**Random mutations, natural selection, and antibiotic resistance**

**Complete the surveys at the following links prior to the activity:**

**Assignment:**

* Read the short selection from Why We Get Sick on Antibiotic Resistance.
* Watch the video of evolution of bacteria on a “mega-plate” Petri dish.
	+ <https://www.youtube.com/watch?v=plVk4NVIUh8>

Natural selection and random mutations together result in populations become adapted to their environment. Organisms can not select how they will adapt to their environment and microbes are no exception. So how does antibiotic resistance arise in a population of bacteria?

1. Genetic variation is always present in individuals within a population, but if there is no selective advantage to this genetic variation it is not likely to increase in the population.
2. If the environmental conditions change and now the reproductive success of one individual in the population increases because of a trait that is due to one of these random genetic mutations, we would expect to see more individuals with that trait in the population.

How can random mutations and environmental conditions result in antibiotic resistant bacteria? We will us Avida Ed software to investigate how random mutations and antibiotics in the environment can give rise to antibiotic resistance in populations of bacteria.

Watch the video tutorial on Avida Ed <https://www.youtube.com/watch?v=x7pISK-eBE8> before beginning the following exercise.

Access the Avida-ED Application at the link below and complete the following activities.

<https://avida-ed.msu.edu/avida-ed-application/>

You can use the web hosted version or a downloadable version.

**Fitness**-the number of offspring an individual contributes to later generations, or lifetime reproductive success.

**Avida Ed exercise:**

**Ancestor Treatment 1- A high fitness organism when antibiotics are absent**

1. In the Population viewer, click Setup in the upper right of the panel.
2. Drag “@ancestor” from the organism menu on the left to the Ancestral Organism(s) box.
3. Set the following parameters:
	1. Dish size 30x30;
	2. 2% per site mutation rate;
	3. place offspring near their parent
	4. **Uncheck all resources**
	5. Repeatability mode set to experimental
	6. Pause **At update 1000**
4. Return to Map view and select **Run**.
5. Once 1000 updates have completed, use the Fitness Scale coloration and the “selected Organism Type” panel to identify an individual with a relatively high fitness (that can not perform the NOT function). It does not matter if this Avidian can of can not perform any other functions.
6. Once selected, freeze the individual by choosing the “Save Selected Organism” option from the Freezer menu. Name the organism “No-antibiotic\_ancestor”.
7. Switch to the Organism viewer to confirm your frozen organism’s phenotype.
8. Drag the organism from the Freezer to the genetic code box. Select Run, then End.
9. A genetic code symbol labeled “Offspring Genome” should appear within the offspring’s genome, and the Details window should indicate that it can perform whichever phenotypes you observed it being able to perform when you chose it. If your frozen organism is either unable to reproduce or unable to perform the phenotypes you should return to the Population viewer and repeat steps 5-9.
10. Record data for your selected ancestor in table 1.

**Select New at the bottom of the population screen to begin the next section.**

**Ancestor Treatment 2- A high fitness organism when the antibiotic notose is present.**

Repeat Ancestor Treatment 1 with the same parameters, except add notose to the environment by marking notose with a check. Leave all the other resources absent (unchecked). Identify an Avidian with a relatively high fitness that has the NOT mutation (click on the NOT function in the population statistics box on the right and it will highlight all the organisms with the NOT mutation). If you do not have an organism that can perform NOT after 1000 updates then continue the run until one occurs in the population. Then confirm its phenotype and ability to reproduce following steps 7-9. The selected frozen organism should be named “Notose-ancestor”.

**Table 1. Starting organismal attributes for competition ancestors in selective enviroments.**

|  |  |
| --- | --- |
| **“No-Resource\_ancestor”**(does not perform function) | **“Notose\_ancestor”**(antibiotic resistant, performs NOT)  |
| **Fitness (absolute)** | **Energy Acq. Rate** | **Offspring Cost** | **Fitness (absolute)** | **Energy Acq. Rate** | **Offspring Cost** |
|  |  |  |  |  |  |

**Select New at the bottom of the population screen to begin the next section.**

**Competition Treatment 1- Competing ancestors in the absence of antibiotics (no notose)**

1. In the Population viewer, click Setup.
2. Drag **both** “no-resource\_ancestor” and “Notose\_ancestor”from the Freezer to the Ancestral Organism(s) box.
3. Set the following parameters:
	1. **Dish size** 30x30:
	2. 1% Per Site **Mutation Rate**;
	3. **Place Offspring Near their parent**;
	4. **Uncheck all resources**;
	5. **Repeatability Mode** Experimental;
	6. **Pause Run** at update 500.
4. Return to Map view. Note: Do not press Run until step #6.
5. Below the Map, **set Mode** to “Ancestor Organism.” In the “Population Statistics” panel select the **“NOT”** button. Change the graph’s Y-axis to “**Number of Organisms**.”
6. In the Control menu choose “**Run**”.
7. Record in Table 1 the (absolute) fitness, energy acquisition rate, and offspring cost for each ancestor by selecting each and using the “Selected Organism Type” panel.

**Competition Treatment 2-Competing ancestors when antibiotic (notose) is present.**

Repeat steps 1-11 of Competition Treatment 1 using the same parameters and procedures, except add notose to the environment by marking notose with a check.

**Table 2-Population Composition after 1000 updates**

|  |  |  |  |
| --- | --- | --- | --- |
| **Competition Environment** | **Number performing NOT** | **Number of viable organisms** | **Percent of viable organisms performing NOT** |
| All resources absent |  |  |  |
| Notose present |  |  |  |

**Questions**

1. How did the antibiotic resistance gene originate? Could this have occurred in the absence of the antibiotic?
2. What role (if any) did the presence of antibiotics in the environment play in the persistence of this mutation in the population.
3. If antibiotics were removed from the environment would you predict the frequency of this mutation to increase, decrease or be unchanged?