scores range from a high acceptance of 100 to a low acceptance of 20. The MATE was administered to the AP Biology class in June 2011 when they picked up their summer assignment for the course and again at the end of May 2012. The biology students completed the MATE during the third week of school in early September 2011 and again at the end of May 2012.

The self-produced assessment was administered directly before and after students worked with Avida-ED. In AP Biology the pre and post-test consisted of seven open-ended questions.

Although the bacterial selection experiment was not specifically addressed during the Avida-ED lessons, it served as the context for six of the questions. The last question was intentionally broad as it was intended to uncover student conceptions about the general mechanism of natural selection. The pre and post-test administered in the biology class was similar in the concepts that it included, but differed from the AP Biology test in that it consisted of six multiple-choice questions that asked generalized questions about how natural selection acts on living organisms as well as the open-ended question about a different life form on another planet. The multiple-choice questions were adapted from the Conceptual Inventory of Natural Selection (CINS) instrument (Anderson et al 2002), but broadened so that they did not refer to a particular species. The questions addressed the source of variation among individuals, inheritance of traits, the types of changes that occur in populations over time, and the concept of fitness. The instrument has been validated with undergraduates, but literature on its use in high school courses is lacking. The CINS was assumed to be suited for the population of high school juniors in this study because of the academic abilities of the students and its similarity to other assessment tools used in the course.

Chapter III: Results

The AP Biology open-ended questions from the pre and post-test were scored according to a rubric (Table 5) that was developed by eliciting answers from a panel of professional teachers and scientists. The ideal response for each question was segmented into two or three critical components. Accurate and complete coverage of the critical component was awarded two points, while accurate but incomplete coverage of the critical component was awarded one point. No points were awarded for incorrect responses or those that did not address the specific critical component. Therefore each open-ended question was worth a maximum of four (two critical components) or six (three critical components) points. Because questions differed in their maximum score, percentages are used in reporting the scores for ease of comparison. The pre and post-test administrations of the open-ended questions were compared using a paired Student's t-test. The sample size for AP Biology was 16 students. Figure 2 shows the AP Biology pre and post-test scores for each question and the total for all seven questions.

*Asterisks indicate a significant difference ($p < 0.05$) between pre and post-test scores.

For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.
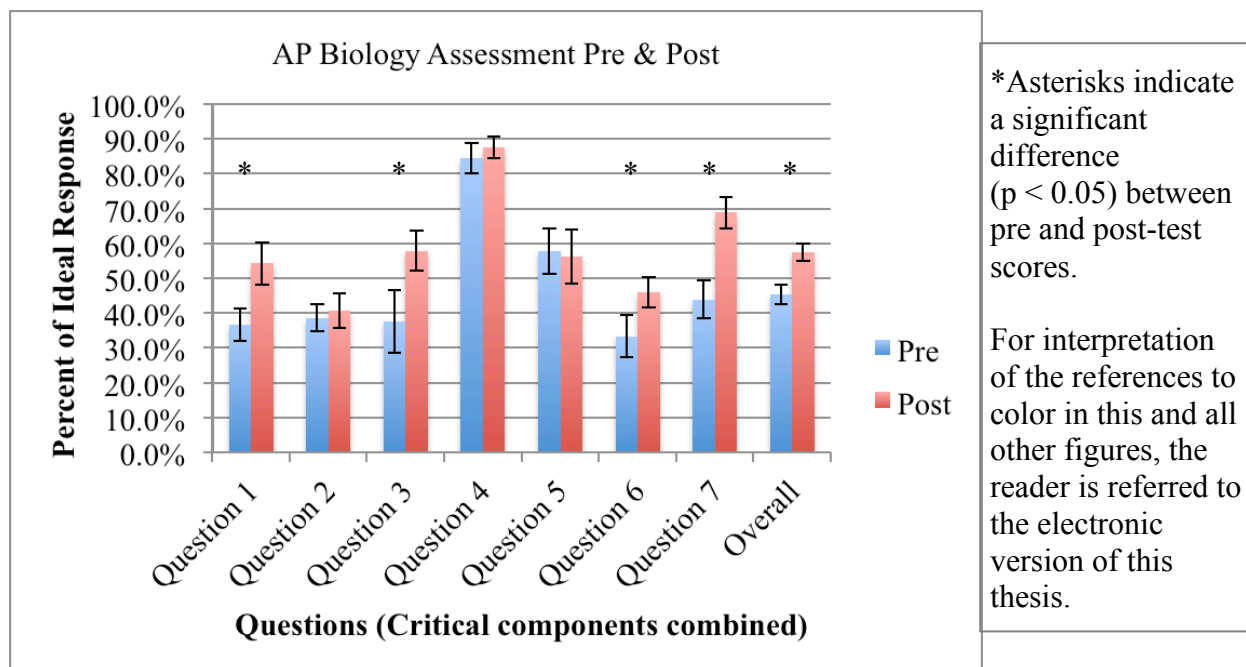
Figure 2. AP Biology pre and post-test scores (n = 16).

Table 5. AP Biology Assessment Questions Scoring Rubric.

Question	Ideal Response	Critical Component Accurate & Complete (2 points each)	Critical Component Accurate & Incomplete (1 point each)
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.	1. mutation	1. NA
		2. random replication error or plasmid	2. NA
		3. link gene to protein	3. mentions gene or DNA
2. Did the change first occur in one or many *E. coli* cells? Explain your reasoning.	The mutation first occurred in one cell because the zone around the disk was initially clear. The probability of the specific mutation occurring was relatively low, but once it occurred it was passed on to all of that cell's offspring creating a resistant colony.	1. one cell	1. NA
		2. zone initially clear	2. NA
		3. probability of a mutation	3. random mutation
3. Explain how this change in the *E. coli* led to the results that were observed (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.	1. competitive advantage or increased reproductive success	1. grow closer to disk (without comparison)
		2. frequency/proportion of resistance in population increased	2. a colony of resistant bacteria resulted
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 resistance gene changed over many generations. The decreased size of the zone of inhibition was a measure of the bacteria's sensitivity and is evidence of a change in gene frequency in the population.	1. yes	1. NA
		2. the frequency of the gene/trait increased over many generations	2. bacteria grew nearer to disk

Table 5 continued.

Question	Ideal Response	Critical Component Accurate & Complete (2 points each)	Critical Component Accurate & Incomplete (1 point each)
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.	1. same mechanism (nonrandom selection among traits)	1. random genetic mutation as in natural selection
		2. specifically chose resistant bacteria or speeds up change	2. some bacteria had a higher chance of survival
6. If you repeated the experiment many times using the same procedure and same antibiotic/antibacterial chemical 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.	1. resistance every time	1. more bacteria survive
		2. probability of mutation	2. timescale may vary
		3. multiple possible mutations	3. NA
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.	1. information that is copied	1. reproduce
		2. mutation	2. differences among individuals
		3. variations lead to differential survival/reproduction	3. competition in general

The maximum score on the AP Biology pre and post-test was 36 points. The total average score increased significantly ($p < 0.001$) from the pre-test score of 45.3% of the ideal responses to the post-test score of 57.3% of the ideal responses. For each of the seven questions the post-test scores were higher than the pre-test scores, but post-test scores increased significantly ($p < 0.05$) only on questions 1, 3, 6, and 7. The significant increases for these questions were due to higher post-test scores on specific critical components of the ideal response. Figures 3, 4, 5, and 7 show the AP Biology pre and post-test scores for the critical components of the ideal response. Because accurate yet incomplete responses were awarded one point and responses that were both accurate and complete earned two points, scores of 50% indicate basic understanding of the concept, while scores nearer to 100% indicate more sophisticated understanding of the concept. The questions were completely open-ended (students were not prompted to include specific components), so omitting a particular critical component does not necessarily indicate a lack of understanding of the concept.
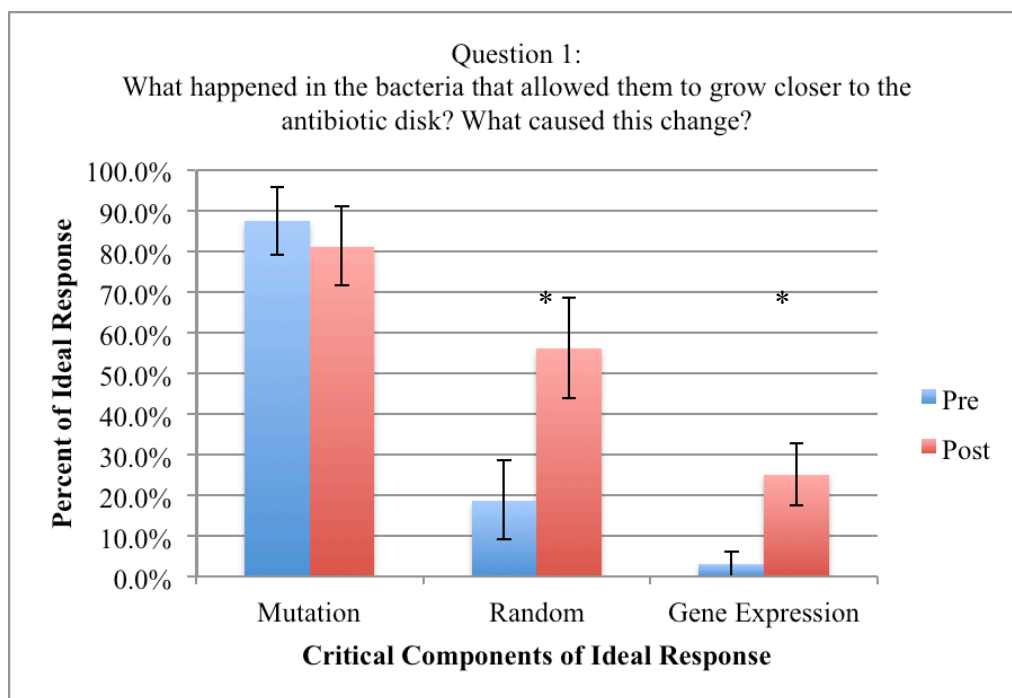
Figure 3. AP Biology assessment question 1 scores (n = 16).
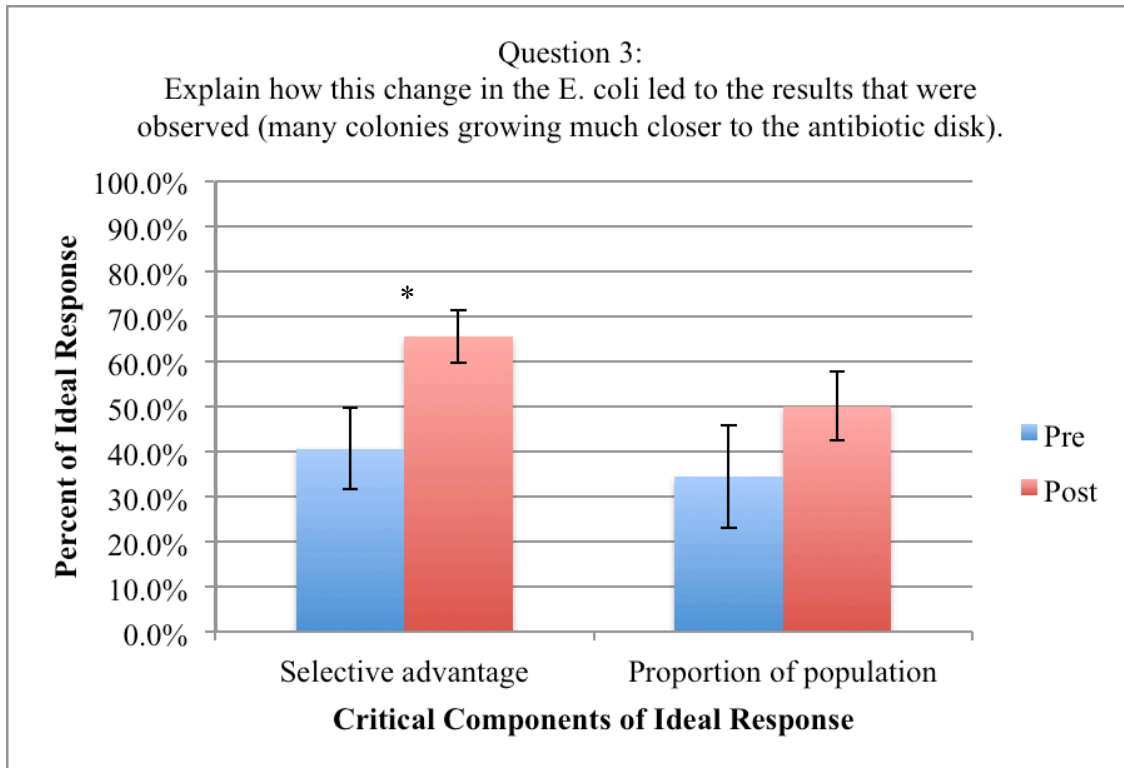
Figure 4. AP Biology assessment question 3 scores (n = 16).
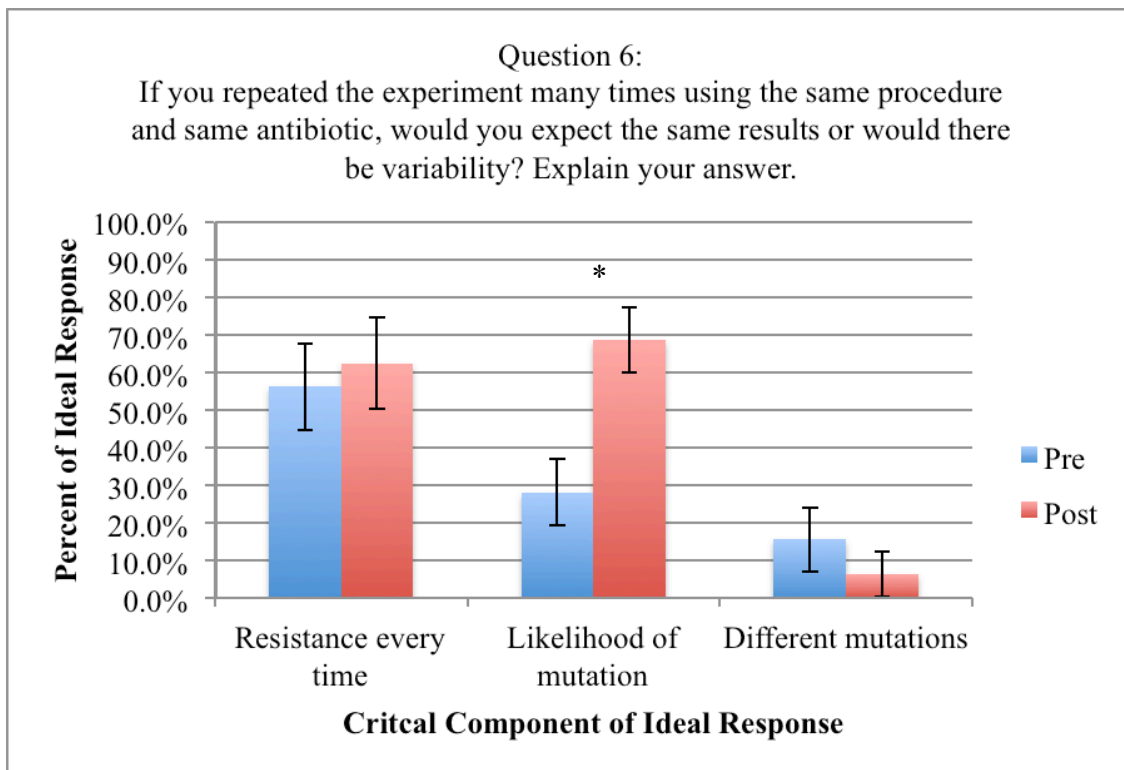
Figure 5. AP Biology assessment question 6 scores (n = 16).

The biology pre and post-test included six multiple-choice questions (Table 6) that were adapted from the Conceptual Inventory of Natural Selection instrument (Anderson et al 2002). These questions targeted important factors in the process of natural selection, but were not specifically aligned with the bacterial experiment or Avida-ED lessons. This assessment was administered in order to determine whether Avida-ED influenced student thinking about the main factors contributing to natural selection. Eighty-eight students completed both the pre and post-test; the results are displayed in Figure 6. The scores on questions 2 and 6 significantly increased from the pre to the post-test, while the percentage correct for question 3 significantly decreased as determined by a paired Student's t-test.

Figure 6. Biology multiple-choice scores (n = 88).

Table 6. Biology Multiple-Choice Assessment.

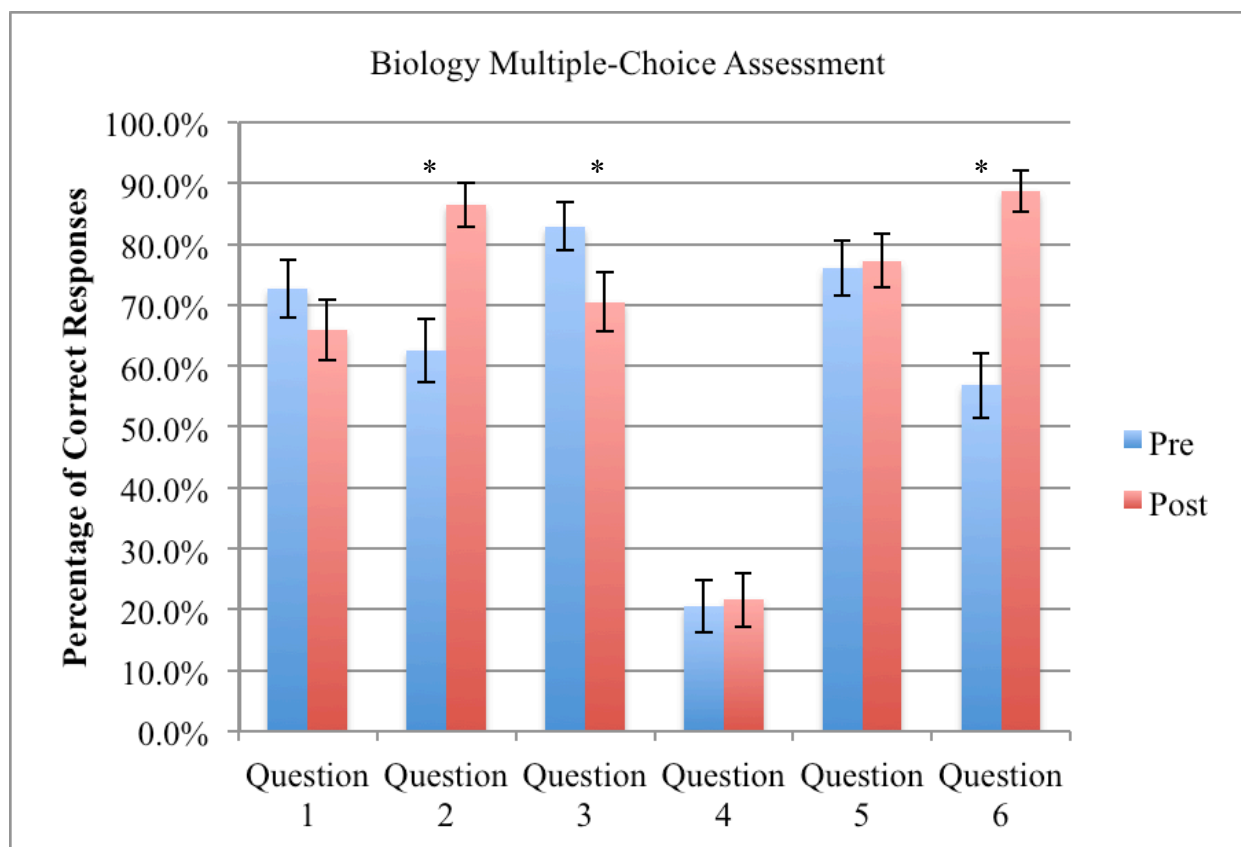| Question	Correct Choice	Choice A	Choice B	Choice C	Choice D
1. Individuals of a population differ in their physical and behavioral traits. Which statement best describes the organisms of a single isolated population?	D	the majority of the individuals share all the same traits but there are always a few that are different	the individuals share the essential traits of their species and the minor variations don't affect them	the individuals are identical on the inside but may differ slightly on the outside	the individuals share many essential traits of their species but also vary in many features
2. How do new traits arise in organisms?	B	new traits arise because organisms require certain characteristics to survive	random mutations in genetic information cause new traits to arise	changes in the environment cause specific mutations which lead to new traits	organisms change a little bit with each generation to more closely fit their environment
3. What types of traits are passed to an organism's offspring?	C	characteristics that an organism was born with and any that were acquired during its lifetime	characteristics that were beneficial in the environment that the organism lived	characteristics that were determined by the organism's genetic information	characteristics that were acted on by the environment
4. In an evolving population of organisms, what are the primary changes that occur gradually over time?	D	the traits of each organism within the population gradually change over its lifetime	successful behaviors learned by organisms are passed on to their offspring	mutations occur to meet the needs of organisms as the environment changes	the proportions of organisms having different traits within a population change

Table 6 continued.

Question	Correct Choice	Choice A	Choice B	Choice C	Choice D
5. The physical and behavioral characteristics of an organism influence what types of food it will be able to find and eat. Which statement best describes the interactions among the different organisms of a population and their food supply?	D	organisms with different traits will cooperate to locate food and share what they find	organisms with different characteristics fight for their food and the physically strongest will win	organisms rarely need to compete for food since they can always just eat something else	organisms that eat the same types of food will compete and not all of them will survive
6. Fitness is a term used by biologists to explain the evolutionary success of certain organisms. Which feature would a biologist consider to be most important in determining which organisms were the "most fit"?	C	physical strength	excellent ability to compete for food	high number of offspring that are able to reproduce	the ability to hide from predators and live for many years

Both the AP Biology and the biology pre and post-tests included question number 7 (Figures 7 and 8). Although AP Biology students scored higher than biology students, the scores on each critical component significantly ($p < 0.05$) increased for both groups from the pre to the post-test. A paired Student's t-test was used for AP Biology and an unpaired t-test for biology (due to differing pre and post-test sample sizes). The writing prompt for question 7 was:

> 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 your reasoning.
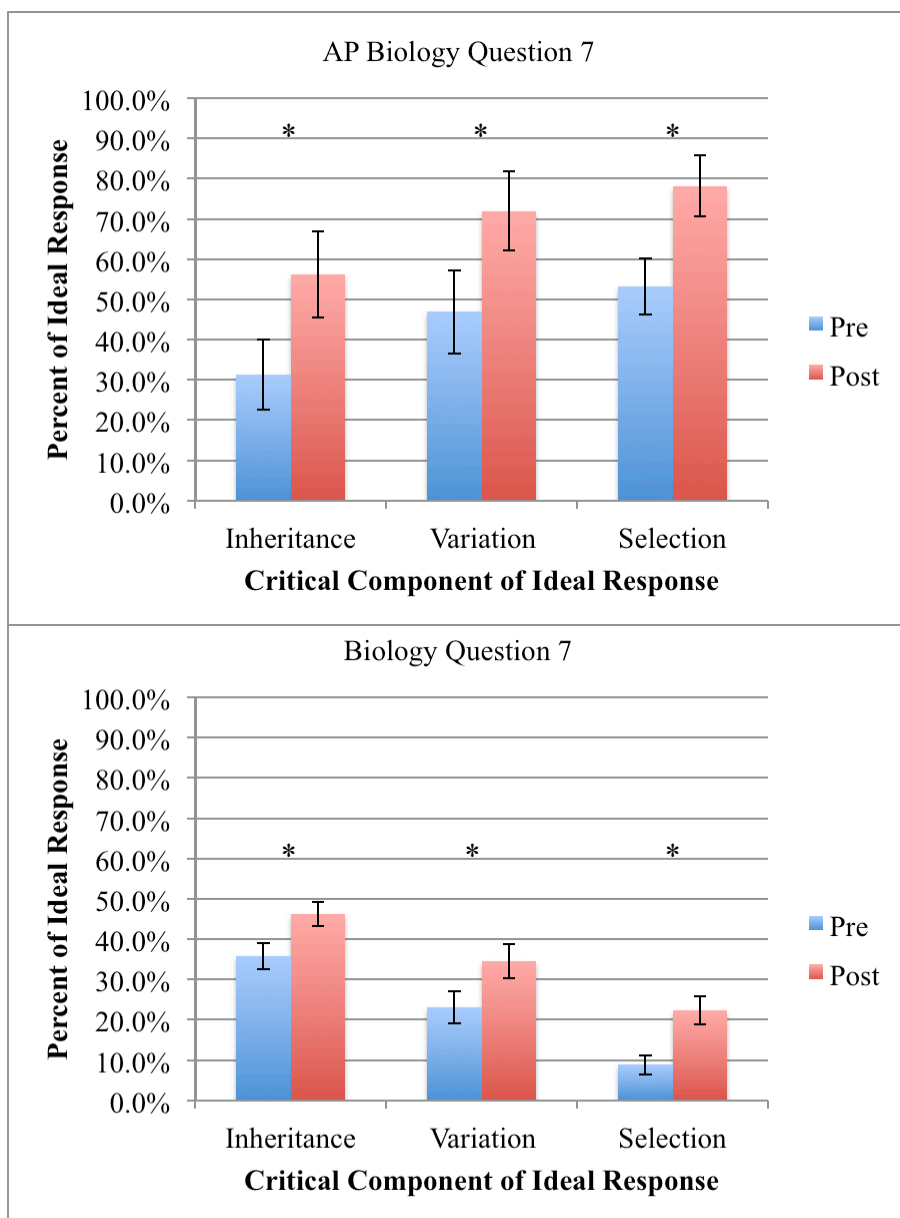
Figure 7. AP Biology assessment question 7 scores (n = 16).

Figure 8. Biology assessment question 7 scores (n pretest = 90, n post-test = 102).

The MATE instrument was administered in both the AP Biology and biology classes (Figure 9). Both groups showed significant increases ($p < 0.001$ paired t-test for AP Biology, unpaired for biology) in their acceptance of the theory of evolution over the course of the school year. Nineteen AP Biology students completed the MATE in June 2011 and May 2012. In the biology class 112 students completed the MATE in September 2011 and 102 of them took it again in May 2012. The average pre-test score in biology was 70.9 and the post-test average was 81.0 out of 100 points. The average pre-test score in AP Biology was 75.6 and the post-test average was 91.7 out of 100 points.
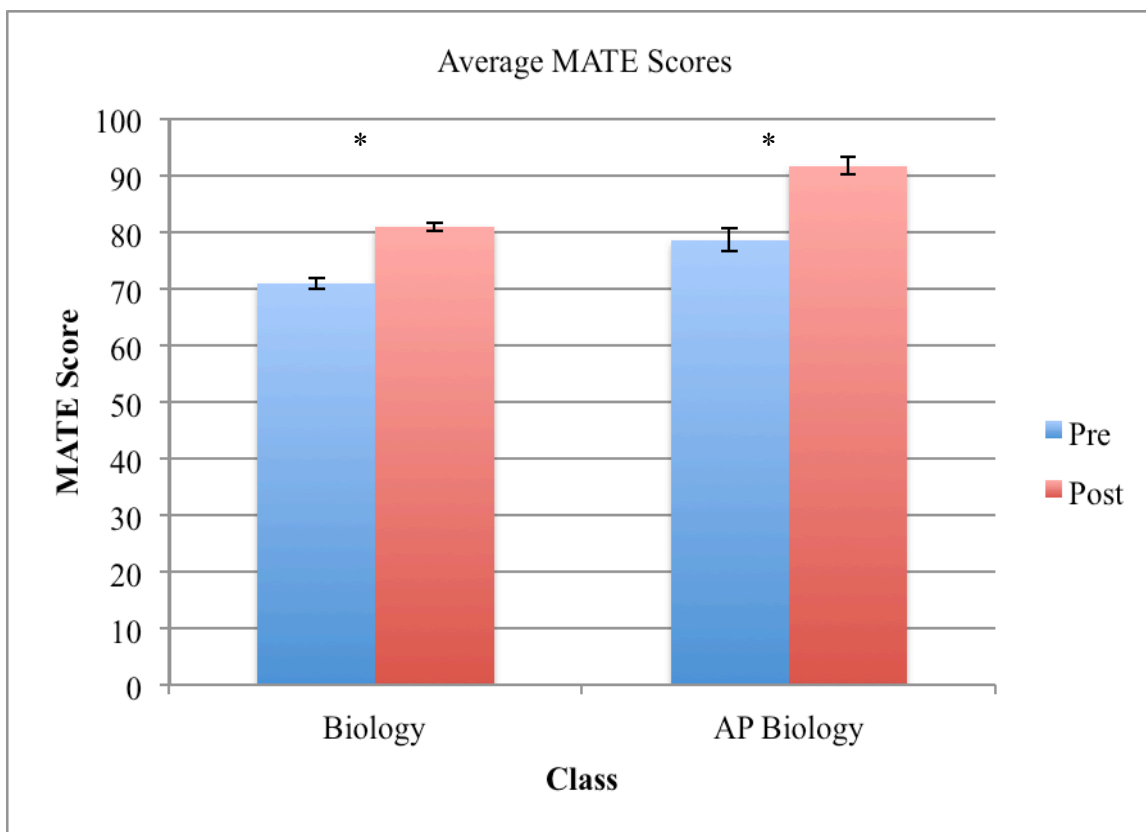
Figure 9. MATE pre and post-test scores
AP Biology (n = 19) and biology (n pretest = 112, n post-test = 102).

Chapter IV: Discussion

The curriculum implemented in this study was unique in that the activities explicitly addressed the nature of scientific inquiry and allowed students to observe the process of evolution by natural selection. The hypothesis that student understanding of evolution is intimately connected to their understanding of the nature of science led to the development of this curriculum in order to provide students with multiple contexts for learning and engage them in scientific practices. The combination of the bacterial selection experiment followed by the use of Avida-ED software has not been previously described, but was predicted to foster conceptual understanding of the process of evolution by natural selection because it actively engaged students in the practices of science to study it. The assessment results documented significant learning gains and increased acceptance of the theory of evolution which indicates that this curriculum has potential for increasing student understanding of evolution and the nature of science.

The students in both AP Biology and biology classes showed significant learning gains in their understanding of the process of natural selection on the pre and post-tests. Both groups demonstrated significant increases in their understanding of the role of random mutation in the process of natural selection as evidenced by the AP Biology students' increased scores on questions 1, 6, and 7 (Figures 3, 4, and 5) as well as the biology students' increased scores on the multiple-choice question number 2 and the open-ended question 7 (Figures 6 and 8). The increase in AP Biology scores on question 1 was due to students' increased reference to the random nature of mutations from 18.8% of the ideal response to 56.3% of the ideal response (Figure 3), even though slightly fewer mentioned mutation in general on the post-test. The increased score on question 6 (Figure 5) was due to the fact that students included a discussion of

the likelihood of a particular mutation occurring much more often on the post-test (68.8% of the ideal response) than on the pre-test (28.1% of the ideal response). Both groups mentioned the importance of the mutation of genetic information to the process of natural selection more often in question 7 (Figures 7 and 8) on the post-test (AP Biology 71.9%, biology 34.5%) than on the pre-test (AP Biology 46.9%, biology 23.1%).

Both groups of students also showed significant learning gains in understanding selection as differential success of organisms in their environment, especially as it relates to reproductive success. On the post-test AP Biology students more often discussed a specific competitive advantage over other organisms when explaining how the bacteria became less sensitive to antibiotics in question 3. Many students specifically named a reproductive advantage as the key factor causing change in the population in question 3 (Figure 4), increasing their scores from 40.6% of the ideal response on the pre-test to 65.6% of the ideal response on the post-test. Multiple-choice question 6 on the biology assessment was similar to question 3 on the AP Biology assessment in that it referred to the evolutionary success of organisms. The percentage of biology students correctly answering multiple-choice question 6 (Figure 6) increased from 56.8% on the pre-test to 88.6% on the post-test demonstrating that more students recognized the importance of reproductive success to the process of evolution.

Interestingly, biology students' average score on multiple-choice question 3 actually decreased from 83.0% correct on the pre-test to 70.5% correct on the post-test (Figure 6). The question asked, "What types of traits are passed on to an organism's offspring?" On the post-test as compared to the pre-test students more often chose option B which indicated that "characteristics that were beneficial in the environment that an organism lived" are passed on to their offspring. It is possible that the Avida-ED activities introduced this misconception because

students were asked to explain how new functions appeared and increased in the population. It is also likely that after learning about the mechanism of natural selection students were thinking about populations rather than individuals like the question asked. Because they had learned that organisms with beneficial traits are more likely to reproduce (as demonstrated by the significant increase in correct responses to question 6), they may have misapplied the same concept to question 3.

In the biology course the scores of the multiple-choice questions number 3, 4, and5 did not change significantly from pre to post-test (Figure 6). The fact that the averages for these three questions did not change much from pre to post-test suggests that these concepts were not addressed in the Avida-ED activities or students did not understand these concepts when they were addressed. Question 5 was not explicitly addressed in the Avida-ED activities, especially because the digital organisms compete for only space and not for food. Question 1 was indirectly addressed in the lessons as students examined individuals from an evolved population. Because the digital organisms are defined by a set of computer commands, the concept of variation within the digital organisms was rather abstract and may have been lost on the students. Question 4 asked, "In an evolving population of organisms, what are the primary changes that occur gradually over time?" The correct option is that "the proportions of organisms having different traits within a population change" was chosen by only 18 of the 88 students on the pre-test and by only 19 of the 88 students on the post-test. The option that the students most often chose was "mutations occur to meet the needs of organisms as the environment changes." This concept was explicitly addressed within the Avida-ED lessons, but it appears that it was not highlighted in a way that changed students' deeply held misconceptions. It is interesting that most students were able to correctly identify random mutations as causing new traits in question

2 and many mentioned random mutations in question 7, but then seemed to contradict that in question 4. It indicates that population-level thinking may be much more difficult for students than thinking about individual organisms.

The students in both AP Biology and biology were better able to connect the concepts of variation, inheritance, and differential success in an environment to explain the process of natural selection on the post-test. The scores for all three critical components of question 7 increased significantly in both groups, although both pre and post-test scores were significantly higher in AP Biology versus the biology classes (figures 6 and 7). The lower average scores in biology reflect differences between the students of the two groups in terms of academic abilities and previous science knowledge, but clearly show that both groups benefitted from the learning experiences. Importantly, both groups were able to transfer their knowledge of the process of natural selection from their experience within the digital context of Avida-ED to the new context of acellular life on another planet.

The similarities between learning gains of AP Biology students and biology students suggests that the activities in the curriculum used in this study promote the development of conceptual understanding of natural selection for students of many backgrounds and ability levels. It is important to note that none of the assessment questions were directly discussed between the pre and post-tests. The fact that students were able to more accurately answer questions about biological systems after using Avida-ED suggests that they were able to generalize from the digital system and transfer their understanding to biological contexts. Their ability to transfer their knowledge suggests that deep understanding was developed and 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

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conceptual change in student understanding of natural selection, especially in the AP Biology class where scores above 50% indicate a basic understanding of the concepts.

In contrast with the pre and post-tests addressing the process of natural selection, the MATE instrument more broadly assessed students' understanding 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 science and therefore is well matched to the goals of this study. Figure 9 shows that students in both groups significantly increased their acceptance of the theory of evolution as measured by the MATE. The average score in regular biology increased from 70.9% to 81.0%, while the AP Biology scores increased from 78.6% to 91.7%. As in the content scores, both the pre and post-test scores were considerably higher in the AP Biology class than in the biology class, but both groups showed significant increases suggesting that the activities promoted conceptual change in all ability levels and backgrounds. 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, while scores of 77 – 88 indicate high acceptance. The biology students thus started in the moderate acceptance range and moved into the high acceptance range. The AP Biology students started in the high acceptance range and moved into the very high acceptance range (89 – 100).

Observations throughout the school year also support the 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 with the same teacher did in previous years. Additionally, on the final exam in the biology class many students wrote that they finally

understood evolution and were not as concerned about it conflicting with their religious faith as they were before the course. 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 practices of science in both activities and throughout the course likely contributed to student learning.

Following the bacterial selection experiment with Avida-ED seems to be especially helpful for student learning because each activity informs the other. Through the bacterial selection experiment students saw that Darwin's idea was testable, and Avida-ED allowed them to interact and manipulate individuals and populations in a way that typical biological experiments do not. These activities engaged students in ways that traditional methods of teaching about natural selection through abstract simulations or explanations of how natural selection has acted on a particular trait (through reading, lecture, video, etc.) cannot. 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 predicted and the fact that Avida-ED was adapted from an actual research tool made their work seem more relevant. Future research is needed to delineate 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

The hypothesis that student understanding of evolution is intimately connected to their understanding of the nature of science leads to the prediction that deep understanding of evolutionary theory requires competency in scientific thinking. In order to help students develop

these competencies this study implemented learning activities that engaged students in explicit discussions of the nature of science as well as the practices of science including generating and testing hypotheses using biological and computer models and analyzing data to draw conclusions. The sequence of activities was specifically targeted to developing an understanding of the methods and goals of scientific inquiry and the process of natural selection, and especially how these two are related to each other. Because there are a multitude of documented misconceptions (Gregory 2009) which are highly resistance to change, this study incorporated many types of learning experiences spread throughout the school year to foster critical thinking and conceptual change in students' ideas about evolution and the nature of science. While pre and post-test scores were higher in the AP Biology class than in the biology class, similar learning gains were observed in both groups. Learning gains were documented in student understanding of the random nature of mutations and their importance to the process of natural selection, explaining selection as a competitive advantage of one variation over another type and specifically linking this differential success in an environment to reproductive success, and in connecting inheritance, variation, and selection to explain the process of natural selection. Acceptance of the theory of evolution and its scientific validity also increased significantly in both groups over the course of the school year. These findings suggest that sequence of activities implemented in this study promote students' conceptual change about the nature of science and the process of evolution by natural selection.

APPENDICES

APPENDIX A

Dear Students and Parents,

Over the past four years I have been studying effective ways to teach biology as part of my Masters in Biological Science degree program at Michigan State University and through multiple research projects that I have participated in. Additionally, I devoted the entire past summer to developing research-based curriculum for use in my biology classes. Throughout this school year I will be studying the effects of this curriculum on student learning. The results of this research will contribute to teachers' knowledge about the best ways to teach science topics and will be presented as my thesis to earn my master's degree.

The curriculum that I developed over the summer builds on what I have been doing in my biology classes over the last four years at Lansing Catholic High School. The course itself will not be significantly different, but I will be collecting data throughout the year to help me determine the effectiveness of my curriculum. I will be focusing on drawing connections that help students better understand the process of science and the interrelatedness of the topics we explore in biology. There are no unique research activities – participation in this study will not increase or decrease the amount of work that students do. I will simply make copies of students' work for my research purposes as well as statistically analyze student assessment data. The focus of this research is not on individual student's understanding, but rather on the effectiveness of the curriculum. Students' names will not be reported in my master's thesis or in any other dissemination of the results of this research. The data will consist of class averages and examples of student work that do not include names.

Participation in this research is completely voluntary. You have the right to say "no". You may change your mind at any time and withdraw. If either the student or parent/guardian requests to withdraw, the student's information will not be used in this study. There are no penalties for saying "no" or choosing to withdraw. On this form you will indicate your decision about whether or not to participate in this study and then turn in the form in a sealed envelope that will be stored in a locked filing cabinet until after grades are issued. Later I will analyze the written work only for students who have agreed to participate in the study and whose parents/guardians have consented.

If you have concerns or questions about this study, you may contact me (wjohnson@lansingcatholic.org or 517-267-2145) or my thesis advisor, Dr. Merle Heidemann (118 North Kedzie Lab, Michigan State University, East Lansing, MI 48824; heidema2@msu.edu; 517-432-2152 x 107). If you have questions or concerns about your role and rights as a research participant you may contact, anonymously if you wish, the Michigan State University's Human Research Protection Program at 517-355-2180 or e-mail irb@msu.edu or regular mail at 207 Olds Hall, MSU, East Lansing, MI 48824.

Please initial on the appropriate lines below to indicate your participation or nonparticipation in this study.

Student's Name: _____

_____ I voluntarily AGREE to participate in this study.

_____ I DO NOT wish to participate in this study.

_____   _____
                (Student Signature)   (Date)

Parental Consent:

_____ I give Wendy Johnson PERMISSION to use data generated from my child's work in this class for her thesis research. All data from my child shall remain confidential.

_____ I DO NOT wish to have my child's work used in this thesis project. I acknowledge that my child's work will be graded in the same manner regardless of their participation in this research.

_____   _____
            (Parent/Guardian Signature)   (Date)

I look forward to an exciting year! Please return this form to Mrs. Johnson sealed in the accompanied envelope. Thank you!

APPENDIX B

Introduction to Digital Evolution

Part I
1.1 Introduction to artificial life and evolution of digital organisms
Read the Discover Magazine article, "Testing Darwin" (Zimmer, Feb 2005). The article is freely available online at http://discovermagazine.com/2005/feb/cover.

Avida-Ed is a software program adapted from the Avida research software described in the Discover Magazine article. Both programs can be described as instances of evolution in a model environment. The evolution itself is real; the digital organisms are subject to the process of natural selection just as biological organisms are. For biologists the main advantages of using digital organisms are that the environment can be precisely controlled and manipulated and the fact that digital organisms reproduce much faster than any biological organisms. Avida-Ed was created to allow students the opportunity to learn about evolution by watching it in action. This powerful tool also allows students to design and perform their own experiments to test hypotheses about evolution in much the same way that researchers use Avida in the lab.

Questions to address about artificial life and evolution of digital organisms
1. Compare and contrast the digital organisms in the Avida environment to biological organisms in the natural world. In what ways are digital organisms similar to computer viruses?
2. What do biologists mean when they use the word "evolution"? Can we observe evolution? Can we experiment with evolution? Explain your answer and give examples from the article or prior knowledge.
3. What makes Avida a useful tool for biologists? What are the strengths and limitations of such an approach?

1.2 Using the Avida-ED software
Avida-ED is freely available for download at http://avida-ed.msu.edu/. Once it is installed on a computer the software can be identified by the Avida-ED icon that looks like a petri dish with colored dots. When the software is opened you will see a blank workspace preloaded with default environmental settings.

Quick Tour
Avida-ED has three panels
    1) Navigation (*selects the lab bench view*)
          Population – view organisms evolving
          Organism – view individual organisms
          Analysis – analyze results
    2) Freezer (*saved materials*)
          Configured dishes – settings / no organisms
          Full petri dishes – settings and organisms (saved by clicking the snowflake icon below
          the dish)
          Organisms – individual organisms (saved by dragging an organism to the freezer panel)
    3) Lab bench (*where things happen*)

To run an experiment drag an organism to the lab bench and click the play button below the
petri dish in the population view or the organism in the organism view. You can also choose
"run" under the control pull down menu at the top of the screen. To start a new experiment in
the population view, choose "start new experiment" under the control pull down menu.

1.3 Examining an organism
Big idea: Digital organisms are defined by a series of commands including instructions for
replication.
The Avida-ED program reads the "genome" of an organism and carries out the commands,
which are symbolized by letters. The default organism has a circular genome of 50 letters, which
includes the instructions for replication.

Questions to address while examining organisms
You may need to go back and observe replication a few times to answer these questions.
    1. How is the instruction set for an organism in Avida-ED similar to a bacterial genome?
    2. At which point of the genome did the program begin reading the instructions? How could
        you tell? Does the program always proceed through the instructions the same way?
    3. If you wanted to determine the function of each letter (command) of the code, where
        could you find that information? At which position of the genome are the instructions for
        replication?
    4. How was the organism's instruction set copied? How did the copy compare to the
        original using the default setting (0% per side mutation rate)?
    5. After you changed the per site mutation rate to 10% how did the copied genome compare
        to the original instructions? What would happen if a mutation occurred in the replication
        instructions?
    6. How did your new organism (replicated with the 10% mutation rate) compare to your
        neighbors' organisms? How do you account for any differences?

    a. Click on 'Organism' in the navigation panel.
       The workspace changes to an empty square with some buttons at the bottom.

    b. Drag the default organism (@ancestor) from the freezer panel to the workspace. *A circle of*
       dots should appear.

c. Note that the genome is circular and made up of colored letters. *Each of the letters is a specific command. Notice the proportion of the organism's instructions (nucleotide bases) that are tan colored c's (these are "no operation" commands and are essentially placeholders).*

d. Click the play button and watch the organism run through its code. *About a quarter of the way through the video, the circle starts to get bigger. The organism is copying its code one instruction at a time to the end of its instruction set. You can grab the slider and drag it more slowly to watch the process step by step.*

e. Click the "Flip to Settings" arrow in the top right corner. *Set the per site mutation rate to 10% by moving the slider or typing in the box. Click the arrow again to return to the genome view and then click play or drag the slider to watch the organism run through its code. After replication is complete compare your new organism to your neighbors'. **When manually changing the mutation rate look carefully at the placement of the decimal to verify you have set it to 10% and not 0.10%. You must press enter and verify that the slider actually moved to 10%.*

d. Save the new organism by hovering over the center of the circular genome to highlight it and then dragging it over to the freezer panel. *The program will ask you to first open an existing workspace or create a new one. Choose "create new workspace" and give it a name. Make sure you save the workspace file in a location (using the "where" pull down menu) where you can retrieve it later. You will then be prompted to "Enter Name of Organism to Freeze". Use any name you like and add 1.3 at the end (referring to section 1.3 of this tutorial so that you can easily identify it later).*

1.4 Growing organisms
Big idea: Digital organisms reproduce asexually but are not identical to their parent due to mutation.
Growing organisms in Avida-ED is similar to growing bacteria in a Petri dish. The virtual dish is divided into a grid where each box can hold one organism. Offspring may be placed in a box adjacent to their parent (default) or randomly on the grid.

Questions to address while growing organisms
1. What do we mean when we say that we are 'growing' Avida organisms? How is this similar to the growth of bacteria? How is it different than the growth of plants or animals?
2. There are nine different resources available in the default setting of Avida-ED. Why might you want to change these in future experiments?
3. What are the default mutation rate and world size? What are the maximum and minimum values for mutation rate and world size in Avida-ED? Why might you want to change these in future experiments?
4. What types of data can you collect in Avida-ED?
5. What do biologists mean when they use the word "fitness" in reference to populations? What does the fitness depend on and why?

6. Based on what you observed in the population statistics and the organism information boxes during the run, what do you think accounts for the increasing fitness of the population and specifically the organism you froze (saved)?

7. In Avida-ED fitness is defined as the metabolic rate divided by gestation. Metabolic rate refers to how fast an organism can execute instructions (letters of its genome) while gestation refers to the number of instructions it takes for an organism to reproduce. How does this compare to fitness in biological populations?

a. Click on "Population" in the navigation panel. *The lab bench changes back to the petri dish.*

b. Drag the default organism (@ancestor) from the freezer panel into the black space of the petri dish viewer. *In the drop down menu below the petri dish choose "fitness." You can adjust the zoom on your petri dish by clicking the arrows next to the zoom box below the petri dish. For now it is best to have the black region (which is the dish itself) filling the entire workspace.*

c. Click the flip to settings arrow in the top right corner of the workspace. *Set the per site mutation rate to 2.0 and the world size to 30 x 30 cells.*

d. Click the flip settings button to return to the petri dish view. *Push the play button below the petri dish and watch as the organisms start multiplying. As you watch them multiply notice that the information in the population statistics box and the graph change. When the dish looks full click on the play button again (which is now a pause button and will stop the growth in the petri dish). Each square on the grid represents an organism.*

d. Click on an organism (a square on the grid). *Its information appears in the organism information panel. Click on a few other organisms and notice how their information differs. You can click on an organism to see its information at any time during a run. Information on the population is displayed in the population information panel and the population statistics graph.*

f. Click the play button again and observe the dish and the population statistics boxes as the run proceeds. *You may also click on different individuals during the run to observe their characteristics in the organism information box. Pause the run when there have been about 1000 updates (unit of time for Avida-ED).*

g. Return to the population view and click the snowflake icon below the petri dish to save the population. *In the box, type a name for this population and add 1.4 to the end of the name.*

h. Click on several organisms, one at a time, to find an individual with a high fitness. *To do this you can use the fitness scale below the petri dish as well as looking in the organism info panel for each organism.*

i. Drag the organism with the highest fitness to the freezer panel. *In the box, type a name for this organism and add 1.4 to the end of the name.*

1.5 Examining an evolved organism
Big idea: Offspring of digital organisms are assigned their parent's fitness value, but are not identical to their parent.

Questions to address while viewing an evolved organism
1. How is the arrangement of the evolved organism's instructions different than the default organism's instructions? Are there any sections that are conserved (stayed the same from the ancestor)? Compare your evolved organism with your neighbors'. How similar are they?
2. Organisms in Avida-ED reproduce asexually similarly to bacteria. What caused the changes in the genome of the evolved organism from the ancestral organism?
3. Occasionally you will freeze an organism in Avida-ED only later to find out that it cannot make copies of itself. Based on what you know about the nature of random mutation how can you explain the fact that some organisms would be incapable of reproduction? What implications does this have for the future genetic information of the population?
4. In Avida-ED an organism inherits its parent's fitness. Knowing this, how certain are you of the fitness value of the particular individual you saved? What could cause an individual to have a fitness value different than its parents?

a. Click on 'Organism' in the navigation panel. *The workspace changes to Organism view.*

b. Drag your saved organism from section 1.4 from the freezer panel to the workspace. *Observe the proportion of the organism's instructions (nucleotides) that are tan colored c's.*

c. Verify that the mutation rate is 0% then click the play button and watch the organism run through its code.

d. If your saved organism does not make an exact copy go back to the evolved population (click population in the navigation panel) and choose another individual to freeze. Give this individual a different name so that you can distinguish it from the other saved organism. Then repeat steps a – c to examine your organism. Repeat this step until you have a saved organism that is capable of replication. **A viable organism will finish its replication at the end of the "video." If it keeps running through its genome over and over (with or without replicating) it has a deleterious mutation and will not perform well in future experiments.

Part II
In part I you observed the genomes (instruction sets) of individual organisms and grew a population of organisms from a single ancestor in Avida-ED. You saw that the default environmental settings include a mutation rate of 2% or 3% (depending on your version of the software) and nine possible resources available, but that each run was unique in terms of the organisms that evolved. Recall that Avida-ED is an instance of evolution in a model environment. Thus the software is not preprogrammed with specific results but instead allows the user to set initial conditions and observe the effects of natural selection acting on organisms of differing fitness. Remember that the fitness of organisms in Avida-ED depends on metabolic rate and gestation time. In part II you will explore the effects of the environmental resources on the fitness of organisms which leads to selection.

2.1 Competing two organisms
Big idea: Digital organisms that obtain and use more energy can reproduce faster.
Open the Avida-ED software and choose "open workspace" under the file menu. Open the workspace that you saved in part I. Alternately, if you are continuing on from part I click on "control" in the toolbar at the top and then choose "start new experiment."

Questions to consider while competing two organisms
1. What is the metabolic rate of the default organism (@ancestor) and what functions can it perform? What is the metabolic rate of your saved organism and what functions can it perform?
2. The metabolic rate is an indication of how fast an organism is able to execute the instructions of its genome. How do the functions that an organism performs influence its metabolic rate? How does this relate to the resources available in the environment (look back at the default environmental settings if necessary)?
3. After 10 – 20 updates how did the descendants of the saved organism compare to the descendants of the default organism in terms of fitness, functions performed, and population size (number of individuals)?
4. After a few hundred updates how did the descendants of the saved organism compare to the descendants of the default organism in terms of fitness, functions performed, and population size (number of individuals)?

5. Given that the default organism starts with a certain metabolic rate (even though it can't perform any functions) and that resources in the environment are unlimited (cannot be depleted), the competition between organisms is not for resources in the environment. What are the organisms competing for? Relate this to the petri dish grid and the world size settings. How does this compare to competition between biological organisms?
6. Did you observe extinction of one of the populations? Why or why not?
7. Explain how selection can account for the differences observed in the populations descending from the two different ancestors.

a. In the organism viewer drag the default organism (@ancestor) into the space and then press play. *As the organism's code is read watch the "function count" in the top right hand corner of the screen. Note the functions performed.*

b. Drag your saved organism (one that was able to replicate) from part I of the tutorial from the freezer panel into the organism viewer and press play. *As the organism's code is read watch the "function count" in the top right hand corner of the screen. Note the functions performed.*

c. Switch to the population view. Drag the default organism into the petri dish. Then drag your saved organism into the petri dish. *You should see two white boxes on the black background of the dish.*

d. In the pull down menu below the petri dish choose "ancestor organism" and click the play button and then immediately press it again to pause the run (do this as fast as you can to pause the run within 10 – 20 updates). *Notice that a color was assigned to each of the starting organisms in the ancestor view. Click on each organism (every square on the plate) and observe the fitness, metabolic rate, gestation time, and functions performed.*

e. Click play to continue the run. *As the population grows watch the population statistics box and note the functions that the organisms are performing.*

f. After a few hundred updates pause the run and save the populated dish as "competition".

2.2 Changing the Environmental Resources
Big idea: Only functions that are rewarded will be selected for in a population.
The environment settings include resources that are available to the organisms in Avida-ED. The resource names end in "–ose" because they are similar to sugars (i.e. glucose, fructose, lactose, etc.) that biological organisms use for food. When one of the nine specified functions is performed by a digital organism it is "using the resource" and is rewarded with an increased metabolic rate. You can highlight individuals on the petri dish performing a certain function by clicking on the function in the population statistics box. If you want to see which organisms are performing multiple functions you can choose multiple functions at once.

Experimental Procedure:
a. Start a new experiment and drag your saved organism into the new petri dish. Make sure you know which functions your saved organism can perform. If you are not sure check it by running it in the organism view before starting this experiment.

b. Flip to the settings and turn off all resources by unchecking the boxes.

c. Change the "Pause Run" setting from "manual" to "At 100 updates" by clicking the button next to "At" and typing "100" in the box.

d. Flip back to the petri dish view and make sure you are viewing fitness (using the pull down menu below the petri dish) and then click play.

e. Observe the population statistics box and the petri dish during the run. When the run pauses at 100 updates write down the functions that organisms can perform and the total population size in the data table titled "No Resources."

f. Flip back to the settings and change the settings to pause the run at 200 updates. Continue doing this every 100 updates until you have collected data for 1000 updates.

g. After 1000 updates save the populated dish (by clicking the snowflake) as "No Resources."

h. Start a new experiment and drag your saved organism into the new petri dish.

i. Flip to the settings and turn off the resources only for the functions that your saved organism is able to perform. Leave the boxes checked for rewards for any functions your organism cannot perform.

j. Repeat steps "e" and "f" as you did for the environment with no rewards and collect data every 100 updates for 1000 updates in the data table title "Limited Resources."

k. Save the populated dish (by clicking the snowflake) as "Limited Resources."

Data Analysis:
You are going to analyze your data in the Analysis Viewer of Avida-ED. It is also possible to export data to analyze in a spreadsheet or text file. In each view an "export" option appears under the "file" menu.

a. Change to analysis view by clicking the analysis button in the navigation panel in the top left corner of the screen.

b. Drag and drop your populated dishes (the one you saved in section 1.4, "No Resources" and "Limited Resources") into the blank space on your graph. You should have three lines on your graph (one named after your organism of high fitness, one called "no resources" and one called "limited resources." The key below the graph will show you which population each colored line corresponds to.

c. In the "variables" box under the graph select "average fitness" in the top drop down menu and select "thick" next to it.

d. In the variables pull down menu select "number of organisms" in the bottom drop down menu and select "thin" next to it.

e. Notice that you now have two different data sets on the same graph (average fitness labeled on the y-axis on the left and number of organisms labeled on the y-axis on the right). Under the file menu at the top choose "export graphics" and save your graph. Then import it into a word processing document and make a legend. You may want to print your graph to analyze later.

Use your data tables and graph to answer the following questions.

1. Compare the shapes of the population growth curves (thin lines) for the three different experiments. Did the populations reach their maximum world size in approximately the same number of updates for each experiment? Why or why not? What accounts for the similarities in the overall shape of the growth curves for each experiment?
2. Compare the shapes of the average fitness lines on the graph. Which one shows the sharpest increase? Would this pattern be the same for every trial?
3. Explain why the graph of average fitness for the "no resources" experiment looks the way that it does.
4. Compare the graph of average fitness for the "no resources" experiment to the data you collected in the table above. Which functions evolved in the population during the experiment? Did the functions that evolved have an impact on fitness? Why or why not?
5. Compare your graph for average fitness in the "limited resources" experiment to your data table. Can the data you collected account for the change in the shape of the line over time (updates)?
6. Did any functions increase in a population for a time only to decrease or disappear later? Explain how this could occur.
7. What do the differences in the shapes of the graphs of average fitness for the three different experiments suggest about the role of the environment in evolution?
8. Did any new functions appear in the population with limited rewards? What caused these functions to appear? Did they increase or decrease in frequency in the population over time? Why?

Conclusion

The process of natural selection is the main driving force of evolution. Natural selection is sometimes broken down into four main components: variation, inheritance, selection, and time (VIST). Explain how each of these components is represented in Avida-ED and how they interact to cause a population to evolve.

Functions Performed With <u>No Resources</u> in the Environment

Note: Since you started with one organism you should put a "one" in each box for the functions that your saved organism could perform, a zero for each function that the organism could not perform, and a "one" for total population in the "update zero" column in both data tables.

Function	Update Number										
	0	100	200	300	400	500	600	700	800	900	1000
Not											
Nan											
And											
Orn											
Oro											
Ant											
Nor											
Xor											
Equ											
Total Pop											

Functions Performed With <u>Limited Resources</u> in the Environment

Note: Since you started with one organism you should put a "one" in each box for the functions that your saved organism could perform, a zero for each function that the organism could not perform, and a "one" for total population in the "update zero" column in both data tables.

Function	Update Number
	0	100	200	300	400	500	600	700	800	900	1000
Not											
Nan											
And											
Orn											
Oro											
Ant											
Nor											
Xor											
Equ											
Total Pop											

Tutorial Discussion Questions Key
These questions and the suggested answers are meant to guide discussion, not as an assessment. It is recommended students answer each question to the best of their ability as they complete each section of the lesson while leaving space to modify and add to their answers during class discussion.

1.1 Questions to address about artificial life and evolution of digital organisms

1. Compare and contrast the digital organisms in the Avida environment to biological organisms in the natural world. In what ways are digital organisms similar to computer viruses?

Digital organisms in Avida have an instruction set similar to the genetic information that biological organisms have in their DNA. Like biological organisms, the instruction set codes for "traits" of an organism as well as replication instructions for reproduction. The genetic information can also be changed by random mutation. Digital organisms are similar to computer viruses because they are short self-replicating computer programs.

2. What do biologists mean when they use the word "evolution"? Can we observe evolution? Can we experiment with evolution? Explain your answer and give examples from the article or prior knowledge.

Evolution refers to change in a population over time (many generations). Modern biologists are specifically referring to change in gene frequencies over generations (whereas Darwin focused on changes in frequencies of traits in a population). We can observe evolution in biological organisms, especially organisms that replicate quickly such as bacteria and many simple plants and animals. The development of antibiotic resistance and the evolution of viruses are important examples. We can experiment with evolution using biological organisms, but we can also use software models such as Avida. The article gives many examples of evolutionary questions that Avida has addressed such as the study of the origin of complex features, the origin of sexual reproduction, and the future course of evolution.

3. What makes AVIDA a useful tool for biologists? What are the strengths and limitations of such an approach?

Avida has been summarized as "an instance of evolution in a modeled environment." Avida is less complex than biological systems and can be manipulated in ways that biological organisms cannot. All of the requirements for evolution by natural selection are present: variation (random mutation), inheritance (replication), selection (differential fitness), and time (many generations). Because digital organisms replicate much faster than biological organisms, data for many generations can be gathered quickly. In addition, the software makes it much easier to track changes in the "genetic" code of digital organisms than in biological ones. The same mechanisms are at work in Avida and in biological organisms, but the actual organisms and the environment are very different.

1.3 Questions to address while examining organisms

1. How is the instruction set for an organism in Avida-ED similar to a bacterial genome?

It is a circular set of instructions for replication and for the traits of the organism similar to the circular DNA of bacteria.

2. At which point of the genome did the program begin reading the instructions? How could you tell? Does the program always proceed through the instructions the same way?

The program began reading at the "r" command. It is at the three o'clock position on the circular genome. You could tell because the circle was outlined (as it runs through the code each letter is highlighted with this outline). The program always proceeds in the same direction (clockwise) through the genome.

3. If you wanted to determine the function of each letter (command) of the code, where could you find that information? At which position of the genome are the instructions for replication?

The boxes in the lower right corner of the organism viewer tell what each letter stands for. You can tell that the instructions for replication of the ancestor organism are in the one o'clock to two o'clock region of the genome because when the cursor reaches those letters it keeps reading them over and over and starts copying each letter of the genome into a new organism.

4. How was the organism's instruction set copied? How did the copy compare to the original using the default setting (0% per site mutation rate)?

As each letter is circled in blue it is copied to a new organism that is attached to the original organism. At the 0% per site mutation rate the new organism was identical to the original.

5. After you changed the per site mutation rate to 10% how did the copied genome compare to the original instructions? What would happen if a mutation occurred in the replication instructions?

The copied genome was not identical to the original. Some letters were substituted for different letters. If a mutation occurred in the replication instructions the new organism would not be capable of reproducing.

6. How did your new organism (replicated with the 10% mutation rate) compare to your neighbors' organisms? How do you account for any differences?

They were not the same. Everyone will see differences between the original and the new organism, but the location and exact substitutions will differ. Even the number of substitutions can differ (at 10% there is a one in ten change of an error as each letter is copied. The exact number of differences will range from one or two errors to more than ten since it is completely random).

1.4 Questions to address while growing organisms

1. What do we mean when we say that we are "growing" Avida organisms? How is this similar to the growth of bacteria? How is it different than the growth of plants or animals?

The digital organisms are replicating. It is the population that is "growing." Usually when we say that a plant or animal is growing we are referring to it increasing in size. The growth of digital organism is more similar to bacteria that do not increase very much in size, but reproduce quickly to form a colony of individuals.

2. Nine different resources are available in the default setting of Avida-ED. Why might you want to change these in future experiments?

You may want to test what effects the availability of resources has on the population.

3. What are the default mutation rate and world size? What are the maximum and minimum values for mutation rate and world size in Avida-ED? Why might you want to change these in future experiments?

The defaults depend on the version. They are either 2.0% or 3.0% mutation rate and 60x60 or 30x30 cells. You may want to test the effect of different mutation rates and world sizes in future experiments.

4. What types of data can you collect in Avida-ED?

You can collect data about individuals (the functions they can perform, their fitness, metabolic rate, and gestation time can be seen in the organism box of the lab bench. You can also save individuals and examine their instruction sets in the organism viewer). You can also collect data about the whole population in the population statistics box on the lab bench (i.e. population size, the number of organisms performing each function, and the average fitness, gestation, and age). The population data can be graphed in the analysis viewer.

5. What do biologists mean when they use the word "fitness" in reference to populations? What does the fitness depend on and why?

Fitness is related to how successful an organism is in its environment. Biologists define fitness in terms of reproductive success, because it is the number of viable offspring that really determine how the organism's traits will be represented in the future generations. Fitness depends on the survivorship of the organism in order to reproduce.

6. Based on what you observed in the population statistics and the organism information boxes during the run, what do you think accounts for the increasing fitness of the population and specifically the organism you froze (saved)?

The ability to perform functions increases an organism's fitness. When a mutation occurs that allows an organism to perform a function it is rewarded with more energy to replicate itself.

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Mutations can also decrease the gestation time, which allows the organism to replicate more efficiently and thus increases its fitness.

7. In Avida-ED, fitness is defined as the metabolic rate divided by gestation. Metabolic rate refers to how fast an organism can execute instructions (letters of its genome) while gestation refers to the number of instructions it takes for an organism to reproduce. How does this compare to fitness in biological populations?

Biologists define fitness in terms of reproductive success. It refers to the contribution that the organism has made to the gene pool. If an organism survives for a very long time but never passes on its genes its genes will not be represented in the population in the future. Therefore, reproductive success is the most important factor in fitness.

The calculation in Avida is very similar to the concept of biological fitness since the only things that influence the reproduction of the organism are how much energy it can utilize (metabolic rate) and how efficiently it replicates (gestation time). Fitness of the digital organisms is maximized by increasing the metabolic rate (i.e. by performing functions that are rewarded) and by decreasing the gestation time.

1.5 Questions to address while viewing an evolved organism

1. How is the arrangement of the evolved organism's instructions different than the default organism's instructions? Are any sections conserved (stayed the same from the ancestor)? Compare your evolved organism with your neighbors'. How similar are they?

There will be many differences in the order of the commands (letters) between the evolved organism and the ancestor organism. Students may notice that the instructions in the replication information (the one o'clock to two o'clock position of the genome) are more highly conserved that in other regions. The evolved organisms will each be unique due to the random nature of the mutations.

2. Organisms in Avida-ED reproduce asexually, similarly to bacteria. What caused the changes in the genome of the evolved organism from the ancestral organism?

Random errors during replication over many generations account for the differences between the evolved organism and the ancestor organism.

3. Occasionally you will freeze an organism in Avida-ED only later to find out that it cannot make copies of itself. Based on what you know about the nature of random mutation, how can you explain the fact that some organisms would be incapable of reproduction? What implications does this have for the future genetic information of the population?

If a mutation occurs in the replication instructions the offspring would not be able to reproduce. Those mutations cannot be passed and will thus not affect future generations (although they will keep popping up because of new mutations in the replication instructions).

4. In Avida-ED, an organism inherits its parent's fitness. Knowing this, how certain are you of the fitness value of the particular individual you saved? What could cause an individual to have a fitness value different than its parent?

Since fitness depends on reproductive success there is no way to calculate absolute fitness until an organism actually replicates (in which case you wouldn't be able to watch it in real time in Avida-ED). The software therefore assigns the fitness value of the parent to the offspring. It will often be a close approximation, but if a mutation has occurred in the replication instructions then the organism has a fitness value of zero (and it will not reproduce to pass on that particular mutation).

2.1 Questions to consider while competing two organisms

1. What is the metabolic rate of the default organism (@ancestor) and what functions can it perform? What is the metabolic rate of your saved organism and what functions can it perform?

The default metabolic rate of the ancestor is 0.2526 and it cannot perform any functions. Evolved organisms will have a higher metabolic rate and may be able to perform one or more functions.

2. The metabolic rate is an indication of how fast an organism is able to execute the instructions of its genome. How do the functions that an organism performs influence its metabolic rate? How does this relate to the resources available in the environment (look back at the default environmental settings if necessary)?

In the default settings all of the functions are rewarded, thus any organism's metabolic rate increases with each function that it performs.

3. After 10–20 updates, how did the descendants of the saved organism compare to the descendants of the default organism in terms of fitness, functions performed, and population size (number of individuals)?

There were more descendants of the saved organism and they had a higher fitness than the ancestor (which may not have replicated at all yet). Note: Sometimes the ancestor doesn't replicate at all because the evolved organism's metabolic rate is so much higher that a colony quickly forms and overwrites the ancestor organism.

4. After a few hundred updates, how did the descendants of the saved organism compare to the descendants of the default organism in terms of fitness, functions performed and population size (number of individuals)?

There were more descendants of the saved organism and they had a higher fitness value. Typically after a few hundred updates the ancestor population is "extinct" because the descendants of the evolved organism outcompeted the ancestor's offspring for space on the grid.

5. Given that the default organism starts with a certain metabolic rate (even though it cannot perform any functions) and that resources in the environment are unlimited (cannot be depleted), the competition between organisms is not for resources in the environment. What are the organisms competing for? Relate this to the petri dish grid and the world size settings. How does this compare to competition between biological organisms?

The organisms are competing for space on the grid. The faster they replicate they more descendants they leave behind (they new organisms begin to overwrite the older ones). Some biological organisms probably compete for space as well, but in the natural world they also compete for many other resources.

6. Did you observe extinction of one of the populations? Why or why not?

The ancestor population went extinct because they were not able to compete (for space on the grid) with the descendants of the evolved organism that could perform functions.

7. Explain how selection can account for the differences observed in the populations descending from the two different ancestors.

We would say that the ability to perform functions (the descendants of the evolved organism) was "selected for" since they had the advantage of increased metabolism. Selection refers to the competitive advantage that one type has over another which leads to its increased reproductive success. In nature we call this "natural selection" which refers to the increase in proportion of adaptive traits over many generations due to the reproductive success of those traits.

2.2 Use your data tables and graph to answer the following questions.

1. Compare the shapes of the population growth curves (thin lines) for the three different experiments. Did the populations reach their maximum world sizes in approximately the same number of updates for each experiment? Why or why not? What accounts for the similarities in the overall shape of the growth curves for each experiment?

The populations all reached the maximum world size eventually, although not always in the same number of updates. Therefore the growth curves all look similar in terms of their shape.

2. Compare the shapes of the average fitness lines on the graph. Which one shows the sharpest increase? Would this pattern be the same for every trial?

Results vary with different runs depending on which functions evolved because the increases in fitness are due to the appearance of new functions that are rewarded.

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3. Explain why the graph of average fitness for the "no resources" experiment looks the way that it does.

The trial with no rewards on will always show a flat line because fitness cannot increase much at all (only the gestation time may get slightly lower) if none of the functions are rewarded.

4. Compare the graph of average fitness for the "no resources" experiment to the data you collected in the table. Which functions evolved in the population during the experiment? Did the functions that evolved have an impact on fitness? Why or why not?

Functions appear due to random mutation but since they are not rewarded they do not increase an organism's fitness and thus are not acted on by selection (they do not increase in frequency).

5. Compare your graph for average fitness in the "limited resources" experiment to your data table. Can the data you collected account for the change in the shape of the line over time (updates)?

Students should compare their graph to the data tables they made in section 2.2. The changes in average fitness on the graph for their runs with no rewards and limited rewards can be directly correlated to their data tables by noting when (how many updates) a new function appeared and how quickly it increased in frequency in the population.

6. Did any functions increase in a population for a time only to decrease or disappear later? Explain how this could occur.

This is common in the run with no rewards because there is no advantage for performing a particular function. A function may appear (by random mutation) and increase in frequency as that organism reproduces, only later to decrease or disappear. This is because later random mutations "knock out" the function and there is no disadvantage for not having it. Functions that are rewarded will also occasionally appear, but because the number of organisms performing the function is low it may be lost when other organisms around it overwrite it.

7. What do the differences in the shapes of the graphs of average fitness for the three different experiments suggest about the role of the environment in evolution?

The environment does not cause certain traits to appear, but the environment determines whether a trait will be selected for (increase in frequency in the population over generations) or against (decrease in frequency in the population over generations). A trait that is advantageous in one environment could have no advantage (or even be harmful) in another environment.

8. Did any new functions appear in the population with limited rewards? What caused these functions to appear? Did they increase or decrease in frequency in the population over time? Why?

The appearance of new functions depends on the random mutations that occurred in the individuals.

Evolving TCE Biodegraders

Scenario:
Your school wants to buy an adjacent piece of property to build new athletic facilities. The property includes a large warehouse that has been used for various industrial purposes over the last fifty years. During the site inspection it was found that the soil and water around the warehouse have been contaminated by trichloroethylene (TCE), a hazardous chemical used as a spot remover in dry cleaning and as a degreaser for metal parts.

The school board has asked our class to team up with an environmental consulting company to help clean up the TCE so that our school can move ahead with purchasing and using the land. The environmental consulting company has informed us that current methods to remove TCE are expensive and require that contaminated soil be removed and disposed in hazardous waste dumps. The company is interested in spiking the soil with bacteria that biodegrade (break down) TCE. The class's goal is to use a model system (Avida-ED) to evolve a bacterial strain that can biodegrade TCE and present a protocol to the company. Your goal is to convince the company (using data) that your protocol is likely to lead to an efficient means of evolving TCE degrading bacteria. They can then use your recommendations to produce the bacteria that will allow the soil to be cleaned up on-site instead of dumped into a hazardous waste landfill.

Before going any further discuss the following questions:

1. What do you <u>know or think you know</u> about this problem?

2. What things do you <u>not know or need to know</u> about this problem?

Background:
Researchers use models to test hypotheses when an experiment would take too long to perform, be difficult to manage, or be too expensive to conduct. For this project we are using a model of bacterial organisms in a Petri dish (Avida-ED). In real life, bacterial populations can double as fast as every 30 minutes. In Avida-ED, doubling times are about one second.

Imagine that the "oro" function of Avida-ED degrades TCE ("oro" is an enzyme). Your job is to evolve organisms that make "oro". You must begin with the default organism (@ancestor) but you may change any of the environmental settings and you may use multiple different environments over the course of your experiment. Your goal is to determine the best procedure for evolving a population that most effectively metabolizes the "orose" resource in an environment with only "orose".

Experimental Design:

Design the experiment to answer the following question: **What conditions will lead to the evolution of the most efficient TCE degrading bacteria?** The following questions will guide you in designing your experiment. Make sure that you answer each question thoroughly (on paper, word processed, or in your lab notebook) before beginning your investigation.

1. What conditions (settings) might you change in order to increase the likelihood of the "oro" function evolving and maximizing the effectiveness at which the organisms degrade TCE? List as many possibilities as you can (these are potential hypotheses).

2. What conditions do you think have the best potential for solving the problem? Explain your reasoning.

3. A hypothesis is a proposed explanation for an observation. You just wrote your hypothesis in answering the preceding question. Now state it concisely in a sentence or phrase.

4. Hypotheses lead to predictions. Based on your hypothesis about the best conditions for evolving efficient TCE degrading bacteria your prediction is that the organisms will have a high fitness value in an environment with only the "orose" resource. Now you will state your hypothesis and your prediction together as an "if… then… statement" where the "if" refers to the hypothesis and the "then" refers to the results that you predict to observe. This should be a single sentence that concisely links your hypothesis (the "why") to the prediction (the "what") that follows the "if… then… format."

5. What data will you need to collect in order to test your hypothesis?

6. How many data points (number of replications, variables, etc.) will you need to be able to confidently accept or reject your hypothesis? Avida-ED is an instance of evolution in a model environment, which means that every run is unique. You must include at least five runs for each treatment to draw any conclusions.

7. Describe your experimental design. This should be a concise description of your methods (including settings, replications, data collection, etc.) that is clear enough for another group to replicate your experiment.

8. What are the dependent and independent variables and the controls? It is often helpful when determining what variables need to be controlled for to go back to the alternative possible explanations to your original question and think of how you will specifically rule those out in your procedure. Another type of control that is often essential is a point of comparison (a "no treatment" group).

9. How will you organize your data as you collect it? Make a data table to fill in during the investigation.

10. How will you present the data? Usually this is a graph or series of graphs. What type of graph will best display the pattern that you expect to show? What are the x (independent variable) and y (dependent variable) axes of the graph? It may be helpful to sketch a quick version of the graph that you would expect to see if your prediction were correct (you can compare your actual data to this later to see if it is consistent).

Data Collection:
For each run, write down the parameters you changed, the number of updates it took to evolve the "oro" function, and what proportion of the population performed the "oro" function and the maximum fitness values. During and after each run record observations that you think might be important, but that are not included in the experimental design. Use these observations to help you explain the results.

Data Analysis:
Display your results as graphs. Make sure to title each graph and label the axes.

Conclusion:
Write a description of how your results support or refute the hypothesis.

Proposal:
Based on the results of your investigation propose a protocol for evolving bacteria to degrade TCE. Prepare a brief presentation to the class to explain your proposal.

Discussion:
As a class evaluate the proposals and determine which protocol most effectively evolves efficient TCE degrading bacteria. Are there any unanswered questions? Your class may find that you have a protocol that you feel confident submitting to the environmental consulting company or you may decide that you need to run more experiments before doing so. It is important that you be able to present data in support of your protocol in order to convince them that your protocol is likely to lead to a feasible plan for evolving TCE degrading bacteria.

Evolving TCE Biodegraders Handout Key

The questions are meant to guide students in planning their experiment, and answers will vary depending on what variables the students choose to test. They may change any settings in Avida-ED including the mutation rate, offspring placement, world size, etc. The instructor may wish to limit students to changing only one variable at a time, or may leave the question more open-ended and allow students to test protocols that involve changing many variables. Students may choose to evolve organisms in several different environments (by saving the organisms and then placing them in a new dish). Their hypothesis must be testable in Avida-ED and they should use observations and data to explain their results. Instructors may wish to check each group's hypothesis before they begin and may need to guide students in relating their manipulated variables to an explanation of the effects on the organism's ability to efficiently metabolize TCE ("orose"). The best indicator of the organism's ability to metabolize TCE is its fitness value in an environment with only TCE.

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