BiteScis | EDUCATOR

Evolution and *E. coli*

Natural Selection in a Stable Environment

Purpose

This lesson is meant to be used early in the evolution unit. Students explore bacterial evolution in a stable environment, which counters the intuitive misconception that environmental change is a necessary component to natural selection. A landmark study provides the backdrop against which students can challenge their thinking about what it means for a population to evolve.

Audience

This lesson was designed to be used in an introductory high school biology course.

Lesson Objectives

Upon completion of this lesson, students will be able to:

- predict the fate of different mutations depending on the type of mutation and the context in which the mutation arises.
- explain that mutations and natural selection happen, even in a constant, stable environment.
- differentiate between types of mutations and be able to explain why the appearance of a mutation does not always guarantee its longevity.

Key Words

adaptation, allele, allele frequency, evolution, fitness, fixation, mutation, natural selection

Big Question

This lesson addresses the Big Question "*What does it mean to observe?*"

Standard Alignments

- **Science and Engineering Practices**
  - **SP 4.** Analyzing and interpreting data
  - **SP 6.** Constructing explanations (for science) and designing solutions (for engineering)
- **MA Science and Technology/Engineering Standards (2016)**
  - **HS-LS4-2.** Construct an explanation based on evidence that Darwin's theory of evolution by natural selection occurs in a population when the following conditions are met: (a) more offspring are produced than can be supported by the environment, (b) there is heritable variation among individuals, and (c) some of these variations lead to differential

fitness among individuals as some individuals are better able to compete for limited resources than others.

- **HS-LS4-5.** Evaluate models that demonstrate how changes in an environment may result in the evolution of a population of a given species, the emergence of new species over generations, or the extinction of other species due to the processes of genetic drift, gene flow, mutation, and natural selection.

- **NGSS Standards (2013)**
  - **HS-LS4-2.** Construct an explanation based on evidence that the process of evolution primarily results from four factors: (1) the potential for a species to increase in number, (2) the heritable genetic variation of individuals in a species due to mutation and sexual reproduction, (3) competition for limited resources, and (4) the proliferation of those organisms that are better able to survive and reproduce in the environment.
  - **HS-LS4-4.** Construct an explanation based on evidence for how natural selection leads to adaptation of populations.
- **Common Core Math/Language Arts Standards**
  - **CCSS.ELA-LITERACY.RST.9-10.1.** Cite specific textual evidence to support analysis of science and technical texts, attending to the precise details of explanations or descriptions.
  - **CCSS.ELA-LITERACY.RST.9-10.7.** Translate quantitative or technical information expressed in words in a text into visual form (e.g., a table or chart) and translate information expressed visually or mathematically (e.g., in an equation) into words.

Misconceptions Addressed

- This lesson addresses many common misconceptions about evolution, including:
  - Evolution only occurs when there are dramatic environmental changes. (Question 10)
  - Evolution acts only on traits you can see. (Questions 1, 6, 9, 13)
  - Populations always change slowly and we cannot see evolution happening. (Questions 7, 9)
  - Evolution and natural selection are the same thing. (Question 2)
  - Only individuals with the highest fitness survive and reproduce. (Question 12)
  - Evolution is entirely random OR evolution is entirely pre-determined. (Questions 2, 9)
  - Mutations arise and evolution occurs because organisms need something. (Question 9)
- Further information about student misconceptions on this topic can be found here as well as on the Understanding Evolution website.

Primary Sources

- **Bite** "Evolution in Real Time: Tracking Mutations in *E. coli* Populations"

  Good, Benjamin H., Michael J. McDonald, Jeffrey E. Barrick, Richard E. Lenski, and Michael M. Desai. 2017. "The Dynamics of Molecular Evolution over 60,000 Generations." *Nature* 551: 45–50.

- **Misconceptions**
  Anderson, Diane I., Kathleen M. Fisher, and Gregory J. Norman. 2002. "Development and Evaluation of the Conceptual Inventory of Natural Selection." *Journal of Research In Science and Teaching* 39(10): 952–978. doi: 10.1002/tea.10053

Materials

Copies of the Student Handout and Science Bite for each student

Time

This lesson should take approximately two to three 50-minute class periods.

Student Prior Knowledge

Students should have a basic understanding and familiarity with the ideas of evolution and natural selection. It will be helpful (but not absolutely necessary) for students to have some basic genetics knowledge, such as with the structure of DNA, the central dogma, and the relationship between mutations and alleles.

Instructions and Teacher Tips

- **General Procedure**
  - Have students read the introduction and answer questions based on the introduction. You may want to have students pause and discuss questions 1–5 as a class before reading the Bite.
  - Students should then read the Bite and answer the questions about the Bite together.
  - Debrief with students, either having them change groups or discuss as a class to make sure the students understand the key points and misconceptions listed above.
- **Tips, Extensions, and Variations**
  - Time can be saved by having students read the introduction before the first class period.
  - This lesson would best be completed in pairs or in groups of three so they can collaborate and discuss with each other. It could also be completed individually depending on the level of your students.
  - Depending on the level of your class, students could complete the introduction and introductory questions individually as homework before you begin this lesson. Then they can get into small groups in class and share their answers to these questions before

reading the Bite together and answering the final questions. If you are doing this in an AP level class, a second year biology course, or if you have already covered the introductory information in class, you may be able to skip the introduction altogether and jump straight into the Bite.

- The experiment described in this Bite was also described in Chapter 5 of Richard Dawkins' book *The Greatest Show on Earth* (2009, New York: Free Press).
- As an extension to this lesson, you could:
  - Discuss methods of DNA sequencing. See the "Big Question Discussion" below for more details.
  - Discuss synonymous and non-synonymous mutations (see Background Information) and ask students to explain whether or not they would expect natural selection to act on these types of mutations in bacteria.

Background Information and Research Details

- The *E. coli* long-term evolution experiment was initiated by Richard Lenski on February 24th, 1988 at UC Irvine, and is now hosted at Michigan State University. It involves twelve cultures of *E.coli* bacteria, all from the same founding strain. These twelve cultures were used for the twelve replicates of the long-term experiment. Six of the populations had a point mutation that gave them the ability to grow on arabinose (Ara+), while the other six were unable to grow on arabinose (Ara−). Other than this one difference, the populations were identical. The reason for beginning the experiment with this one point mutation has to do with making it easier to visualize the bacteria. Ara+ colonies appear white or pink on a growth plate, while the Ara− ones appear red. This difference allows researchers to do direct competition experiments. For example, they can mix an evolved colony of either Ara+ or Ara− with the original population of the opposing type, use the color differences to distinguish between the two populations and compare rates of growth. Since they alternate the colonies, it also allows them to immediately see if there was contamination between strains.
- The Bite includes this line, in explaining why bacteria are so useful to work with: "First of all, humans and most animals reproduce sexually, they do not clone themselves, which complicates the picture significantly." Students may ask how sexual reproduction complicates the study of evolution. Sexual reproduction increases variation through many methods, including independent assortment and crossing over in meiosis, as well as through random fertilization. It is much easier to study changes in the allele frequency among asexual reproducers in which a small number of factors influence whether or not an allele will be passed on to offspring, and chance plays a more limited role.

- Additional information about experimental methods:
  - Each day, 1% of the culture (0.1 ml) is transferred to a new flask. The researchers know the 0.1 ml is representative of the population, since they've been shaking the flask all day which distributes the bacteria evenly. By transferring 0.1 ml to a new flask filled with nutrients, the bacteria have space to keep dividing. If they left the bacteria in a single flask and didn't do this transfer, they'd run out of space!

- Every 75 days (~500 generations), samples from each population are collected and frozen. Freezing the bacterial samples prevents them from dividing any more, creating the “fossil record” that researchers can go back to for their studies. If the researchers ever want to propagate cultures from those generations, they can simply get out their tubes of bacteria from the freezer and inoculate new cultures! Along with freezing samples, researchers also compare the growth rates of each population alongside ancestral populations to assess fitness.
- Your students might wonder how the researchers detected when a mutation arose, and the answer is that they didn’t! Rather, they detected when mutations had spread to at least 10% of a population in at least two sampled time points. The pipeline that the researchers developed was also able to differentiate between point mutations and insertions or deletions!

- A few major findings from this study:
  - Six populations developed defects in their abilities to repair DNA, which lead to an increase in their rate of mutations (“mutator” phenotypes). Within the six “non-mutator” populations, only 20 mutations reached fixation during the first 10,000 generations, and between 60–100 total mutations reached fixation over 60,000 generations. This finding is the basis for Question 11g.
  - One interesting finding is that there appears to be a trade-off between cell size and fitness advantage. Each population of bacteria had an increase in average size (which also meant that each flask had a lower maximum number of bacteria it could support). Researchers found that when they changed the environment of the bacteria, this increase in size corresponded with a lowered resistance to osmotic stress—changes to the cell wall of the bacteria made them less tolerant to salt stresses. This is an example of a case where the bacteria might have had fitness advantages in this one environment, but would not have advantages in saltier environments!
  - One of the most exciting, and headline-grabbing findings was the evolution, in one of the populations, of the ability to grow on a new medium. These bacteria evolved an ability to use an entirely new source of nutrients and energy! *E. coli* typically cannot grow on citrate (this is a defining feature of the species!), but the researchers put both citrate and glucose in the liquid media they were growing the bacteria in. After about 30,000 generations, one population evolved the ability to use citrate (Cit+), giving that population an advantage, since it could use another resource previously unavailable to it. This was really exciting, because the scientists calculated that during the first 30,000 generations every possible mutation that could occur in the the genome would have happened—the fact that the ability to use citrate took so long to evolve implies that this wasn’t just a one-mutation trait: it’s difficult to evolve, and likely requires earlier “potentiating” mutations. It was also really exciting because the Cit+ population increased greatly in cell size, but also coexisted with a population that remained unable to use citrate (Cit−).

- Some mutations that reached fixation did so without any benefit to the bacteria. For example, if a mutation just happened to occur close to a beneficial mutation that was under selection, it could "hitchhike" to fixation. That hitchhiking mutation didn't give the bacteria any kind of fitness advantage, it just happened to be close to the mutation that did. The scientists also found that *when* a mutation arose was an important factor influencing fixation. When the mutation arose was important because whether that mutation is beneficial or not depends on what other mutations are already present. A mutation may be beneficial only in the presence of another mutation. An example of this is the citrate digestion mutation described above. Scientists have inferred that the ability to digest citrate was gained through a biochemical pathway that had more than one step. If a bacterium gains one mutation, that may not be helpful, but if the bacterium was "primed" with the first mutation when the second one happened to arise, the mutations would have a combined benefit.

- Some genes accumulated mutations early in the experiment but then didn't mutate much at all later on. Other genes followed the opposite pattern. The researchers hypothesize that some of these late mutations might depend on other mutations to actually provide a fitness benefit, and might test this by introducing the "late" mutations into other strains of *E. coli* that don't have the earlier mutations.

- Scientists also found that in line with their predictions, far more nonsynonymous mutations occurred relative to synonymous ones. This makes sense because synonymous mutations (or "silent" mutations) lead to the same amino acid sequence in the resulting protein, and therefore also usually lead to the same protein structure and function. A synonymous mutation would most likely not be acted on by natural selection because it would have a neutral effect on phenotype.

Big Question Discussion

This lesson should get students thinking about the Big Question "*What does it mean to observe?*" In particular, how do we know there are differences among organisms that look the same? If you choose to delve into the Big Question, consider the following ideas:

- You could have a wrap-up discussion with the students about different ways to make observations. Observations are not just things we can see with our naked eyes. In this experiment, scientists used technology to sequence the DNA of these bacteria and look at their genetic code.

- In an upper level class this could dovetail with a discussion about biotechnology, including how DNA sequencing works, like the Sanger method or Maxam-Gilbert sequencing. You could also discuss (or demonstrate) PCR, gel electrophoresis, and other ways to tell two organisms are different at a molecular level.

Answers

1. Answer the following questions about alleles:

a. Define "allele" in your own words

Alleles are different versions of a particular region of DNA (this is quoted from the reading.

Students should use their own words). Be aware that some students believe that the term allele only applies to genes, when in fact it applies to all regions of the DNA.

b. Describe the relationship between mutations and alleles.

Mutations lead to different alleles by changing the sequence of nucleotides in a region of DNA.

c. A friend claims that if two mice have different alleles, they will look different. Explain two reasons why that might not be the case.

One possible reason that two mice with different alleles may appear the same is that DNA can also be different in regions of the DNA that do not code for traits. In this case, even if the two individuals have different nucleotide sequences (different alleles), they would still appear the same. A second possible reason why mice may appear similar when they have different alleles is due to the fact that not all differences in the DNA code will lead to a different trait. Some mutations are silent (or synonymous). In that case, the DNA sequence would be different, but the trait may appear the same. A third possible reason is that sometimes alleles occur in genes that code for traits that are not *visible* to the naked eye. The mice may be different in ways that we cannot see (like the ability to digest food efficiently).

2. Explain how evolution by natural selection is different from other ways a population can evolve. Give examples.

Evolution is when the allele frequency changes over generations. Evolution by natural selection is in-part predictable as alleles become more common when they are associated with beneficial traits and less common when they are associated with harmful traits. Populations can also evolve when new alleles appear randomly through mutation. Another way is through random chance, like when a forest fire burns through half of a forest (genetic drift). Finally, allele frequency can change through migration, like when individuals move into or out of a population.

3. Explain the role of competition in evolution by natural selection.

Individuals within a population have to compete because not all organisms within a population can survive and thrive, due to limited resources, presence of predators, or limited mating opportunities. Some individuals are better suited to a particular environment than others (have higher fitness). The ones who are better at accessing resources can pass their “good” alleles on to the next generation in higher numbers.

4. Why does reproductive success matter in evolution by natural selection?

Reproductive success is very important in evolution by natural selection. The definition of evolution is a change in allele frequency *over generations*. If an individual is really great at accessing resources, but then does not pass his good traits down to the next generation, he will not impact the frequency of the alleles in the next generation. Allele frequency is affected when one individual passes his or her traits down to more offspring than another individual.

5. Consider a population of lions.

a. Brainstorm some reasons why some lions might have a higher fitness than other lions. List at least three.

Sample answers: some lions might be better at hiding while hunting for food, be faster when chasing prey, have larger body size to protect offspring or mate, etc.

b. Reflect on your examples in part a. Did you have any examples in the first part where the lions were not competing for a limited resource? If so, circle them. If not, try to think of a few now, add them to your list, and circle them.

Sample answers: sperm count or quality, ability to process food more efficiently so it doesn't need to hunt for as much food, better immunity to disease, etc.

6. Now, consider bacteria. Brainstorm some reasons that among a population of bacteria living in a Petri dish, some might have higher fitness than others. List at least three. (*Hint:* Think about your answers to Question 5.)

Sample answers: ability to process food in the Petri dish more efficiently, ability to survive in the presence of antibiotics, size difference may affect their ability to access nutrients, the ability to use different sources of food, ability to reproduce at a faster rate, etc.

7. What are some of the benefits of using bacteria in a study on evolution? Explain your answer, citing specific information from the Bite.

Bacteria reproduce asexually, producing genetic clones; they don't have brains so they don't care if they're in a lab; they reproduce very quickly (about six generations per day); they don't take up a lot of room; and they don't have a lot of requirements. [This answer is verbatim from the reading, so you should check for students to use their own words.]

8. In the Bite, the scientists say that the bacterial *mutations* were competing with each other.

a. How is competing in this sense different from the direct competition we often think of in our everyday lives?

We often think of competition as being direct. In this case, the mutations are not fighting with each other. They are not conscious and they are not competing directly.

b. If it's not direct competition, what do the scientists actually mean when they say that the mutations were competing?

The scientists mean that certain mutations were better than others, or led to a higher fitness. The mutations themselves were leading to traits that gave one individual bacterium an advantage over the other.

9. Think about your population of lions again from Question 5. A mutation arises that enables the lions to digest a common type of plant that they couldn't digest before (lions usually eat only meat). You read an article in the news that says the deer "got the ability to digest the plants because they needed a new food source." Make a claim about whether mutations arise because they are needed. Explain your reasoning.

Mutations occur at random, they do not arise because they are needed. In this case, the mutation arose randomly, which gave that particular lion an advantage. Since that lion had access to more food, he or she was able to reproduce more, passing that beneficial trait to more offspring, causing that trait to become more common in the lion population.

10. We often think that evolution happens when an environment changes. In this study, the scientists kept the *E. coli* in a constant, stable environment.

a. How does evolution occur if the environment doesn't change? Remember that evolution is **any** change in allele frequency over generations. (*Hint:* Did the environment change in the situation described in Question 9?)

Evolution can occur in a stable environment if a new allele arises due to mutation or if migration occurs into or out of that population from surrounding areas.

Let's consider the 60,000th generation from this experiment. What if the scientists took two samples of the bacteria from this generation, sample A and sample B. If you were to compare the genes present in each of these two populations, they'd be identical. But then what if the scientists kept sample A in the same stable environment as usual, and placed the sample B into a different, but still stable, environment. Maybe the temperature was a few degrees cooler, or the humidity a bit higher. They otherwise continue to follow the same experimental procedure that they've been doing for 60,000 generations.

b. A year later, after about 2,000 generations, they compare samples. Would sample A and sample B still be genetically identical? Explain your reasoning.

The two populations would likely not be identical. Each trait or adaptation is beneficial in that particular environment. The same traits will likely not be beneficial in a different environment.

Now consider a copied 60,000th generation kept in the same environment as the original population. This time, though, both sample A and sample B are kept in the same stable environment as the original population. So you have identical sets of genes in the populations and identical environments.

c. A year later, the scientists compare the two populations and find that they are no longer genetically identical. How can that be? Explain your answer.

Since mutations occur randomly, you would not expect the same mutations to occur in both populations (even though they have identical environments).

11. How is the experiment in the Bite similar to what actually occurs in nature? How is it different from what occurs in nature?

This is similar to what happens in nature because populations evolve and there will always be some individuals within the population that are better fit than others. This is also realistic because mutations occur at random. This experiment is different from nature because in nature, the environment is never so consistent, or unchanging. In the lab, when the dish runs out of food, the experimenter adds more so they start at the same level of food each day. That does not happen in nature.

12. Use **Figure 2** in the Bite to answer the next set of questions.

a. Examine mutations A and B. At what generation did each mutation arise and what was the fate of each mutation?

Mutation A arose at about generation 1,000 and became present in 100% of the population by about generation 7,000. Mutation B arose at about generation 2,500 and was completely gone by about generation 9,000.

b. What caused the frequency of mutation A to rise so quickly from generation 0 to 7,000?

Refer to reproductive success (fitness) in your answer.

Mutation A increased in frequency because it led to a beneficial trait. The bacteria that developed this mutation reproduced more frequently, leading to more bacteria with this mutation in the next generation.

c. Both the C and D mutations are beneficial in this environment. Which mutation arose first?

Mutation C (at around generation 6,000 vs. generation 14,000)

d. Which mutation, C or D, had a higher frequency at generation 30,000?

At generation 30,000, mutation D had a higher frequency (about 70% versus mutation C, which had a frequency of about 40%).

e. If both C and D mutations are beneficial, explain the patterns of frequency over the first 30,000 generations.

Mutation C was beneficial and became more common over time. Once mutation D appeared at generation 14,000, the rate at which mutation C became more frequent started to slow down. At about generation 27,000, mutation D started to become more common and mutation C started to become less common (until it eventually disappeared). Mutation D appears to be *more* beneficial than mutation C).

f. What might have happened with mutation C if mutation D never appeared?

It looks like mutation C might have continued to become more common in the population. It's possible that it would have stayed at about 80% frequent, it could have reached 100%, or it could have disappeared altogether.

g. Assume a new mutation arises at generation 30,000 that increases the number of errors that occur during the copying of DNA. What would you expect to happen from generation 30,000 to 60,000? Draw your predictions on the graph.

Students should show lots of new mutations appearing.

13. Connect to the Big Question. If you were to compare the bacterial cells in the first generation with the bacterial cells in generation 60,000, you may not be able to see any physical differences. Scientists have many tools they use to observe organisms. What observations did these scientists make that allowed them to see evolution happening in these bacteria?

Scientists could not necessarily see the changes in the bacteria with their naked eyes. They had to "observe" using technology to sequence the DNA and find mutations at certain generations.