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**Exercise 3 – Exploring Fitness and Population Change
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?

**Student Learning Goals**

* Students will be able to explain the concept of fitness as used in biology, and that an organism’s fitness may change, depending upon its environment.
* Students will be able to explain the process of adaptation by natural selection, leading to genetic frequency change in a population over many generations.
* Students will be able to explain that natural selection is non-random.

**Questions to Consider While Doing Exercise #3**

* What does “fitness” mean, in an evolutionary context? Why is this a useful term for biologists studying evolutionary patterns and processes?
* Does the fitness of an organism ever change? Explain.
* What do biologists mean by, “adaptive evolution”? How do we know when adaptive evolution has occurred?
* Why is natural selection considered to be a “non-random” process?
* Can we observe evolution occurring?

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 reproduce more than others; these individuals have greater fitness relative to organisms lacking such advantageous traits. These advantageous traits, or adaptations, are favored within the selective environment and will become more frequent in the population over generations of change. An individual’s fitness is dependent upon the interaction between its phenotype and the selective environment, and is relative to other individuals in the population. In other words, when you measure the fitness of an organism, you get a value specific to the environment in which you measured it. If you then move the organism to a different environment, its fitness can change.

In Exercise #3, we will observe the process of natural selection in action: beneficial mutations will randomly occur for some individuals, these mutant organisms will have a greater fitness due to their adaptation to the environment and will tend to increase in frequency in the population over successive generations.

**Measuring Fitness**

In biology, the concept of fitness has crucial importance for how evolution by natural selection occurs, but the term fitness is often difficult to define and even more difficult to measure. For our purposes we will simply state that fitness is the number of offspring an individual contributes to later generations, or lifetime reproductive success. 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 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 quicker. 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 needing to be executed before reproduction is completed.

**Frequency Change in a Population**

Evolution can be defined as change in genetic frequencies over successive generations. 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 “gone to fixation”, which is the maximum possible frequency in the population (frequency = 1.00). Evolution is what occurs between these frequency extremes. Unlike 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 frequency in an evolving Avidian population. Thus we can discuss the fitness of an organism as equivalent to the fitness of its genotype, with all identical Avidians having the same fitness.

To understand how natural selection operates, we need to consider fitness both of the individual organism, and the larger population. Populations tend to have a great deal of genotypic variation (ultimately due to mutation), and thus phenotypic variation, and thus the individuals present have a range of fitness values. A specific genotype will change in frequency at a rate directly proportional to the ratio of its fitness compared to the average member of the population. The greater the value’s magnitude, the faster the population will change due to selection. A fitness ratio greater than one indicates that the genotype will increase in frequency, 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 calculated as the average fitness of all distinct genotypes that confer the phenotype.

In this exercise, we explore relationships between variation, selection, and fitness by competing organisms against each other that are adapted to different environments. Organisms that are used as ancestors in these competitions are adapted to different selective environments in Ancestor Treatments 1 & 2. In Ancestor Treatment 1, we evolve an Avidian population with no resources present (all resources are absent), while in Ancestor Treatment 2, only the resource nanose 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”!

Competitions such as these allow us to explore the concepts of genotypic or phenotypic frequencies changing in a population over time. 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.

To begin Exercise #3, we will “grow” Avidians both in the absence and in the presence of the resource, nanose. At the end of 1000 updates, we will “freeze” one organism grown in each of these sets of conditions. In the second part of Exercise #3, we will determine the fitness of each of these organisms in the two different environments (absence of all resources; presence of nanose only). Then, to finish up, we will “battle” the two organisms against each other, both in the no resource environment and in the nanose-only environment. Who do you think will win these battles?

**Freezing individual Avidians evolved in different selective environments**

**Ancestor Treatment 1 – Evolving a high fitness organism when no resources are present (No Resource Environment).**

1. In the Population viewer, flip to Set-up.
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 (your choice; does not have to be the organism in the population with the highest fitness). This Avidian *should not perform any function.*
6. Freeze the individual by choosing the “Save Selected Organism” option from the Freezer menu. Name the organism **“No-Resource\_ancestor”**.
7. Record in Table 1 (below) the Fitness, Energy Acquisition Rate, and Offspring Cost of your selected Avidian. These values should go into the three upper left cells of the table.
8. **Checking for reproductive ability**: Switch to the Organism viewer to confirm your frozen organism’s phenotype.
9. Drag the organism from the Freezer to the genetic code box. Select Run, then End.
10. A genetic code symbol labeled “Offspring Genome” should appear within the offspring’s genome, and the Details window should indicate that it cannot perform any functions. If your frozen organism unable to reproduce, return to the Population viewer and repeat steps 5-10. (Note: right-clicking a Freezer item allows you to rename or delete an organism.)

Avidian Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Fitness: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Energy Acquisition Rate: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Offspring Cost: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Record these data in Table 1 in the upper left shaded squares.**

**Ancestor Treatment 2 – Evolving a high fitness organism when *only* nanose is present (Nanose environment)**

Repeat Ancestor Treatment 1 with the same parameters, ***except*** add nanose to the environment by **marking nanose 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 NAN**. If you do not have an organism that can perform NAN after 1,000 updates then continue the run until an individual that can perform NAN occurs in the population. Confirm its phenotype and ability to reproduce following steps 8-10, making sure that your organism successfully performs NAN.

The selected frozen organism should be named **“Nanose\_ancestor”**. Record the following data below:

* Avidian Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
* Fitness: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
* Energy Acquisition Rate: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
* Offspring Cost: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Record these data in Table 1 in the lower right shaded squares.**

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

|  |  |  |
| --- | --- | --- |
|  | “**No-Resource\_ancestor**”(does not perform NAN function)  | “**Nanose\_ancestor**” (performs NAN) |
| Selective Environment | Fitness (absolute) | Energy Acq. Rate | Offspring Cost | Fitness (absolute) | Energy Acq. Rate | Offspring Cost |
| All resources absent |  |  |  |  |  |  |
| Nanose present |  |  |  |  |  |  |

**Finding the fitness of the two Avidian ancestors in the *other* environment**

To complete Table 1, we need to determine the fitness of the No-Resource\_ancestor in the Nanose-only environment and the fitness of the Nanose\_ancestor in the No Resource environment. We can do this as follows:

1. In the Control menu choose “Start New Experiment”.
2. In the Population viewer, flip to Set-up.
3. Drag the “No-Resource\_ancestor” from the Freezer to the Ancestral Organism(s) box.
4. **Set the mutation rate to 0.2%.**
5. Set the other parameters as follows:
Dish Size 30x30; Place Offspring Near their parent; **Uncheck all resources except Nanose**; Repeatability Mode Experimental; Pause **At update 1**.
6. Return to Map view. Below the Map, set Mode to “Ancestor Organism.” In the “Population Statistics” panel select the “NAN” button. Change the graph’s Y-axis to “Number of Organisms.”
7. In the Control menu choose “Do one update”.
8. Select your organism, and record in Table 1 its Fitness, Energy Acquisition Rate, and Offspring Cost. These values should go into the three lower left cells of the table (Nanose present, No-Resource Ancestor)
9. Repeat Steps 1-8 for the Nanone\_ancestor. Be sure to **uncheck ALL resources**.
10. After one update, record your Avidian’s Fitness, Energy Acquisition Rate, and Offspring Cost in the three upper right cells of Table 1 (All resources absent, Nanose\_ancestor)

***In the spaces provided, respond to the following questions about the data in Table 1***

* Are the fitness values for the No-Resource ancestor the same, or different, in each of the two environments in Table 1? How about the Nanose\_ancestor? Do you think these data make sense? *Why or why not?*
* Which ancestor has a higher fitness in the All resources absent environment? How about the Nanose present environment? Do you think these data make sense? *Why or why not?*
* If you competed the two evolved ancestors in the No Resources environment, which ancestor do you predict will “win” (that is, contribute a greater frequency of descendants to the population)? Explain your reasoning.
* If you competed the two evolved ancestors in the Nanose Only environment, which ancestor do you predict will “win” (that is, contribute a greater frequency of descendants to the population)? Explain your reasoning.

**Competing individuals in their ancestral selective environments (The Battles): Let’s test our ideas!**

Now it’s time to test our ideas! In this part of Exercise #3, we will compete the two evolved ancestors in the No Resources environment and in the Nanose Only environment.

**Competition Treatment 1 – Competing ancestors when all resources absent**.

1. In the Control menu choose “Start New Experiment”.
2. In the Population viewer, flip to Set-up.
3. Drag **both** “No-Resource\_ancestor” and “Nanose\_ancestor” from the Freezer to the Ancestral Organism(s) box.
4. **Set the mutation rate to 0.2%.**
5. Set the other parameters as follows:
Dish Size 30x30; Place Offspring Near their parent; **Uncheck all resources**; Repeatability Mode Experimental; Pause **At update 300**.
6. Return to Map view. Below the Map, set Mode to “Ancestor Organism.” In the “Population Statistics” panel select the “NAN” button. Change the graph’s Y-axis to “Number of Organisms.”
7. Press Run.
8. 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 NAN over time (outlined on the Map and graphed in green, as per step #6).
9. After 300 updates, record in **Table 2** the number of Avidians on the plate who descended from the Nanose\_ancestor. (This will be a bit of a pain, but you only have to do it once!)

***After completing Competition Treatment 1:***

* Compare the relative frequencies of the descendants of the ancestors by observing the two colors on the Map. Does this match your prediction? Explain.
* Who won the battle?

**Competition Treatment 2 – Competing ancestors when nanose present**.

Repeat steps 1-9 of Competition Treatment 1 using the same parameters and procedures, except add nanose to the environment by **marking nanose with a check**. After 300 updates, record in **Table 2** the number of Avidians on the plate who descended from the Nanose\_ancestor. (This should be easy this time!)

***After completing Competition Treatment 2:***

* Who won the battle this time?
* Compare the relative frequencies of the descendants of the ancestors by observing the two colors on the Map. Does this match your prediction? Explain.
* How does this differ from what you observed in Competition Treatment 1?

**Table 2. Frequency of individuals performing NAN after 300 updates of competition in two different selective environments.**

|  |  |  |  |
| --- | --- | --- | --- |
| Selective Environment | Number performing NAN | Number of viable organisms | Percent of viable organisms performing NAN |
| All resources absent |  |  |  |
| Nanose present |  |  |  |

**Recording your data.** Experiments in biology often involve investigating processes or phenomena with lots of variation, we will therefore be examining the data generated by all students in the course.

Enter your *final column of Table 2 data*, the percentage of viable Avidians performing NAN in each experimental treatment, in the document linked here:

**INSTRUCTORS - IF YOU WISH TO USE OUR DATA ANALYSIS SPREADSHEET PLEASE CREATE A NEW LINK FOR YOUR CLASS. THE ORIGINAL FULL DATA ANALYSIS SPREADSHEET CAN BE FOUND HERE AND SHOULD BE COPIED FOR YOUR COURSE USE.**

[Exercise 3 - Data Analysis](https://docs.google.com/spreadsheets/d/1EO1Eb90cZ-b5tdAfcYK6wS0Hu7FNFsyFBCbDShugTqg/edit#gid=1097002526)

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 final column of Table 2 data in the following rows. The instructors will periodically collect these data, anonymize it, and add it to the course data set.

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

* Use the concept of adaptation to describe and compare the starting fitness values of each ancestor in each of the two environments.
* On average, how does the final frequency of individuals performing NAN compare in the two different environments?
* Describe a mechanism that could explain this result.
* Explain adaptation in the context of the *all resources absent* environment.
* Explain adaptation in the context of the *nanose present* environment.
* **Brain Buster –** Even after many thousands of updates of evolution in a selective environment for NAN performance, why might this phenotype never quite reach 100% frequency in the population? Describe your hypothesized mechanism. Hint: observe the number of individuals performing NAN over time (outlined on the Map and graphed in green) near the end of Competition Treatment 2, and allow the experiment to run longer if you wish.

***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?