# Lab 10: Microevolution and Natural Selection

## Purpose

The purpose of this activity is to investigate the microevolution of drug-resistant bacteria.

## Learning Objectives

At the conclusion of this exercise, students will be able to:

* Simulate and observe the microevolution of bacteria.
* Determine the factors that drive changes in allele frequency.
* Calculate the allele frequency in a population.
* Determine an organism’s fitness.
* Identify the mode of natural selection in a population.
* Describe the impact of evolution on human society.

## Why It’s Relevant

Evolution is a fundamental theme in biology that helps us understand the incredible biodiversity on Earth. Much like the study of history, it allows us to make sense of the present, learn from the past, and inspire positive changes in the world. For instance, the Irish potato famine taught us a valuable lesson about the importance of genetic diversity in crops to ensure a stable food supply. Similarly, protecting keystone species helps maintain population balance within ecosystems, supporting species interaction as well as the health of all species—including humans. Furthermore, understanding how quickly infectious diseases can evolve has enabled us to develop vaccines and other treatments to protect the health of our communities. By studying evolution, we can better safeguard biodiversity and gain insight into what the future might hold for the evolution of the human species.

## Introduction

**Evolution** is the genetic change in populations across generations and is a central theme at all levels of biological organization. It can be studied on two different scales: **microevolution**and **macroevolution**. Microevolution focuses on changes in allele frequencies within a population over time, while macroevolution examines broader patterns of change that occur among species over long evolutionary periods. In other words, microevolution serves as the foundation for macroevolutionary processes, since hereditary traits are passed down from parent to offspring or genetically transferred between microorganisms, and consequently, population changes over generations.

One of the key mechanisms driving evolution at both scales is **natural selection**. Natural selection occurs when individuals with certain heritable traits have greater **reproductive success** than those with other variations of the trait.

As a result, natural selection leads to increased**fitness**—an organism’s ability to survive and reproduce—and the development of **adaptations**, which are heritable traits that improve survival and reproduction in a given environment. If a trait provides an individual high fitness, the associated **alleles** are more likely to be passed on to future generations, helping maintain the population’s characteristics. Conversely, if a trait results in low fitness, those alleles may decrease in frequency or disappear entirely, reducing genetic diversity within the **gene pool** or **allele frequency** in the population.

A current health concern we are facing is the emergence of highly drug-resistant bacteria, such as *Acinetobacter baumannii*. According to the World Health Organization (WHO), *A. baumannii* is classified as a critical priority pathogen and is a significant global health threat that causes hospital outbreaks worldwide (Atunes et al. 2014). The bacteria cause infections in wounds and can spread into the blood, lungs, and urinary tract of humans (Antunes et al. 2014). Other species in the*Acinetobacter* genus are found in natural environments, but *A. baumannii* is almost exclusively associated with hospital settings, suggesting it has evolved to survive in highly sterile environments (Antunes et al. 2014). **Genetic** and **epidemiological** studies suggest that its evolution is driven by a rapid ability to spread, acquire drug resistance genes, and accumulate adaptive **mutations** (Antunes et al 2014, Diancourt et al 2010, Scoffone et al 2025).

You and your lab partner (if applicable) will perform a simple simulation of the evolution of *A. baumannii* and apply your knowledge of microevolution and natural selection to the results.

## Curiosity and Inquiry

You and your lab partner (if applicable) are going to model the evolution of drug-resistant bacteria. The hypothetical population will include three different alleles of the immunity trait and will be exposed to antibiotics. With this in mind, the question you are asking is:

“Why and how does a bacterium evolve to become a drug-resistant bacteria, like *A. baumannii*?”

## Formulating a Hypothesis

1. Develop a hypothesis based on the question above.
2. You will model 10 generations of a bacterial population with three alleles: least drug-resistant, drug-resistant, and most drug-resistant. The population will be exposed to antibiotics in each generation.

Prediction #1: Based on the scenario above, what do you predict the allele frequencies will look like by generation 10? In other words, will genetic diversity increase or decrease in the population?

## Testing the Hypothesis: Designing an Experiment

### Materials for In-Person Courses

* Bacteria **phenotype**/**genotype** population:
	+ Least resistant (allele: A) individuals: 40 large steel paper clips
	+ Resistant (allele: B) individuals: 30 small steel paper clips
	+ Most resistant (allele: C) individuals: 30 coated paper clips
* Application of antibiotic:
	+ Dice or rolling dice app

### Alternative Materials for Online Course

* Bacteria phenotype/genotype population (Note: Objects should be different from one another for you to distinguish the bacterial trait):
	+ Least resistant (allele: A) individuals: 40 of your chosen objects
	+ Resistant (allele: B) individuals: 30 of your chosen objects
	+ Most resistant (allele: C) individuals: 30 of your chosen objects
* Application of antibiotic
	+ Dice or rolling dice simulation app
1. What does the collection of objects represent in the bacteria population?
2. What evolutionary mechanism does the antibiotic (dice) represent?

### Procedure

1. Create two groups that each include:
* 13 least resistant bacteria (allele: A) individuals
* 6 resistant bacteria (allele: B) individuals
* 1 most resistant bacteria (allele: C) individual

One group will be exposed to antibiotics and the other group will not be exposed to antibiotics.

1. Calculate and record starting population allele frequency for the generation. Allele frequency is the proportion of the alleles occurring in the population. Below is the equation:

# of individual(s) with specific genotype / total # of individuals

Your instructor will inform you if they wish for you to manually or automatically calculate the measures. Refer to Appendix 1 for instructions on using Google Sheets to automatically calculate allele frequency. However, you can use any program that you are most comfortable with. to calculate allele frequency.

1. For group 1: roll the dice.
* The number of dots facing up represents the number of bacteria that die within the generation and are removed from the population.
* Survivorship of antibiotic exposure:
	+ The least resistant bacteria have a 1/6 (16.7%) chance of surviving.
	+ The resistant bacteria have a 3/6 (50%) chance of surviving.
	+ The most resistant bacteria have a 5/6 (83.3%) chance of surviving
* The first individuals to remove are the A individuals. If there are no more A bacteria, then remove the B individuals. If there are no more B bacteria, then remove the C individuals.
* Example (**Figure 7.1**):
	+ Bacteria population: 3 A, 4 B, and 2 C.
	+ Six dots face up – Remove the 3 A and 3 B individuals to represent death.
	+ The surviving individuals are 0 A, 1 B, and 2 C.

**Figure 7.1**

1. For group 2: Do not roll the dice.

For both groups and each generation:

1. Record the number of individuals that died and survived in the data collection tables 10.1 or 10.2 below.
2. Add two more objects to each bacterial trait.
3. Record the total number of objects in the end population column and in the starting population for the next generation in the data collection tables 10.1 or 10.2 below.
4. Repeat steps B-G until you have completed 10 generations.

### Questions

1. What are the**independent** and **dependent variables**?
2. Which group represents the**control group**?
3. Which group represents the **experimental group**?
4. What are the starting **sample sizes** of your control and experimental groups?
5. How many replications are done in both the control and experimental groups?
6. What does procedure step #6 represent in the bacteria population?

## Testing the Hypothesis: Data Collection

1. **Table 10.1: Control Group Data Table**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Generation | Bacteria Trait and Allele | Start Population Allele Frequency (%) | # of individuals that died | # of individuals that survived | End population Allele Frequency (%) |
| 1 | Least resistant (A) |  |  |  |  |
|  | Resistant (B) |  |  |  |  |
|  | Most resistant (C) |  |  |  |  |
| 2 | Least resistant (A) |  |  |  |  |
|  | Resistant (B) |  |  |  |  |
|  | Most resistant (C) |  |  |  |  |
| 3 | Least resistant (A) |  |  |  |  |
|  | Resistant (B) |  |  |  |  |
|  | Most resistant (C) |  |  |  |  |
| 4 | Least resistant (A) |  |  |  |  |
|  | Resistant (B) |  |  |  |  |
|  | Most resistant (C) |  |  |  |  |
| 5 | Least resistant (A) |  |  |  |  |
|  | Resistant (B) |  |  |  |  |
|  | Most resistant (C) |  |  |  |  |
| 6 | Least resistant (A) |  |  |  |  |
|  | Resistant (B) |  |  |  |  |
|  | Most resistant (C) |  |  |  |  |
| 7 | Least resistant (A) |  |  |  |  |
|  | Resistant (B) |  |  |  |  |
|  | Most resistant (C) |  |  |  |  |
| 8 | Least resistant (A) |  |  |  |  |
|  | Resistant (B) |  |  |  |  |
|  | Most resistant (C) |  |  |  |  |
| 9 | Least resistant (A) |  |  |  |  |
|  | Resistant (B) |  |  |  |  |
|  | Most resistant (C) |  |  |  |  |
| 10 | Least resistant (A) |  |  |  |  |
|  | Resistant (B) |  |  |  |  |
|  | Most resistant (C) |  |  |  |  |

1. **Table 10.2 Experimental Group Data Table**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Generation | Bacteria Trait and Allele | Start Population Allele Frequency (%) | # of individuals that died | # of individuals that survived | End population Allele Frequency (%) |
| 1 | Least resistant (A) |  |  |  |  |
|  | Resistant (B) |  |  |  |  |
|  | Most resistant (C) |  |  |  |  |
| 2 | Least resistant (A) |  |  |  |  |
|  | Resistant (B) |  |  |  |  |
|  | Most resistant (C) |  |  |  |  |
| 3 | Least resistant (A) |  |  |  |  |
|  | Resistant (B) |  |  |  |  |
|  | Most resistant (C) |  |  |  |  |
| 4 | Least resistant (A) |  |  |  |  |
|  | Resistant (B) |  |  |  |  |
|  | Most resistant (C) |  |  |  |  |
| 5 | Least resistant (A) |  |  |  |  |
|  | Resistant (B) |  |  |  |  |
|  | Most resistant (C) |  |  |  |  |
| 6 | Least resistant (A) |  |  |  |  |
|  | Resistant (B) |  |  |  |  |
|  | Most resistant (C) |  |  |  |  |
| 7 | Least resistant (A) |  |  |  |  |
|  | Resistant (B) |  |  |  |  |
|  | Most resistant (C) |  |  |  |  |
| 8 | Least resistant (A) |  |  |  |  |
|  | Resistant (B) |  |  |  |  |
|  | Most resistant (C) |  |  |  |  |
| 9 | Least resistant (A) |  |  |  |  |
|  | Resistant (B) |  |  |  |  |
|  | Most resistant (C) |  |  |  |  |
| 10 | Least resistant (A) |  |  |  |  |
|  | Resistant (B) |  |  |  |  |
|  | Most resistant (C) |  |  |  |  |

## Interpreting and Visualizing Your Data

1. Now plot the data that your group collected. Label the **X** and **Y axes** and choose the appropriate number scale for each axis. Display the end population allele frequency for each generation between the control and experimental groups. Your instructor will inform you if they wish for you to manually or automatically create the graph. Refer to Appendix 1 for instructions on using Google Sheets to graphs with standard deviation bars. However, you can use any program that you are most comfortable with. *Helpful Tip: Apply your knowledge about independentand dependent variables when labeling the axes.*



1. What is the allele frequency from the starting population in generation 1?
2. What is the allele frequency from the end population in generation 10?
3. Do you notice patterns in the allele frequency throughout all generations?

## Interpreting and Visualizing the Class Data

### Class Data Spreadsheet Entry

Once you have collected all your data, enter your data in the class spreadsheet on Google Sheets. Your instructor will provide you with the link.

Complete the simple statistical analysis of the class data in the table below.

1. What are the **mean** and**standard deviation** of the control group? (Your instructor will inform you if they wish for you to manually or automatically calculate the measures. Refer to Appendix 1 for instructions on using Google Sheets to automatically calculate measures. However, you can use any program that you are most comfortable with. to calculate allele frequency.)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Start Population Allele Frequency (%) of Least Resistant generation 1 | End population Allele Frequency (%) of Least Resistant generation 10 | Start Population Allele Frequency (%) of Resistant generation 1 | End population Allele Frequency (%) of Resistant generation 10 | Start Population Allele Frequency (%) of Most Resistant generation 1 | End population Allele Frequency (%) of Most Resistant generation 10 |
| Mean |  |  |  |  |  |  |
| Standard Deviation |  |  |  |  |  |  |

1. What are the mean and standard deviation of the experimental group?

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Start Population Allele Frequency (%) of Least Resistant generation 1 | End population Allele Frequency (%) of Least Resistant generation 10 | Start Population Allele Frequency (%) of Resistant generation 1 | End population Allele Frequency (%) of Resistant generation 10 | Start Population Allele Frequency (%) of Most Resistant generation 1 | End population Allele Frequency (%) of Most Resistant generation 10 |
| Mean |  |  |  |  |  |  |
| Standard Deviation |  |  |  |  |  |  |

### Class Data Analysis and Graphing

1. Now plot the data that the class collected. Label the X and Y axes and choose the appropriate number scale for each axis. Graph the mean and the standard deviation bars of both the control and experimental groups. Your instructor will inform you if they wish for you to manually or automatically create the graph. Refer to Appendix 1 for instructions on using Google Sheets to graphs with standard deviation bars. However, you can use any program that you are most comfortable with.



1. Do you notice patterns between the independent and dependent variables? Explain the relationship.

## Making a Conclusion

1. Do your results validate or reject your group’s hypothesis? Why or why not?
2. Do your data display the same relationship as the class data? If not, describe the difference(s).
3. When analyzing the data, would you use your data or the class data to formulate your conclusions? Why?
4. Does the class data validate or reject your group’s hypothesis? Why or why not?

Using the class data, answer the following questions:

1. Which population had the greatest genetic diversity by the 10th generation?
2. Which population had the greatest allele C frequency by the 10th generation?
3. Which organism had the greatest fitness by generation 10 in the experimental group? How did the organism achieve this?
4. What was the selective pressure or agent in the activity and which group was not under selective pressure?
5. Which microevolution mechanism favored the increased fitness and adaptiveness of the bacterial trait?
6. Which mode of natural selection does the phenotype/genotype best represent in both the control and experimental groups?
7. Does a prevalent trait in the experimental group make this organism adapt perfectly to its habitat? Why?
8. Do you feel that doctors should be prescribing antibiotics for pathologies other than bacterial infections? Justify your stance with an evidence-based argument based on this activity.
9. Based on what you have learned in this activity, how would you educate others about why evolution is important?
10. According to Diancourt, et al., their study suggests that one of the *A. baumannii*clones may spread through the bottleneck effect. What is the bottleneck effect and how would this microevolutionary mechanism cause outbreaks in hospitals?