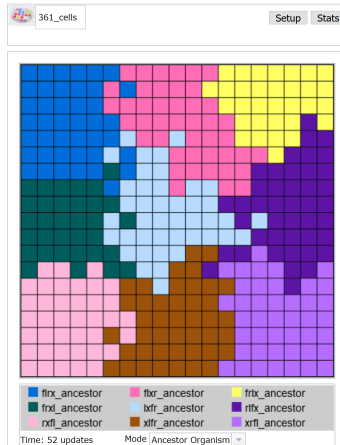




## Exercise 4 – Exploring Population Change without Selection



This experiment began with nine Avidian ancestors of identical fitness; the mutation rate is zero percent. Since descendants can never differ in fitness, will evolution occur in this population?

### Student Learning Goals

- Students will be able to explain how non-adaptive evolutionary change can occur.
- Students will be able to explain the relationship between population size, population diversity, and genetic drift.
- Students will be able to graph and explain how population size and genetic drift influence the frequency of traits or alleles over evolutionary time.

### Questions to Consider While Doing Exercise #4

- Is natural selection the only process that produces evolutionary change?
- What does “random sampling” mean in the context of biological evolution?
- How does population size affect diversity in a population in the absence of selection?
- How does population size impact the frequency of any single ancestor lineage over time?
- Why do we refer to the pattern created by random sampling as “genetic drift”?

### Genetic Drift

Evolution, ***changes in traits or alleles in a population over generations***, can occur as the result of a number of different processes or mechanisms. The Introduction and Exercise 1 of the lab book explored the role (and nature) of *mutations* to the evolutionary process. Exercise 3 explored how *selection*, created by the presence of resources that reward specific functions, can influence an Avidian’s ability to survive and reproduce. There are other factors, not associated with an Avidian’s ability to perform a function, that impact which ones survive and reproduce. In fact, in Avida-ED as well as in biological systems, this occurs every update or generation unless a population is infinitely large. *Random sampling* alone will cause changes in the numbers or types of Avidians in the population over time. We refer to these observed changes in the frequency of traits (or



types of Avidians) in the population as genetic drift. These changes are not the result of selection but instead are nonadaptive and often described as resulting in neutral evolution.

In Avida-ED, random sampling effects are created by random differences in drawing genotypes during reproduction and influence the survival and/or reproduction of individuals irrespective of their fitness. ***In Avida-ED when multiple Avidians reproduce on the same update, their offspring will be randomly chosen for placement onto a random grid location on the Map, with each location only able to contain one organism. This offspring placement can result in one offspring precluding the existence of another when instantly placed at the same location despite which one might have a higher fitness. In addition an offspring can overwrite (“kill”) an aging Avidian, preventing it from completing its reproduction.*** The random fluctuations in the number of any particular Avidian ancestor and its descendants is genetic drift.

In this exercise you will investigate the effects of genetic drift alone by ensuring that adaptive evolution as a result of selection cannot occur. Since natural selection requires phenotypically expressed genotypic variation, we must eliminate the source of this variation by setting the mutation rate to 0%. ***Your experiments will begin with nine Avidian ancestor types that vary at four loci (or genome positions); the name of each indicates its genotype at position 14, 20, 27, and 33. For example, “rxfl\_ancestor” has an “r” instruction at locus 14 and “l” at locus 33.*** In order to visualize genetic drift in Avida-ED, your experiments will use these nine unique ancestors whose descendants we can easily track over the course of evolution by using the “Ancestor Organism” map mode. Note that although each organism (or genotype) is distinct, they all have an identical fitness, so natural selection will not favor one ancestor type over another. In this exercise you will explore how random sampling impacts populations from one generation to the next and how it influences overall diversity in populations over time. You will investigate these effects by starting each experiment with all nine distinct ancestor Avidians, observing any changes in the frequency of these ancestor types over time, and recording how many of the original ancestor Avidians are present after 300 generations. Changing the size of the population (i.e. 3 x 3; 9 x 9; 19 x 19) allows you to explore how random sampling impacts the evolutionary change in populations.

### **Visualizing evolutionary change without natural selection**

Your instructor will provide the Workspace file “Ex4.avidaedworkspace.zip”.

Verify that you have downloaded this to your computer -- it cannot be directly used from a cloud server. ***Note you do not need to extract, open, or edit this file.***

In Avida-ED, choose the File menu option “Open Workspace,” and navigate to and select this downloaded file, “Ex4.avidaedworkspace.zip”.





**Part I.**

1. Go to Setup. Drag each of the **nine unique organisms** from the Freezer to the ancestor Organism(s) box; the order does not matter. Note if you do not see nine organisms in your Freezer then you have not successfully loaded the Exercise 4 workspace. If you fail to place all nine organisms then you might receive an error message!
2. Set the following parameters:  
**0%** Per Site Mutation Rate; Place Offspring Near their parent; **Uncheck all resources**; Repeatability Mode Experimental; Pause Run **At update 300**. For the first series of replicates, set the Dish Size to **3x3** (total of 9 cells).
3. In the Freezer menu choose “**Save Experiment Configuration,**” and enter the name “**9\_cells**” to save this setup.
4. Go to the Map. Change Mode to **Ancestor Organism**. Run the experiment and record *the total number of ancestor organisms* (total number of different colors) present at the end of 300 updates in Table 1 for Replicate 1, Population size 3 x 3.
5. To create a new replicate, start new experiment, drag “9\_cells” from the Freezer’s Configured Dishes section to the small box next to the Avida-ED symbol above the Map (see top left of image at the beginning of this exercise). **Visually confirm your experiment looks correct in the Setup and Map views**. Run. Record final number of ancestor types present after 300 updates.
6. Repeat the experiment for a total of 5 replicates.
7. **Start a new experiment, repeat steps 1-5 for population size 81 (Dish Size 9x9). Remember to save your experimental configuration (“81\_cells”) for use with each replicate.** Record the total number of ancestor organisms (total number of colors) present at the end of 300 updates in Table 1.
8. **Start a new experiment, repeating steps 1-5 for population size 361 (Dish Size 19x19). Again, save your experimental configuration (“361\_cells”) for use with each replicate.** Record the total number of ancestor organisms (total number of colors) present at the end of 300 updates in Table 1.

**Table 1. Diversity of descendant types (number of colors) across experimental replicates for various population sizes.**

Number of <u>descendant types</u> present after 300 updates						
	Replicate					
Size	1	2	3	4	5	Average
<b>9</b> (3 x 3)						
<b>81</b> (9 x 9)						
<b>361</b> (19 x 19)						





**Recording Your Data.** Calculate the average for each experimental set-up. Enter your Table 1 data in the spreadsheet provided by your instructor, in the tab labeled T1-Diversity. Follow the “Example” in column B. Find the first column on the right that does not contain data; enter your name in row 5, and your averages from Table 1 in the following rows.

[Exercise 4 Data Collection Spreadsheet](#)

**Examining Class Data.** The instructors will periodically collect these data, anonymize them, and add them to a larger course data set. Once you have entered your data, and compared and discussed your group’s data, your instructor will share a compiled spreadsheet showing an analysis of the data collected by other courses.

[Exercise 4 Data Analysis Spreadsheet](#)

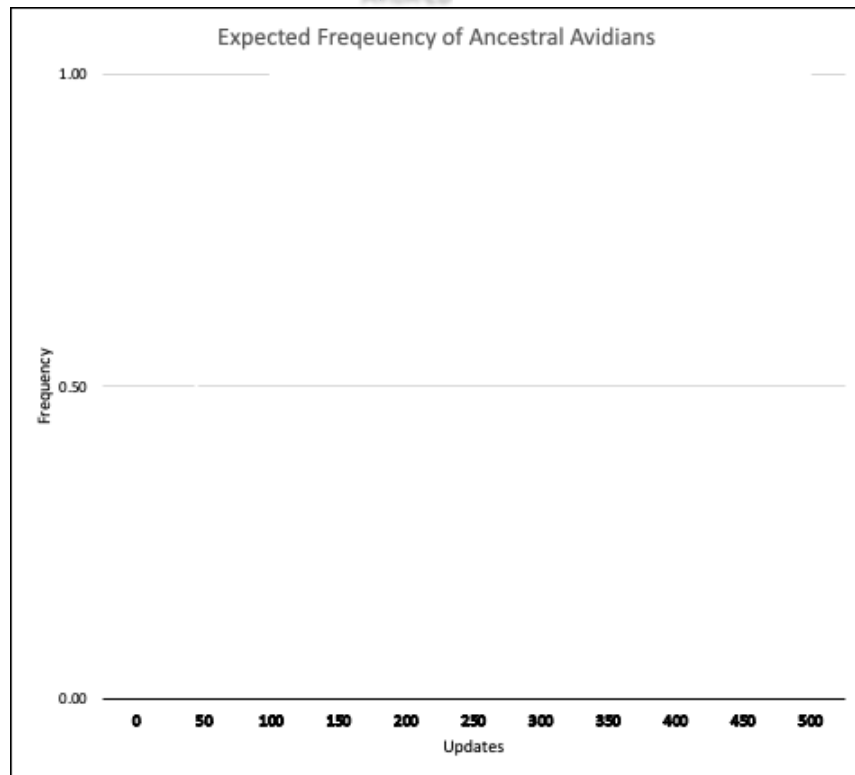
## Part II

In biological systems, evolutionary change can be observed by tracking the frequency of a particular allele (different forms of a gene) in a population over time. As we’ve observed in earlier exercises, these changes can be the result of new mutations or selection for particular traits. In Part I of this exercise we have also observed that random sampling from one update to the next can cause random changes in a population, and that the impact of this random sampling is greater in small populations. In Part II we investigate how the frequency of any single ancestor and its descendants changes over time. The *random fluctuations in these frequencies result in what is called genetic drift.*

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

- The population always begins with 9 different individual Avidians, and hence the initial frequency of any single individual (i.e., flrx\_ancestor) is  $1/9 = 0.11$  or 11%. If you tracked the frequency of this single ancestor type over time would you expect the **frequency** to stay the same, increase, or decrease?
  
- Do you think this will be the same or different across the **different population sizes? Why?**
  
- Please draw your predictions on the graph below**, Figure 1. You can graph your prediction for each population size on the same graph using different types/colors of lines.





**Figure 1. Graph of the frequency of a chosen ancestor organism at each of the time updates (every 50 updates).** Create three different lines on the graph, one for each of the population sizes. Note: **Initial Percentage** of any single ancestor at the beginning =  $1/9 = 11\%$ ; **Extinction** = 0%; **Fixation** = 100%.

## Instructions

1. In the Map viewer, drag the 3 x 3 configured dish from part I. This will repopulate your dish with the appropriate dish size and conditions necessary to carry out Part II.
2. All parameters should be set to the same values as in Part I (**0%** Per Site Mutation Rate; Place Offspring Near their parent; **Uncheck all resources**; Repeatability Mode Experimental). The only change you will make is to **manually stop** the updates at 50, 100, 150, 200, 250, 300, 350, 400, 450, 500. Return to Map view and make sure Mode is set to "Ancestor Organism" (see bottom of image at the beginning of this exercise).
3. Choose **one Avidian ancestor organism** (e.g. *rxfl\_ancestor*) and record the number of **that ancestor organism** in the population at 0 updates in Table 2. The initial frequency of any single ancestor at time 0 is 1 out of 9, or 0.11. Run the program for 50 updates, pause, and record the number of the ancestor organism you have chosen to track (e.g. *rxfl\_ancestor*) in Table 2. Repeat, stopping every 50



updates. It may be difficult to stop at exactly 50 updates but get as close as possible and then record the number. *NOTE: It is possible that any single ancestor type can be lost entirely, resulting in the number to go to zero. If this occurs you can stop the experiment as the ancestor will not return.*

- Repeat the above protocol for a population size of 9x9 and 19x19.

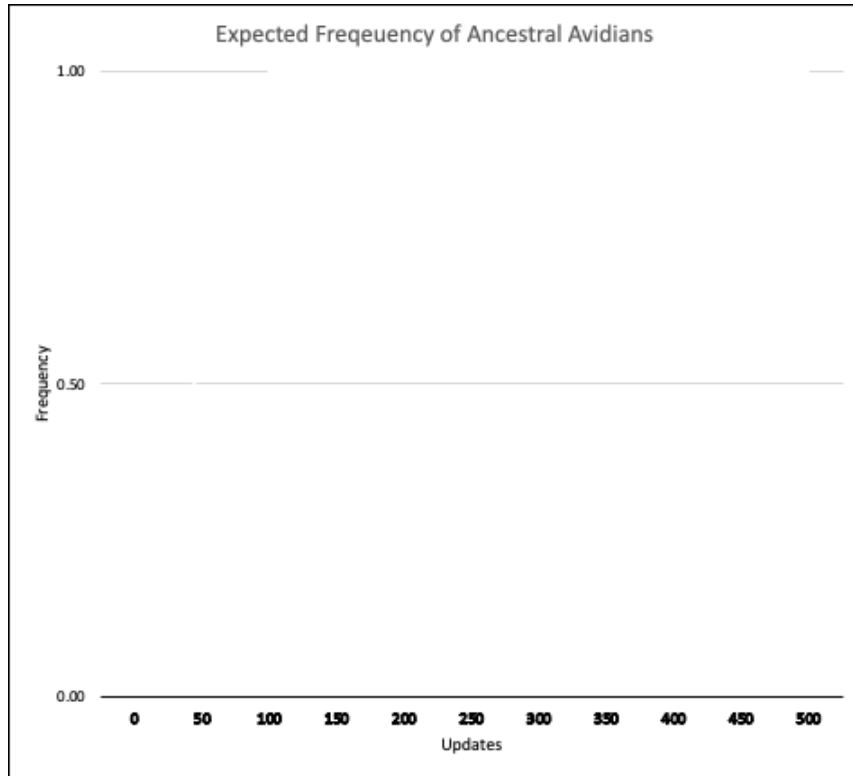
**Table 2. Number and frequency of one particular descendant type at intervals of every 50 updates.**

Updates	Number of single ancestor type present for 3 x 3 dish	Freq. = No./Total (9)	Number of single ancestor type present for 9 x 9 dish	Freq. = No./Total (81)	Number of single ancestor type present for 19 x 19 dish	Freq. = Total (361)
<b>Ancestor</b>	e.g. rxfl_ancestor		e.g. rxfl_ancestor		e.g. rxfl_ancestor	
<b>0</b>	<b>1</b>	<b>0.11</b>	<b>1</b>	<b>0.11</b>	<b>1</b>	<b>0.11</b>
<b>50</b>						
<b>100</b>						
<b>150</b>						
<b>200</b>						
<b>250</b>						
<b>300</b>						
<b>350</b>						
<b>400</b>						
<b>450</b>						
<b>500</b>						

- Calculate the frequency by dividing the number of the ancestor organism you tracked by the total number of organisms in the population.
- Graph the frequency of the ancestor for each population size in **Figure 2**.

**Entering Your Data.** Using the frequencies you calculated in Table 2, enter your data into the class spreadsheet tab titled T2-Frequency provided by your instructor. Follow the “Example” in column C. Find the first column on the right that does not contain data; enter your name in row 5, and the **frequencies you calculated in step 5** in the spreadsheet.





**Figure 2. Graph of the frequency of a chosen ancestor organism at each of the time updates (every 50 updates).** Create three different lines on the graph, one for each of the population sizes. Note: **Initial Percentage** of any single ancestor at the beginning =  $1/9 = 11\%$ ; **Extinction** = 0%; **Fixation** = 100%.

[Exercise 4 Data Collection Spreadsheet](#)

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[Exercise 4 Data Analysis Spreadsheet](#)





**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 the sole influence of genetic drift on the evolution of a population?
  
- What impact does population size have on diversity in an evolving population?
  
- How does population size influence the effects of genetic drift?
  
- Have you seen the effects of genetic drift in any previous Avida-ED experiment(s)? If so, describe what occurred and why you think genetic drift was a cause. If not, why don't you think so?
  
- Thought experiment** – How much final descendant type diversity would you expect to find if you ran your replicates of population size 361 for 10,000 more updates (i.e., a much longer time)?
  
- If each replicate represents an isolated population, how would genetic drift impact the diversity between small isolated populations? (Would the diversity between populations increase, decrease or stay the





***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?



