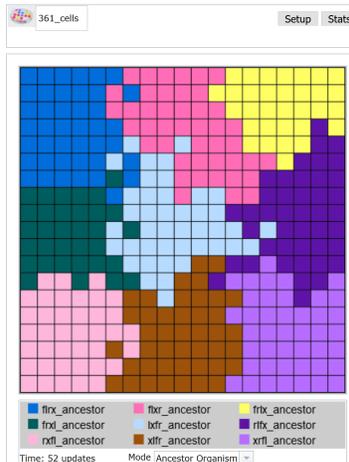# Exercise 4 – Exploring Population Change without Selection

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 and genetic drift, and the relationship between population diversity and genetic drift.

Questions to Consider While Doing Exercise #4
- Is natural selection the only process that produces evolutionary change?
- What does "random sampling" mean in this biological context?
- What does population size have to do with genetic drift?
- How does genetic drift affect diversity in a population?

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 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
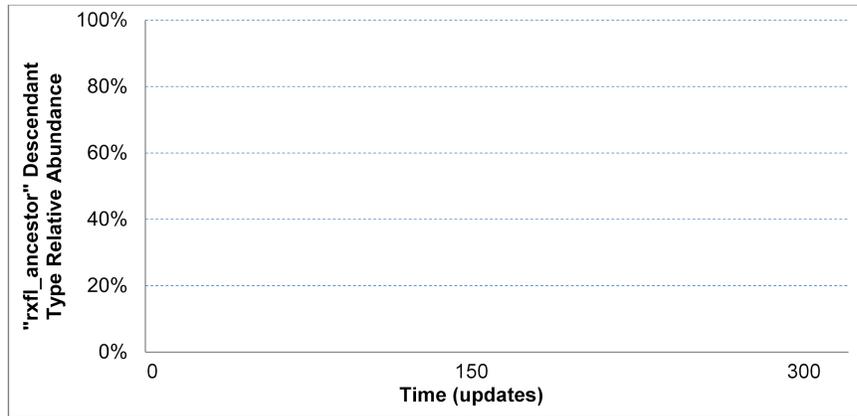
irrespective of their fitness. In Avida-ED when multiple Avidians reproduce on the same update, their offspring will be randomly chosen for placement onto a random grid location on the Map, with each location only able to contain one organism. This offspring placement can result in one offspring precluding 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.

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 ancestral avidians, observing any changes in the frequency of these ancestral types over time, and recording how many of the original ancestral 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.

Before you begin collecting data answer the following questions:

The population always begins with 9 different individual Avidians. Do you expect the relative frequency of each descendant type to stay the same, increase, or decrease <u>over the course</u> of each experimental replicate? Do you think this will be the same or different across the different population sizes? Why? Please draw your predictions on the graph below. You can graph your prediction for each population size on the same graph using different types/colors of lines.

The population always begins with 9 different individual Avidians. Do you expect the <u>diversity of descendant types</u> (number of colors) present at the end of each replicate of 300 updates to stay the same, increase, or decrease? Do you think this will be the same or different across the different population sizes? Why?

If you repeated the experiment do you think you would get the same result? Why?

Visualizing evolutionary change without natural selection

1. Your instructor will provide the Workspace file "Ex4.avidaedworkspace.zip".

   Exercise 4 Avida-ED Workspace

2. 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.***
3. In Avida-ED, choose the File menu option "Open Workspace," and navigate to and select this downloaded file, "Ex4.avidaedworkspace.zip".

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4. In the Population viewer, flip to Setup.
5. Drag each of the **nine unique organisms** from the Freezer to the Ancestral 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!
6. Set the following parameters:
   0% Per Site Mutation Rate; Place Offspring Near their parent; **Uncheck all resources**; Repeatability Mode Experimental; Pause Run **At update 50**.
7. For the first series of replicates, set the Dish Size to **3x3** (total of 9 cells). In the Freezer menu choose "Save Experiment Configuration," and enter the name "9_cells" to save this setup.
8. Return to Map view. Below the Map, set Mode to "Ancestor Organism" (see bottom of image at beginning of this exercise). Each ancestor will be uniquely colored and this "descendant type" will visually denote each ancestor's descendants.
9. For the first replicate you will choose one avidian ancestor organism (i.e. rxfl_ancestor) and record the number of that ancestral organism in the population every 50 updates. Run the program for the first 50 updates, record number of ancestral organism present, press run, repeat until you have data for 300 updates. **Record data in Table 1**. Calculate frequency by dividing number of one particular ancestral organism present in the population by the total number of organisms. Note: it is possible that any single ancestral avidian will be completely lost from the population (so the frequency will be 0) or go to fixation (take over the population) so the frequency will be 1.
10. Graph these data in **Figure 1**.
11. In **Table 2** record only the number of remaining descendant types (colors) at the end of 300 updates.
12. Creating replicates: In the Control menu choose "Start New Experiment" and then "Discard." Drag "9_cells" from the Freezer's Configured Dishes section to the small box next to the Avida symbol above the Map (see top left of image at beginning of this exercise). Visually confirm your experiment looks correct in the Setup and Map views.
13. Repeat experiment 4 more times, but this time you do not have to record the number at 50 interval updates. Record *the total number of ancestral organisms* (colors) present at the end of 300 updates in Table 2.
14. Start a new experiment, repeat steps 5-10 for population size 81 (Dish Size 9x9). Remember to save your experimental configuration ("81_cells") for use with each replicate. Note: For replicate #1 it may be difficult to get an exact count of the ancestral organism you have chosen to track so you can estimate the number based on the size of the dish. For example, if the ancestral organism you are tracking takes up ¼ of the dish you can estimate the frequency as 0.25. Record data in **Table 2**.
15. Start a new experiment, repeat steps 5-10 for population size 361 (Dish Size 19x19). Again, save your experimental configuration ("361_cells") for use with each replicate. Note: For replicate #1 it may be difficult to get an exact count of the ancestral organism you have chosen to track so you can estimate the number based on the size of the dish. Record data in **Table 2**.

Table 1. Frequency of one particular descendant type at 50 update intervals.

Updates	Number of single ancestral type present (i.e. rxfl_ancestor) for 3 x 3 dish	Number of single ancestral type present (i.e. rxfl_ancestor) for 9 x 9 dish	Number of single ancestral type present (i.e. rxfl_ancestor) for 19 x 19 dish
0			
50			
100			
150			
200			
250			
300			

Table 2. Diversity of descendant types (number of colors) across experimental replicates for various population sizes.

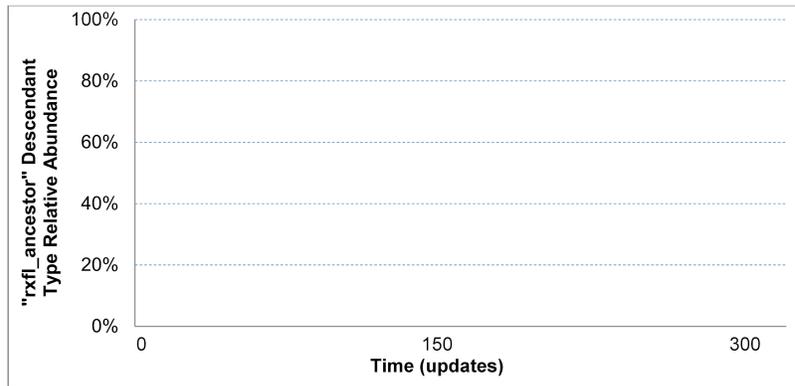| Number of descendant types present after 300 updates						
	Replicate					
Size	1	2	3	4	5	Average
9 (3 x 3)						
81 (9 x 9)						
361 (19 x 19)						

Figure 1. Graph of the frequency of a chosen ancestral organism (from Table 1) in the population at each of the time updates. Create three different lines on the graph, one for each of the population sizes. Note: **Initial Frequency** = one of nine ancestors = 0.11; **Extinction** = 0.0; **Fixation** = 1.0.

Recording your data. Experiments in biology often involve investigating processes or phenomena with lots of variation, we will be examining the data generated by the entire class/course. Enter your *Table 2 data*, the average final descendant type diversity in each experimental treatment, in the spreadsheet provided.

Exercise 4 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 averages from Table 2 in the following rows. The instructors will periodically collect this data, anonymize it, and add it 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, your instructor may wish to share a compiled spreadsheet showing an analysis of the data collected by other courses.

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?

Did the same descendant types (colors) go extinct for each of your runs?

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)?

What impact does genetic drift have on diversity in an evolving population?

How does population size influence the effects of genetic drift?

Thought experiment – How would Figure 1 be different for a population of smaller size (for example, 5 organisms)? And for a much larger population size?

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?

Why does genetic drift always occur in an evolving population?

We used Avida-ED and this experimental protocol to model what occurs when biological populations experience genetic drift. 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?