Experimental Evolution in Avida-ED
Michigan State University
NSF Beacon Center for the Study of Evolution in Action

YouTube tutorial: https://www.youtube.com/watch?v=mJwtgOso4BA&feature=youtu.be
Launch Avida-ED: https://avida-ed.msu.edu/app/AvidaED.html

These activities focus on the mechanisms of evolution (mutation, natural selection, and genetic drift) by highlighting the basic components of evolutionary change—variation, inheritance, selection, and time. A population of organisms can exhibit both genotypic (genetic information) and phenotypic (expressed trait) variation. Mutations generate genotypic variation that can be expressed phenotypically. Only phenotypic variation is acted upon directly by natural selection; differences in expressed traits allow some individuals in a population to reproduce more than others. An individual’s fitness is dependent on its environment, and is relative to other individuals in the population. Genetic drift (evolutionary change due to random sampling in a population) and gene flow (the transfer of genetic information from one population to another) are also important influences on genotypic and phenotypic frequencies. Time plays a fundamental role throughout all of these processes.

Helpful tips:
• This software is optimized to work on Chrome and Firefox, but does work on Safari and Explorer.
• Do not refresh or close your browser without saving what you need.
• This software will run more slowly with larger populations and higher mutation rates. Take care with setting these two parameters.
• There is a glossary at the end of this document.

Exercise 1: Genetic variation by random mutation

During the process of reproduction, the parent Avidian's instruction c at position 23 mutated to instruction q in its offspring. Is this mutation more likely than another?

Learning outcome: Explain how mutations arise in different genes throughout the genome and how this contributes to genetic variation in a population.

Summary: Biological organisms can exhibit both genotypic and phenotypic variation. Similarly, the organisms in Avida-ED—called Avidians—also have genotypes and phenotypes. An Avidian’s genotype is
the entire sequence of instructions in its genome, and its phenotype is its ability to reproduce and perform functions. Mutation is crucially important because it generates genotypic variation that might be expressed as phenotypic variation. In this exercise, we will focus on inheritance and genotypic variation by investigating the fundamental source of variation – mutation.

**Random mutation and genetic variation:** Like bacteria, Avidians have single parent reproduction and a circular genome composed of simple genetic instructions that can undergo mutation. If mutations did not occur, an offspring would inherit the exact genetic sequence of its parent. Though an organism largely resembles its parent, it is rarely exactly identical because during the reproduction process, a few genetic sites may change due to mutations occurring at random. Mutation creates genotypic variation in a population of organisms because different genetic sequence changes may occur during each individual’s reproduction. For simplicity, Avida-ED allows only substitution mutation, which is a random change from one instruction to another in an organism’s genome. Other types of mutation (insertions and deletions, chromosomal mutations), and the processes of transcription, translation, recombination, and horizontal transfer do not occur in Avidian genetics. Therefore, an Avidian genome in Avida-ED will always contain the exact same number of instructions or ‘genes’ (50).

In this exercise, we will explore how mutations produce genotypic variation. In addition, we will ask whether each individual mutation event is a random event. You will guide an Avidian through its reproduction process and record all of the mutations that occur in the offspring individual. By carrying out this process for three independent replicates, each person in the class will contribute the results of their three replicates to a class data set. With this much larger sample, we can investigate as a class whether or not there are trends in the occurrence of mutations. Where in the Avidian genome did the mutations occur? Did mutations occur such that certain mutant states were preferred? Finally, how many mutations occurred during Avidian reproduction?

**Before you begin collecting data:** On the graph axes provided below, draw your expectations for the frequency distribution of the three features of mutation described in the previous paragraph. These distributions represent data you would expect to observe from very, very many (thousands of) experiments. Later, after we’ve examined the data collected by the entire class, we will return to this page to draw updated expected distributions.

**Recording Mutant Avidians:** Observe how substitution mutations during reproduction change the genetic sequence from parent to offspring.

1. In the Organism viewer, select Settings.
2. Set the Per Site Mutation Rate to 10%. Keep Repeatability Mode as Experimental.
3. Drag the “@ancestor” from the Freezer to the genetic code box.
4. Select Run to observe the Avidian executing its genomic instruction sequence, including the process of reproduction. Each mutation that occurs will be highlighted by a black outline around its instruction circle. You can display the genome position (number) of any instruction by selecting it. It is possible that no mutations occur. Note whether the offspring has acquired the ability to perform a new function by referencing the table in the top right corner. We will explore the adaptive value of the functions in the next module.

5. Record your observations in the first tab of the Google Spreadsheet associated with this lab. This is a shared document where everyone will be able to enter and edit information simultaneously. Once you observe a specific mutation, simply increase the number of times that a mutation location and type were observed in the document. We will graph the pooled data when everyone is done with their runs. Repeat steps 1-5 two more times.

Questions for discussion:
A. How does this experimental setup demonstrate that mutations are random?
B. How would you describe each of the three relative frequency distributions (genome position, instruction identity, total number of mutations) for the class data set?
C. How would you reconcile your responses to the above two questions when considering each person’s individual experimental data versus data from the entire class?
D. How would results change if mutation rates were lower/higher?
E. What proportion of mutations resulted in a gain in function? What does this suggest about the effects of most mutations? Are populations still evolving when mutations do not result in a gain of function?

Exercise 2: Mutation and Selection

Learning outcome: Explain how mutations occur at random and do not occur because they are needed by an organism to succeed in its environment.

Summary: 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 (the phenotypes of Avidians) and even its ability to reproduce. In this exercise you will use Avida-ED to explore whether random mutations can generate phenotypic variation that can be targeted by selection in the environment. 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.
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. An Avidian with a particular sequence of instructions (‘genes’) can perform a function, but the individual Avidian performing this function is only rewarded if the corresponding resource is available in the 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. The “@ancestor” organism cannot perform any of these functions, but random mutations over multiple generations might produce a descendant with a genome that codes for one or more functions (e.g., the NOT function). If the corresponding resource is in the environment (e.g., notose), then this Avidian can utilize this resource.

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. 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.

In this exercise, we will test whether mutations are random versus directed. We will examine 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, you will record how many updates (generations) it takes for a mutation that confers the ability to perform the NOT function 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 your result, you will then perform the same procedure in a second treatment, but this time with the resource notose present; in this case, 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: What are the two competing 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 or second treatment? Why?

Treatment 1 – First occurrence of NOT when all resources absent.

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. There are two ways you can watch for this:
   a. Basic method: Closely watch the number next to the NOT function in the “Population Statistics” panel, and be very quick on the Pause button or else record an approximate update value.
b. Advanced method: Select the “NOT” button to the left of the number in method (a), 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 the second tab of the Google Spreadsheet associated with this lab.

**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 5.
6. Record the update number of this occurrence in the second tab of the Google Spreadsheet associated with this lab.

**Questions:**

A. How does this experiment test whether mutation is random versus directed by the selective environment?

B. On average, did the first occurrence of the NOT phenotype happen earlier or later in Treatment 1 compared to Treatment 2?

C. What pattern would you have expected to observe in the class data if mutations occur in response to the presence of a selective environment?

D. How would evolution be affected if mutations did not occur at all?

E. Why is mutation essential to the evolutionary process?

**Exercise 3: Fitness and population growth under selection**

Avidians descended from ancestors with different adaptations are competing in a selective environment. Can we predict how natural selection will influence the evolution of this population?
**Learning outcomes:** Explain the process of adaptation by natural selection, leading to genetic frequency change in a population over many generations. Explain the concept of biological fitness, and how fitness is dependent on the environment. Explain how natural selection is non-random.

**Summary:** The type of variation in a population acted upon by natural selection is phenotypic or trait variation, which changes gradually over successive generations. Differences in traits may be beneficial, allowing some individuals in a population to differentially reproduce more than others; these individuals have greater fitness relative to organisms lacking such advantageous traits. These adaptations are favored within the environment and will become more frequent in the population over generations of change. This is guaranteed to occur because individuals possessing the adaptations contribute more offspring on average than do individuals in the population lacking the advantageous traits. An individual’s fitness is therefore dependent upon the interaction between its phenotype and the selective environment, and is relative to other individuals in the population.

In Exercise #3, you will observe the process of natural selection in action. Beneficial mutations will randomly occur. Mutant organisms will have a greater relative fitness due to their adaptation to the environment and will tend to increase in frequency in the population over successive generations.

**Fitness:** The concept of biological fitness has crucial importance for how evolution by natural selection occurs. Fitness is incredibly difficult to measure for most organisms, so instead biologists typically use one or more related measures, called fitness components or proxies. For some study systems, biologists might use foraging success, mating success, survival, or other types of fitness proxies. When choosing which to use, a biologist needs to weigh the ease of collecting data with how strongly correlated the measure is to the true evolutionary fitness of the organisms under study.

In Avida-ED, fitness is the number of offspring an individual contributes to later generations, or lifetime reproductive success. We call our fitness proxy “Fitness,” but it is more precisely a measure of reproductive rate. This is a good measure of an organism’s fitness because the quicker an organism can reproduce, the more offspring it can contribute to the population over time. 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. In Avida-ED, fitness is calculated as the ratio of energy acquisition rate divided by offspring cost, in terms of energy necessary to complete the reproduction process. The energy aspects of the ratio cancel out, so fitness is the rate of reproduction. An Avidian rewarded for performing a function will have an increased fitness, producing offspring more quickly. Importantly, Avidians can also have higher fitness by reducing offspring cost, so populations in Avida-ED are always under selection to reduce offspring cost no matter the presence of resources in the environment. Note that performing a function tends to increase offspring cost, because to do so generally requires more instructions (‘genes’) needing to be executed (‘expressed’) before reproduction is completed.

Fitness can be further thought of as having two related measurement types, called absolute fitness and relative fitness. Absolute fitness is that which we have discussed; in Avida-ED a displayed “Fitness” value for an organism is precisely the absolute reproductive rate – the number of offspring an organism can reproduce in an amount of time. Relative fitness is the ratio of two absolute fitness values, the fitness of the focal organism relative to a reference fitness. The denominator of the ratio, the reference fitness, is often the average fitness of all organisms in the population. In Avida-ED you can calculate the relative fitness of an Avidian by dividing its “Fitness” from the “Selected Organism Type” panel by “Average Fitness” from the “Population Statistics” panel.

**Frequency change in a population:** Evolution can be defined as change in genetic frequencies over time. Genetic variation in a population can be described in terms of the relative frequencies of the different genotypes present in the population. When a mutation occurs, a new genetic variant is introduced into
the population (a new genotype). When the mutation appears in only a single individual, it occurs at the minimum possible (non-zero) frequency in the population. If the new genetic variant increases in frequency and becomes present in all individuals over successive generations, we say that the mutation has “become fixed,” which is the maximum possible frequency in the population (frequency = 1.00). This is evolution. Unlike with most biological systems, the simple genetic machinery in Avida-ED allows us to simplify genetic transmission concepts; an Avidian’s entire genomic sequence is its genotype, and it is this genotype that can change in frequency in an evolving Avidian population. Thus, the fitness of an organism is equivalent to the fitness of its genotype, with all identical Avidians having the same fitness.

Populations tend to have a great deal of genotypic variation (ultimately due to mutation). This genetic variation gives rise to phenotypic variation, which results in individuals within a population having a range of absolute fitness values. A specific genotype will change in frequency at a rate directly proportional to the genotype’s relative fitness value. The greater the value’s magnitude, the faster the population will change due to selection. A relative fitness greater than one indicates that the genotype will increase in frequency due to selection, and a value less than one indicates that the genotype will decrease in frequency due to selection. The frequency of a phenotype can be similarly described as increasing or decreasing in a population. The concept of relative fitness can be applied to phenotypes as well, and is related to the average fitness of all distinct genotypes that confer the phenotype.

In this exercise, you will explore relationships between variation, selection, and fitness by competing organisms against each other that are adapted to different environments. Ancestors in these competitions are adapted to different selective environments in Ancestor Treatments 1 & 2. In Ancestor Treatment 1, you will study an Avidian population with no resources present (all resources are absent), while in Ancestor Treatment 2, only the resource notose is present. Selected organisms will then serve as the ancestors in Competition Treatments 1 & 2, in which the ancestors compete within each of these two selective environments. By selecting and freezing organisms from each of these two environments, and competing them against each other in both of these environments, we can test whether there is a “home-field advantage” in these “battles.” Thus, this experiment will test for local adaptation.

By synthesizing data recorded near the beginning and at the conclusion of each competition experiment, we can explore whether or not a population will evolve to have a greater proportion of individuals better adapted to that environment – i.e., that natural selection is non-random.

**Ancestor Treatment 1:** Evolving a high fitness organism when no resources are present.

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 At update 1,000.
4. Return to Map view and select Run.
5. Once 1,000 updates have completed, use the Fitness Scale coloration and the “Selected Organism Type” panel to identify an individual with a relatively high fitness. It does not matter if this Avidian can perform any functions.
6. Once selected, freeze the individual by choosing the “Save Selected Organism” option from the Freezer menu. Name the organism “No-Resource_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 unable to reproduce, return to
the Population viewer and repeat steps 5-9. (Note: right-clicking a Freezer item allows you to rename or delete an organism.) Don’t proceed until you have frozen an organism that is capable of reproduction and successfully produces a daughter cell.

**Ancestor Treatment 2:** Evolving a high fitness organism when only 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 other resources absent (unchecked). At step 5, you must identify an Avidian with a relatively high fitness that can perform NOT. If you do not have an organism that can perform NOT after 1,000 updates then continue the run until an individual that can perform NOT occurs in the population. Then confirm its phenotype and ability to reproduce following steps 7-9, again confirming that your organism successfully performs NOT. The selected frozen organism should be named “Notose_ancestor”.

**Table 1.** Fitness attributes of organisms chosen for use as ancestors in competitions in two different selective environments.

<table>
<thead>
<tr>
<th>Selective Environment</th>
<th>“No-Resource_ancestor” (may or may not perform function)</th>
<th>“Notose_ancestor” (performs NOT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fitness (absolute)</td>
<td>Energy Acq. Rate</td>
</tr>
<tr>
<td>All resources absent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Notose present</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Frequency of individuals performing NOT after 300 updates of competition in two different selective environments.

<table>
<thead>
<tr>
<th>Selective Environment</th>
<th>Number performing NOT</th>
<th>Number of viable organisms</th>
<th>Percent of viable organisms performing NOT</th>
</tr>
</thead>
<tbody>
<tr>
<td>All resources absent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Notose present</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Competition Treatment 1:** Competing ancestors when all resources are absent.

1. In the Control menu choose “Start New Experiment”.
2. In the Population viewer, flip to Setup.
3. Drag both “No-Resource_ancestor” and “Notose_ancestor” from the Freezer to the Ancestral Organism(s) box.
4. Set the following parameters: Dish Size 30x30; 0.2% Per Site Mutation Rate; Place Offspring Near their parent; Uncheck all resources; Repeatability Mode Experimental; Pause At update 300.
5. Return to Map view. NOTE: Do not press Run until step #9 (on next page).
6. 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.”
7. In the Control menu choose “Do one update”.
8. Record the (absolute) fitness, energy acquisition rate, and offspring cost for each ancestor in Table 1 by selecting each and using the “Selected Organism Type” panel.
Before proceeding: Answer the following questions. How would you describe and compare the starting fitness values of each ancestor? Predict which ancestor will contribute a greater proportion of descendants to the population after 300 updates.

9. After responding to the two questions on adaptation and fitness (above), press Run.
10. Observe the number of descendants of each ancestor over time (colored differently on the Map, as per step #6). Also observe the number of individuals performing NOT over time (outlined on the Map and graphed in green, as per step #6).
11. After 300 updates, consult the “Population Statistics” panel and record in Table 2 the number of Avidians performing NOT and the number of viable organisms. Use these to calculate the percent of viable Avidians performing NOT and record this in Table 2. Note: We are focusing on only the viable organisms because only these have the ability to perform NOT and be rewarded for this by reproducing quicker.

Competition Treatment 2: Competing ancestors when notose is present.
1. Repeat steps 1-11 of Competition Treatment 1 using the same parameters and procedures, except add notose to the environment by marking only notose with a check.
2. Enter all of the data in Table 2 to the third tab of the Google Spreadsheet associated with this lab.

Questions:
A. What does fitness mean in an evolutionary context?
B. Does the fitness of an organism ever change?
C. How do we know when adaptive evolution has occurred?
D. Why is natural selection considered to be a non-random process?
E. How does this experiment test the influence of selection on the frequency of a phenotype?
F. On average, how does the final frequency of individuals performing NOT compare in the different environments?
G. Even after many thousands of updates of evolution in a selective environment for NOT performance, why might this phenotype never quite reach 100% frequency in the population?

Exercise 4: Population change without selection
**Learning outcomes:** Explain how non-adaptive evolutionary change occurs. Describe the relationship between population size and genetic drift, and the relationship between population diversity and genetic drift.

**Background:** Evolution can occur as the result of a number of different processes or mechanisms. Exercise 1 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 affect 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 random changes in the frequency of traits in a population as genetic drift. These changes are not the result of selection but instead are non-adaptive and are often described as resulting in neutral evolution (although genetic drift can affect a population’s average fitness).

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. 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.

Here 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 observing changes in the frequency of 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 proceeding:** The population always begins with 9 different individual Avidians.

A. Do you expect the relative frequency of each descendant type to stay the same, increase, or decrease over the course of each experimental replicate?
B. Will this expectation be the same or different across various population sizes? Why?
C. Do you expect the diversity of descendent types present at the end each replicate of 300 updates to stay the same, increase, or decrease?
D. Will this expectation be the same or different across various population sizes? Why?
E. If you repeated the same experiment, do you think you would get the same result? Why?
Visualizing evolutionary change without natural selection

1. Download the Workspace file “Ex4.vidaeedworkspace.zip” to your desktop. Note you do not need to extract, open, or edit this file. If your computer automatically unzips the folder, right click it and compress it in to a .zip folder again.

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

3. In the Population viewer, 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.

4. 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. This will save all of your settings for your replicate runs, although you’ll have to add organisms to the population each time.

5. Flip to Map view. 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. 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.

7. 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 below. Calculate frequencies by dividing the 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.

8. Graph these data in Figure 1 below.

9. In Table 2 record only the number of remaining descendant types (unique colors) at the end of 300 updates.

10. 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.

11. 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.

12. 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: If it is difficult to get an exact count of a particular ancestral organism, 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.

13. Start a new experiment by repeat steps 5-10 for population size 361 (Dish Size 19x19). Again, save your experimental configuration (“361_cells”) for use with each replicate. Note: If it is difficult to get an exact count of a particular ancestral organism, you can estimate the number based on the size of the dish. Record data in Table 2.

14. Enter your data from Table 2 to the fourth tab of the Google Spreadsheet.
Table 1. Frequency of one particular descendant type at 50 update intervals.

<table>
<thead>
<tr>
<th>Updates</th>
<th>Number of single ancestral type present (i.e. ref_ancestor) for 3 x 3 dish</th>
<th>Number of single ancestral type present (i.e. ref_ancestor) for 9 x 9 dish</th>
<th>Number of single ancestral type present (i.e. ref_ancestor) for 19 x 19 dish</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150</td>
<td></td>
<td></td>
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<td>200</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

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

<table>
<thead>
<tr>
<th>Number of descendant types present after 300 updates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicate</td>
</tr>
<tr>
<td>Size</td>
</tr>
<tr>
<td>9 (3 x 3)</td>
</tr>
<tr>
<td>81 (9 x 9)</td>
</tr>
<tr>
<td>361 (19 x 19)</td>
</tr>
</tbody>
</table>

Questions:
A. How does this experimental setup test the sole influence of genetic drift on the evolution of a population?
B. Did the same descendant types (colors) go extinct for each of your runs?
C. What impact does genetic drift have on diversity in an evolving population?
D. How does population size influence the effects of genetic drift?
E. 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?
F. 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?

Glossary


Death: In Avida-ED, an Avidian will die of “old age” if it does not self-replicate within a given number of instructions, set here as 20 times its genome length. It may die earlier if another Avidian divides and one of the daughter cells is placed by chance onto its spot on the grid, overwriting it.

Energy Acquisition Rate: Each organism in Avida-ED has a value associated with it that indicates how fast the organism is able to acquire the “energy” (SIPs) that it uses to execute instructions. Avidians acquire energy more rapidly by performing metabolic functions, which let them process resources in the environment. Energy acquisition rate is meaningful relative to other organisms—if organism X has twice the energy acquisition rate of organism Y than X should execute twice as many instructions in any given time frame.

Fitness: Measured in Avida as energy acquisition rate divided by offspring cost. Holding the environment constant, if organism X has twice the fitness of organism Y, it means that, on average, X will reproduce twice as quickly as Y. It is important to realize that, in terms of natural selection, the fitness of an organism must be compared to the organisms it competes with. An organism with a fitness of 0.3 will have a selective advantage in a population where every other organism has a fitness of 0.1, whereas this same organism with a fitness of 0.3 will be at a selective disadvantage in a population where every other organism has a fitness of 1.4.

Genome: This is the genetic code of an Avidian. It consists of a ‘string’ (list) of instructions comprised of the instruction set. Its biological analogy is an organism’s DNA. In AvidaED the size of the genome is fixed at a length of 50 instructions.

Gestation time/length: The number of instructions it takes for an organism to reproduce. This term was used in versions 1.0 through 2, but has been dropped in version 3 and replaced by offspring cost, which expresses reproduction in terms of the amount of energy required to replicate.

Instruction Set: The genome of an Avidian is comprised of a series of simple computer instructions. There are 26 instructions in the basic set. Different instructions are symbolized by the lower-case letters “a” through “z” and by different colored dots in the organism viewer.

Metabolic Functions: Organisms receive a base amount of energy, but may gain additional energy by evolving abilities to perform special “metabolic” functions or tasks. These tasks are logic functions performed on either one or two 32-bit numbers. The organism must manipulate these numbers with the instructions in its genome. In Avida-ED there are 9 functions that may be evolved (NOT, NAND, AND, OR_NOT, OR, AND_NOT, NEITHER_OR, EXCLUSIVE_OR, EQUALS). All functions require some complex sequence of instructions to perform. The simplest of these functions (NOT) takes a minimum of 6 instructions (we think). The most complex tasks (EXCLUSIVE_OR, EQUALS) take a minimum of 19
instructions (we think). The ability to perform a task multiplies the organism’s basic energy acquisition rate by the task’s energetic value.

**Metabolic Rate:** In Avida-ED versions 1 through 2, “metabolic rate” was a unitless number used to indicate how fast an organism is able to execute instructions. Version 3 replaces this with “energy acquisition rate” to highlight the energy metabolism.

**Mutation Rate:** The percent chance that each instruction will be copied incorrectly. So, if the per site mutation rate is 1%, there is a 1% chance that when an instruction is copied, it will end up as any one of the 26 possible instructions (one of which is itself, so it could ‘mutate’ back to itself). With a 1% per site mutation rate, if 100 instructions are copied one of them will be mutated on average (although this number could be higher or lower in any instance). What mutations will occur is determined by a random number generator and will be different each run unless the demo mode is checked to force exact repeatability. In Avida-ED 1.0 - 3.0 only point mutations are allowed but no deletions or insertions in order to keep the genome at a uniform size to preserve legibility.

**Offspring Cost:** The amount of energy it takes for an organism to reproduce (i.e. to execute the number of instructions it takes for it to self-replicate).

**Time:** Avida time is internally constant and is measured in “updates”. Avida standard time is not constant relative to real time; a single update for a large population takes longer in real time than an update for a small population.

**Update:** An “update” is the unit of time in Avida-ED. Avida time is internally constant, but is not constant relative to real time; a single update for a large population takes longer in real time than an update for a small population. “Update” is defined as the passage of enough SIPS (executions of single instructions) such that each organism, on average, has executed 30 instructions (however, more fit organisms will most likely have executed more and less fit organisms will have executed fewer).

**Viability:** Organisms that have the ability to develop and reproduce are viable. Inviable organisms are those that are unable to replicate. In the fitness mode of the population viewer, inviable Avidians are shown colored grey.