Lab 6: Avida-ED

Prepare for this lab by reading the Discover magazine article by Carl Zimmer, “Testing Darwin” linked to the lab website.

Genetic Basis of Evolution

*Evolution* is defined as change in the genetic characteristics of populations over time. More specifically, evolution is *change in allele frequencies in a population over time*. Recall that a *gene* is a sequence of DNA nucleotides that codes for a particular protein. All members of a species may share a common set of genes, but there can be many versions of any given gene – these alternative versions of a gene are referred to as *alleles*. New alleles are introduced into populations when existing alleles experience mutations (random changes in the sequence of DNA nucleotides). Because alleles vary in their specific nucleotide sequences, the resultant proteins that are produced as a result of their coding will also vary.

Environment Acts on Phenotypic Variation

Evolution is a process that can only be observed at the level of populations. Despite the fact that we frequently speak colloquially about an individual’s “personal evolution” or the “evolution of our thinking” – individuals don’t evolve, populations do! However, attributes of individuals are extremely important for our understanding of how populations evolve.

In class and throughout this course, you have observed that individuals within a population can demonstrate tremendous variation in *phenotype*. An organism’s phenotype results from both expression of the organism’s alleles and the influence of its environment. For example, a plant may have the alleles for tall height, but if grown in the shade or left unwatered, it may remain short and never become tall. In our discussion of evolution, phenotypes are of critical importance because it is the phenotype that interacts with the environment. In our plant example, the short plant and tall plants will interact with their environment differently – they may have different abilities to access sunlight, secure water and nutrients, or gain visibility to pollinators. As a result, they will differ in their ability to *survive and reproduce*. Individuals who have the greatest potential to reproduce are said to be the fittest, or have the highest fitness. *Fitness* is defined as an individual’s reproductive potential, or their capacity to produce viable and fertile offspring. Evolutionarily, reproduction is key because it is only through reproduction that individuals’ alleles are represented in future generations. In fact, in the context of evolution and fitness, survival is only important to the extent to which it impacts an organism’s ability to reproduce!

Phenotypic differences may be visible (e.g., height) but many are not (e.g., disease resistance, drought tolerance). A wide variety of physical, behavioral, and physiological traits are not necessarily visible, but have critical roles in an organism’s survival and reproduction. Individuals that have phenotypes that enhance their fitness (improve their chances of reproduction) are said to be “selected”, or favored, by the environment. *Selection* is differential reproduction among individuals that vary phenotypically. Because phenotypes have a genetic basis, individuals that are selected by their environment are most likely to contribute alleles to future generations.

How Can We Study Evolution?

In order to quantify “change in allele frequencies” for a population, researchers must follow the transfer of alleles across multiple generations. In many cases, traits of interest change on the order of tens to tens of thousands of generations. Researchers at MSU have successfully measured evolutionary change in populations of hyenas, trees, and other organisms, but this can take a very long time. For some organisms and some traits, we simply may not live long enough to witness the change we are interested in measuring.

Microbial biologists may have an edge in the study of evolution due to the rapid life cycles and unique genetic characteristics of their study organisms. Unlike eukaryotic organisms, prokaryotes (bacteria) possess only a single loop of DNA for a chromosome. As a result, bacteria are haploid – their alleles occur individually rather than in pairs. One consequence of having only a single chromosome is that all alleles in a bacterial genome have the potential to be expressed in the phenotype – there is no masking of recessive alleles by dominant ones.
Bacterial reproduction is asexual. Bacteria replicate their genome and pass copies of their DNA on to daughter cells through a process that resembles mitosis. As in mitosis, the daughter cells are genetically identical to one another (i.e., clones). However, whenever DNA is replicated, the potential exists for random mutations to occur. Mutations that occur during bacterial DNA replication will be present in the generation of daughter cells that immediately follows division of the bacterial cell. This rapid appearance of mutations introduces variation very quickly in a bacterial population. A steady stream of new variation through random mutations creates tremendous potential for bacterial populations to respond quickly to environmental change.

Despite the advantages of bacteria as model organisms for evolutionary research, even bacteria pose difficulties. Some evolutionary research questions, such as the issue of irreducible complexity discussed in the Zimmer article, require extremely large numbers of individuals and many, many generations in order to reach confidence in conclusions.

**How Can Avida-ED Facilitate the Study of Evolution?**

“Avida-ED” is the name for the educational version of “Avida”, a software application developed by evolution researchers at Michigan State University. Digital organisms, called Avidians, share many attributes of living organisms and may be thought of as analogous to bacteria.

The Avidian genome contains instructions that allow Avidians to consume resources from their environment and reproduce asexually by copying their genome to their offspring. Resources in the Avidians’ environment vary in their complexity; more complex resources are associated with greater rewards (more energy), but are more difficult to process. During replication, there is a small chance that an Avidian’s genome will acquire a random mutation that will be passed on to its offspring and contribute to variation in the population. Avidians that inherit mutations that allow them to consume more complex resources or to consume resources faster will survive and reproduce more, and thus have higher fitness than Avidians who cannot.

Perhaps the most difficult conceptual leap associated with using Avida-ED is to understand that it is not a simulation of evolution, it is an instance of it occurring in real-time!

**What you will do:**

During this week’s lab, you will work with your group to explore the Avida-ED software through a series of guided activities. In next week’s lab, you will work together to design and conduct an original experiment that tests a question you have about evolution using Avida-ED.

**Objectives**

*In this lab, you will work with your group (or in pairs) to:*

1) become acquainted with Avida-ED, a research platform developed by evolutionary biologists at Michigan State University for studying a variety of evolutionary processes,

2) use Avida-ED to explore simple questions about evolution, and

3) design an original experiment to test a question about evolution using the Avida-ED software. During next week’s lab, you will carry out your experiment.
Methods
Part 1. Get Familiar With the Avida-ED Interface.

A. Open Avida-ED by clicking on the link from the desktop (or by downloading Avida-ED from http://avida-ed.msu.edu/). Your interface will look much like the one below. Note the layout and specific location of the following:
- Viewers Panel – we will stay in the Population view for most or all of our work.
- Freezer – just as a freezer in a lab would allow a researcher to save sample organisms or entire Petri dishes containing whole populations of organisms, the Avida freezer allows you to save individual specimen Avidians (organisms) or Petri dishes containing populations of Avidians.
- Petri Dish – the gray screen in the center represents a Petri dish where Avidians will be transferred and allowed to reproduce.

B. Start a run.
1. Click on the “Control” menu near the top of the screen and select “Start New Experiment”.
2. Click on the “@ancestor” icon in the Freezer and drag it to the Petri dish. An outline of a small square should appear in the black area. This is your ancestral Avidian!
3. Note the Time (Updates) counter – it will default to “-1” before a run. In Avida-Ed, Time/Updates are equivalent to generations.
4. Select “Fitness” from the dropdown menu to the right of the counter.
5. Start a run by clicking the Play/Pause button.
6. Stop the run at approximately 200 updates (this will only take a few seconds).
C. Process the run with your TA.
There are now many Avidians on your plate – this is a result of reproduction from the original ancestor. In the biological world, Avidians are most analogous to bacteria. How is bacterial reproduction similar/different from other organisms, such as mammals?

1. **Population Statistics.** This panel (upper right) provides information about the whole population of Avidians currently occupying your plate, including:
   - **Population Size** – indicates the number of Avidians currently in your population. *How many Avidians are in your population?*
   - **Avg. Fitness, Avg. Metabolic Rate, Avg. Gestation, Avg. Age** – average information for each of these parameters is provided. We will focus primarily on fitness.
   - **Functions/Orgs Performing** – this table provides a list of functions or “substrates” that are available to the Avidians in their environment. Think of these substrates as “food” or different types of sugars that the Avidians can make use of for their metabolic needs. The substrates are organized in order of their complexity – “Not” (notose) is the simplest substrate to process; “Equ” (equose) is the most complex and difficult substrate to process. Organisms that have the function that enable them to process more complex substrates receive greater metabolic rewards (i.e., more energy) compared to organisms that are only able to process simple substrates. *How many individuals on your plate are able to process Notose? Are any individuals able to process more complex substrates?*

2. **Org. Clicked on Report.** This panel (left of Population Statistics) provides similar information to the Population Statistics panel, but for specific individual Avidians selected on the plate. Click on an Avidian in the plate to illustrate the features in this panel.
   - **Name** – each Avidian on the plate is associated with a unique identifier. You may find that some clusters of Avidians have exactly the same name – you may think of them as clones (no genetic differences).
   - **Fitness** – represents the fitness value for an individual. If the dropdown menu under the Petri dish is set to display “fitness”, then the color spectrum indicates the range of fitness values for the population. Individuals that appear in different colors will have different fitness values associated with them.
   - **Metabolic Rate, Gestation, and Age** – values for selected individual.
   - **Ancestor** – indicates the ancestor from which a particular Avidian descended. At this point, we have only been working with a single ancestor, so all Avidians will be descendents of the same ancestor.
   - **Functions/Times Performed** – indicates the functions that an Avidian has evolved and the number of times that it has used the function in its lifetime. To quickly find out which Avidians have evolved one of the functions, click on the function (e.g., “Not”) in the Population Statistics panel – all Avidians that have the ability to use the Not substrate become highlighted. Note that this button remains selected until you deselect it.

3. **Environmental Settings.** Click on the “Flip to Settings” arrow at the top right of the Petri dish. This panel displays a number of settings that can be manipulated within the Avida-ED software. In a later activity you will be asked to change some of the settings, but for now, use the default settings:
   - **Per Site Mutation Rate = 2%**.
   - **World Size = 60 x 60 cells.** Changing the world size changes resolution on the Petri dish. A large world size allows you to view more organisms at a time, but it will be more difficult to distinguish among neighboring individuals.
   - **Offspring will be positioned in the plate near their parent.** *Why is this more realistic than random placement?*
   - **Resources.** When all resources are checked, they will all be present in the environment. Deselecting a resource removes it from the environment.
   - **Repeatability = experimental.** This mode reflects a realistic progression; “demo mode” does not allow for natural variation.
   - **Pause run.** If you want to stop the run at a specific number of updates, you may enter that value here.
Part 2. Can we predict the path of evolution for a population?

A. Clear your Petri dish by selecting “Start New Experiment” from the Control Menu. You may discard your previous dish.

B. Drag the “@ancestor” Avidian onto the Petri dish and run Avida-ED for 1000 updates using the default settings (mutation rate 2.0%, all resources present, experimental mode). Stop at intervals of 100 updates and record data in the table below.

Table 1. Path of Evolution, Run 1.

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C. Make a prediction. Do you expect the results to be the same if you run the experiment again in exactly the same way. Why/why not?

D. Clear your Petri dish and run the experiment a second time. Record your data for Run 2.

Table 2. Path of Evolution, Run 2.

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E. Compare Run 1 and Run 2. How were they similar/different? Explain your results in terms of your current understanding of mutation, inheritance, and evolution.
*Before clearing your Petri dish, put some Avidians in your freezer!*

Freeze several (5 or 6) Avidians with the “Not” function. In addition, freeze several Avidians with higher-level, more complex functions (e.g., Ant, Nor, Xor).

How to freeze Avidians with the “Not” function:
1. Display Avidians with the “Not” function by clicking on the function button for “Not” in the Population Statistics panel.
2. Click on one of the highlighted Avidians.
3. Check to ensure that the Avidian ONLY has the “Not” function and not additional functions, such as “Nan” or “And”. (Remember, data for individual Avidians is in the “Org. Clicked on Report” panel.)
4. Drag the Avidian to the Freezer area.
5. You will be prompted to “Create a Workspace” if you haven’t done so already. Your workspace archives the files, data, and records that you save within Avida-ED.
6. You will then be asked to assign a name to the Avidian you are saving. Include the name of the function in the name so that it will be easy to identify later (e.g., Not1).
7. Repeat for 4 or 5 additional Avidians that only have the “Not” function and name them: Not2, Not3, etc.

Note: You will only need one “Not” Avidian for the next experiment, but it is not uncommon for an Avidian to be “sterile”! When you transfer a sterile Avidian to the plate, it simply doesn’t replicate. Saving several replicates will ensure that at least one of them will be able to reproduce successfully.

Repeat steps 1-7 above for a few types of Avidians with higher-level functions (e.g., Ant, Nor, Xor).

### Part 3. How does competition affect evolutionary outcomes?

A. Clear your Petri dish by selecting “Start New Experiment” from the Control Menu.

B. Drag an “@ancestor” and an Avidian you froze that has the “Not” function onto the Petri dish. Two Avidians should appear on your plate. Recall, “Not” is the simplest of the resources available. An Avidian with the “Not” function can only process this low-level resource; the @ancestor is not able to process any of the available resources. In a sentence or less, predict what you expect to happen when you run this experiment.
C. Run the experiment for 500 updates. Stop at 100-update intervals to record your population data.

Table 3. Run 1: @Ancestor vs. “Not” Ancestor – All Resources Present.

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D. Use the dropdown menu under the Petri dish to select “Ancestor Organism”. This displays the population color-coded to show which Avidians descended from each of the 2 ancestors. Estimate the proportion of the total population that descended from each starting organism.

% Population descended from @Ancestor: ______ % Population descended from “Not” Ancestor: ______

Are they approximately equal? Why/why not?

E. Clear your Petri dish to start a new experiment (if you like, you may save the Petri if you want to look at it again later). Choose the “Flip to Settings” and uncheck the “Notose” resource – this removes “Notose” from the environment. Predict what will happen if you repeat the experiment in an environment without notose. Do you expect the outcome to be the same? Why/why not?

F. Repeat the experiment using both the @Ancestor and “Not” ancestor to begin. Run the experiment for 500 updates. Stop at 100-update intervals to record your population data.

Table 4. Run 2: @Ancestor vs. “Not” Ancestor – Notose removed from environment.

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G. Estimate the proportion of the total population that descended from each starting organism for this run.

% Population descended from @Ancestor: ______ % Population descended from “Not” Ancestor: ______

Are they approximately equal? Why/why not?

H. Compare Run 1 and Run 2 for this experiment – how were they similar/different? Explain your results.
Part 4 (Optional, TA’s discretion). How does an ancestor affect evolutionary outcomes?

A. Clear your Petri dish by selecting “Start New Experiment” from the Control Menu.

B. Choose an Avidian from your freezer that is able to process a complex resource (e.g., ant, nor, xor). Use this as an ancestor and allow Avida to run for several updates.

As Avida runs, focus your attention on the Population Statistics panel. Pay attention to: 1) the average fitness of the population as the experiment runs, and 2) the appearance of specific functions within the population and number of organisms performing them.

C. Repeat step “B”, but using the default ancestor (capable of no functions).

D. How does using an ancestor with advanced functions compare with using the default ancestor in terms of: 1) the time required to evolve a diversity of functions, and 2) the average fitness of the population?

Part 5. What other questions would you like to test?

Brainstorm several questions you could ask and answer using the Avida software. Think about questions that are both interesting and feasible with the software. Are there variables you will need to control in the experimental design? How many replicates will you need to reach meaningful conclusions? Next week in lab, you will choose one of the questions and design a simple experiment in Avida to test it.

*Show your completed group work to your TA before leaving lab today. There is only 1 postlab due for the 2-week Avida-Ed lab. The Avida-Ed postlab is due at the end of next week’s lab and will represent your original question, experimental design, results, and conclusions (see lab website). You do not have to write a postlab based on today’s work.