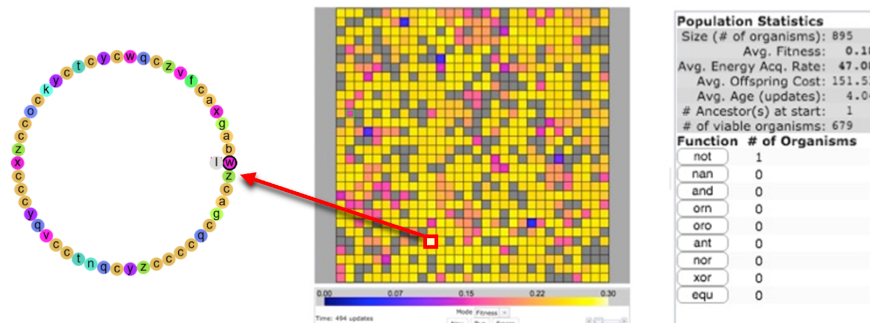




## Exercise 2 - Exploring Random Mutation and Selection



Only one Avidian in this population can perform the function NOT. Did a mutation conferring this phenotype occur because performing NOT is rewarded in this notose environment?

### Student Learning Goal

- Students will be able to explain that mutations occur at random, and do not occur because they are needed by an organism to succeed in its environment.

### Questions to Consider While Doing Exercise #2

- What does it mean to say that mutations occur at random? In other words, do mutations occur in an organism in response to a need, or do they occur spontaneously?
- Is there a pattern to when mutations occur?
- Can we predict whether an organism's offspring will have mutations suitable for living and reproducing in its environment?

Mutation is essential for evolutionary processes because it is the ultimate source of genotypic variation – variation that can then be expressed phenotypically. Alterations to the instructions in an Avidian's genome can affect its ability to perform certain functions and even its ability to reproduce (the phenotypes of Avidians). In this exercise we use Avida-ED to explore the consequences of random mutation generating phenotypic variation that can be under selection in the environment.

Avida-ED provides a way to test whether mutations occur at random, or if mutations are directed to occur in response to natural selection within the environment. In some sense, we are testing directly what Salvador Luria and Max Delbrück (1943) did in their elegant Nobel Prize winning experiments<sup>1</sup>. We also consider a reason why time is fundamental to the process of evolution; if mutations do not generate a phenotype, then that trait cannot evolve in a population.

<sup>1</sup>Luria SE, Delbrück M. 1943. "Mutations of Bacteria from Virus Sensitivity to Virus Resistance." *Genetics* 28:491-511.



## Phenotypic Variation and Selection

Random mutations create genotypic diversity within a population. In Avida-ED, mutations can allow some Avidians to perform functions. For our purposes we will simply note that these are logic functions involving the comparison of numbers Avidians encounter in their digital environment. In Avida-ED there are nine functions – **NOT, NAN, AND, ORN, ORO, ANT, NOR, XOR, EQU**; and nine corresponding resources – *notose, nanose, andose, ornose, orose, antose, norose, xorose, equose*. An Avidian with a particular sequence of instructions can perform a function, but the individual Avidian performing this function is only *rewarded* if the corresponding resource is available in the environment. For example, the “@ancestor” organism cannot perform any functions, but random mutations over multiple generations might produce a descendant with a genome that codes for one or more functions (e.g., NOT). If the corresponding resource is in the environment (e.g., notose), then this Avidian will have an increased energy acquisition rate and be favored by natural selection within its environment.

Multiple types of variation between individuals in a population are possible. Phenotypes are the observable characteristics of an organism; genotypes are the specific genetic sequences that encode for that individual. In Avida-ED, we define the phenotype of an organism based on which of nine different logic functions the organism can perform. Whether or not the individual can perform the function is the phenotype. If the corresponding resource -- which here are the words ending in -ose -- is present, that phenotype will be rewarded. But note that, for example, if the organism consumes *ornose*, it has the **ORN+** phenotype; *ornose* is the resource, and **ORN** is the function.

Natural selection acts upon phenotypic variation in a population of organisms. Individuals whose phenotypes are better suited to a particular environment tend to have greater reproductive success. For understanding Avidian phenotypes in selective environments, it is illustrative to use an analogy to bacteria processing sugar resources as food. When an individual bacterium is able to metabolize a sugar in its environment, it receives energy to be used for growth and reproduction; bacteria that can metabolize the sugar will be favored due to natural selection. Similarly, when an Avidian is able to perform a function corresponding to a resource in its environment it is rewarded with an increased energy acquisition rate, producing offspring more quickly; Avidians that can perform a function associated with an available resource will be favored due to natural selection.

## Random versus Directed Mutation

Before scientists understood the nature of genetic mutation in biology, mutations were thought to be non-random or directed. Scientists hypothesized that bacteria could develop specific mutations depending on the circumstances or environment in which the bacteria lived; if a specific mutation provided an advantage, it would occur. For example, bacteria exposed to a selective environment were thought to be able to generate the necessary mutations that would allow them to evolve accordingly. To test if mutations were random versus directed Salvador Luria and Max Delbrück devised an elegant experiment that allowed them to differentiate between these two hypotheses<sup>1</sup>. We can investigate this same question in Avida-ED.





## Timing the occurrence of phenotype-conferring mutations in the absence and presence of a selective advantage

In this exercise, we will test the relationship between mutation and selection by measuring the time of appearance of an Avidian function (phenotype) in a population in the presence or absence of a selective agent (i.e., resource). In the first treatment, each individual in the class will record how many updates (a measure of time) it takes for a mutation that confers the ability to perform NOT to occur in an Avidian population living in an environment with **all resources absent**, (i.e., there is no reward for performing NOT). After recording their result, each student in the class will then do the same procedure in a second treatment, but this time with **notose present**; performing NOT will be rewarded with increased energy acquisition rate. The NOT phenotype is only selectively advantageous in the second treatment.

**Before you begin collecting your data answer the following questions:**

- ☐ What are the hypotheses being tested in this experiment?
  
- ☐ How does this experiment address them?
  
- ☐ Do you predict that an Avidian performing **NOT** will appear *sooner* in the first treatment when notose is not present or the second treatment when notose is present? Why?

### **Treatment 1 – First occurrence of NOT when all resources absent (no notose).**

1. In the Population viewer, flip to Setup.
2. Drag “@ancestor” from the Freezer to the Ancestral Organism(s) box.
3. Set the following parameters:  
Dish Size 30x30; 2% Per Site Mutation Rate; Place Offspring Near their parent;  
**Uncheck all resources**; Repeatability Mode Experimental; Pause Run Manually.
4. Return to Map view and select Run.
5. Pause your experiment right after the first occurrence of an organism that can perform the NOT function. Select the “NOT” button in the Population Statistics Panel, turning it green. A green line near the x-axis will appear on the Population



Graph. Wait until the green line increases, then hover your cursor over its initial rise and note the exact update highlighted along the x-axis.

6. Record the update number of this occurrence in **Table 1**.

### **Treatment 2 - First occurrence of NOT when notose present.**

1. In the Control menu choose "Start New Experiment."
2. In the Population viewer, flip to Setup.
3. Keep all parameters set as in Treatment 1 except add notose to the environment by **marking notose with a check**. Leave all other resources absent (unchecked).
4. Return to Map view and select Run.
5. Pause your experiment at the first occurrence of an organism that can perform the NOT function, following one of the methods described in Treatment 1, step 4.
6. Record the Update number of this occurrence in **Table 1**.

**Table 1. Updates (time) until the first occurrence of an Avidian performing NOT, with and without notose present (a selective advantage or reward) in the environment.**

Environmental Treatment	Update number at first occurrence of NOT
All resources absent (No reward for NOT)	
Notose present (Reward for NOT)	

**Recording your data.** Experiments often involve investigating processes or phenomena with lots of variation, we will examine the data generated by the class. Enter your *Table 1 data*, the number of updates for NOT to occur in each experimental treatment, here:

### [Exercise 2 Data Collection Spreadsheet](#)

Follow the "Example" in column B. Find the first column on the right that does not contain data; enter your name in row 4, and your Table 1 data on the following rows. The instructors will periodically collect these data, anonymize them, and add them to the course data set.

Once you have entered your data, compared and discussed your group's data, and completed all responses and data entry except the Discussion Questions on the next page, you may view the analysis below.

### [Exercise 2 Data Analysis Spreadsheet](#)





**Data Analysis.** In this exercise, we are comparing whether a particular phenotype first occurs when the environment rewards that phenotype, or when there is no reward for that phenotype. How, though, can we tell? The answer, as is often true in science, lies with statistics.

There are many numbers we could be looking at with this data. The best approach is to decide what evidence is the most important to answer the question we want to ask. In this exercise, we take advantage of the fact that we can pair up data into many separate trials. Each trial consists of one experiment in an environment with the reward, and one in the environment without the reward. For any given trial, there is a roughly 50% chance that the function will first occur when there is a reward, and a 50% chance that it will first occur when there is no reward. (*It is very rare for the first appearance to be on the exact same update, so we will ignore that for now*). By comparing the results of these trials, we can ask whether it is more likely that we will first see the phenotype occur when it is rewarded, or when it is not rewarded. The relative timing – which comes first? – is more important for our question than the specific updates involved.

Because the data for this experiment consist of many replicated trials, each of which has two possible outcomes, the most appropriate statistical test is one called a binomial test. This test will allow us to determine whether our results are best explained by there being a real difference between the two environments, or instead by random chance. But because there are only two possible results of each trial, we will need many replicates to be able to make a meaningful comparison.

**Discussion Questions and Wrap-up.** After examining the course data, work with your lab team to respond to the following questions.

- ☐ How does this *experimental setup* test whether mutation is random versus directed by the selective environment?
- ☐ For your results as shown in Table 1, did the first occurrence of the NOT phenotype happen earlier or later in Treatment 1 compared to Treatment 2?
- ☐ Was this the same for each person in your group, and for each person in the class?



- ☐ What pattern would you have expected to observe in the class data *if mutations occur in response to the presence of a selective environment*?
- ☐ Given the data you have examined across **all** Avida-ED experiments you have performed, how would you describe what it means to say that mutations occur at random?
- ☐ **Thought experiment** – How would evolution be affected if mutations did not occur at all (that is, a zero percent mutation rate)?
- ☐ Why is mutation essential to the evolutionary process?
- ☐ We used Avida-ED and this experimental protocol to model what occurs when biological populations experience mutation. What are some limitations or constraints to our modeling in this exercise?

### ***Reflection and Metacognition***

Think-Pair-Share: Work with your lab team to answer the following questions.

- ☐ What did you learn from this exercise?
- ☐ What are you still wondering about?
- ☐ What would you change in this exercise?