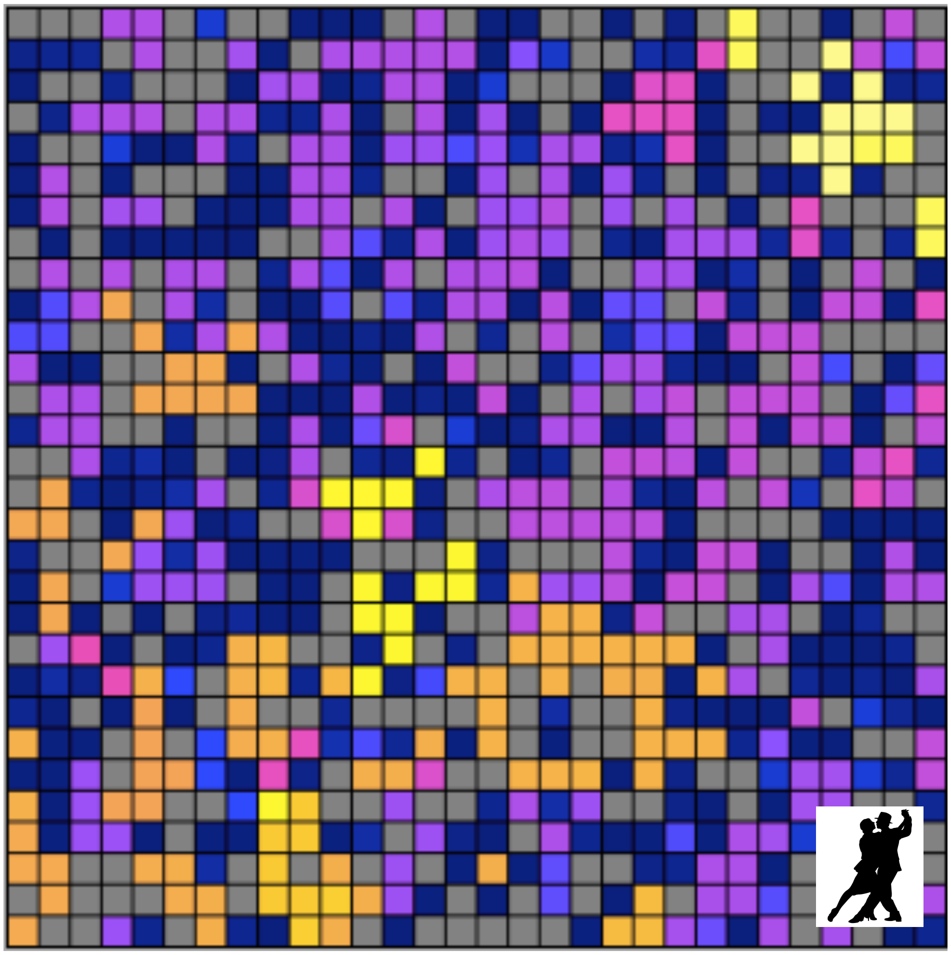
Avida-ED Lab Book

Argentina 2019
Workshop Version



Avidian Tango?
(freeimages.com)

Avida-ED Project Curriculum Development Team

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Preface

This version of the Avida-ED Lab Book was prepared for the Argentina 2019 Avida-ED Workshop at the Faculty of Exact and Natural Sciences of the University of Buenos Aires. The Lab Book represents the cumulative work many people, including the present Avida-ED Project Curriculum Development Team (Robert T. Pennock, Louise Mead, Jim Smith, and Mike Wiser), with significant contributions from former members Cory Kohn, Amy Lark, and Wendy Johnson. The Lab Book, as presented here, was assembled from component files by Jim Smith on May 9, 2019.

Though the activities in this version of the Avida-ED Lab Book are similar to their past iterations, experienced Avida-ED instructors should re-familiarize themselves with the content. Of particular note, the Lab Book now contains a new Exercise #4, which investigates population change due to random processes – genetic drift.

Based on feedback from Active LENS faculty development workshop participants, beginning with the 2016 edition we incorporated terminology changes to help students better understand the factors that lead to changes in an organism's fitness. In particular, what was previously called "metabolic rate" is now called "energy acquisition rate" and "gestation time" is now called "offspring cost." Fitness is defined as the *energy acquisition rate divided by the offspring cost*, which equals the rate of production of offspring.

This edition has updated screenshots and instructions for the web-based version of Avida-ED (vers. 3.0), released in 2016. This version was developed by the Avida-ED software group, Robert T. Pennock, Charles Ofria, Richard Lenski, Diane Blackwood and Matt Rupp. Diane Blackwood is the lead programmer for this version.

The Avida-ED Lab Book contains materials modified from versions originally developed for various undergraduate biology curricula. The Introduction activity was originally produced by Wendy Johnson, Robert T. Pennock, and Louise Mead. Exercise 1, Exercise 3, and the Independent Research activity were originally produced by Robert T. Pennock and Amy Lark. Exercise 3 as presented here has been modified significantly from its original form by Jim Smith and Cory Kohn. Exercise 2 was originally produced by Jim Smith, and Exercise 4 by Cory Kohn.

Getting the Avida-ED software

Avida-ED 3.0 is the current version of the software and its use remains free of charge. With this release, Avida-ED now runs in a browser window instead of as a stand-alone application. This solves the problem of developing and maintaining separate applications for MacOS, Windows, and other operating systems. Eventually, it will run fully in all major browsers, but at the moment *full functionality requires Firefox, Safari, or Chrome*.

- Access the latest software, curriculum, and news from the Avida-ED home page: http://avida-ed.msu.edu/ (mirror at: http://avida-ed-mirror1.beacon-center.org/

  The direct URL for Avida-ED 3.2 is:
  https://avida-ed.msu.edu/app/AvidaED.html

  We also have a mirror location for Avida-ED 3.2:
  http://avida-ed-mirror1.beacon-center.org/AvidaED.html

- Once Avida-ED has loaded in the browser, it runs entirely on the computer's CPU, not on any remote server. This means it requires an internet connection only to initially load, but not to run.

- For users who want to keep a local copy on their computers for occasions when they have no internet access at all, the Avida-ED web site also has links for self-hosted versions for Macs and PCs.

Note: Previous stand-alone versions of the software—Avida-ED 2.0 and 1.2 for Mac OSX and Avida-ED 1.2 for Windows—are no longer maintained but remain available for historical reference.

a. Go to http://avida-ed.msu.edu/
b. Click on "Vintage Archive" on the bottom left.
c. Select the operating-specific version to begin your download.
d. Open the file and drag the Avida-ED icon to the desktop. Open the application.

An Avida-ED Curriculum

Introduction – Avida-ED and Digital Evolution

This introductory activity demonstrates how Avida-ED can be used, while initiating an exploration of biological concepts further addressed in the four Exercises. See the *Discover* Magazine article "Testing Darwin" by Carl Zimmer for background on Avida-ED, and the Avida-ED Quick Start User Manual and an online video <https://avida-ed.msu.edu/support/> for further help.

Exercise 1 – The Introduction of Genetic Variation by Random Mutation
Exercise 2 – Exploring Random Mutation and Selection
Exercise 3 – Exploring Fitness and Population Change under Selection
Exercise 4 – Exploring Population Change without Selection

This series focuses on the processes of mutation, natural selection and genetic drift by highlighting the basic components of evolutionary change – variation, inheritance, selection, and time. If these components are present in a system then evolution will proceed, whether the system is biological or digital. A population of organisms can exhibit both genotypic (genetic information) and phenotypic (expressed trait) variation. Mutation is fundamental to the inheritance process because it generates 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. Natural selection is not the only fundamental evolutionary process; genetic drift, or evolutionary change due to random sampling in a population, is also an important influence on genotypic and phenotypic frequencies. Time plays a fundamental role throughout all of these processes.

Exercise 1 focuses on inheritance and genotypic variation by investigating mutation, the ultimate source of variation. Exercise 2 explores how genotypic variation influences phenotypic variation and how time plays a fundamental role in the process. Exercise 3 synthesizes the components of natural selection by focusing on how relative fitness differences lead to population change. Exercise 4 expands upon processes that produce evolutionary change via a model visualization of genetic drift.

Independent Research – Experimental Evolution Project

Exercises 1-4 prepare students to propose and carry out scientific experiments in digital evolution using Avida-ED. The suite of activities described in the "Independent Research – Experimental Evolution Project" section includes brainstorming research interests, formalizing a proposal, performing experiments with replication, and collecting, analyzing, and interpreting data. The final product is a research poster that students can present to peers, instructors, and passersby.

Introduction - Avida-ED and Digital Evolution

Avida-ED is the educational version of Avida, a software platform created by a group of computer scientists and software engineers interested in the experimental study of digital organisms in order to better understand how biological evolution works. Both Avida and Avida-ED provide an instance of evolution in a model environment. The evolution itself is real; the digital organisms are subject to the same processes as biological organisms, such as reproduction, mutation, and natural selection. Scientists use Avida to study how digital organisms evolve, and examine questions related to the evolution of complex features, sex, parasitism, cooperation, and foraging behavior. Avida has even been used to confirm the outcomes of ongoing biological experiments. This is possible because the process of evolution is “substrate neutral,” meaning that when a system possesses three key characteristics – variation, inheritance, and selection – evolution will inevitably result.

Using the powerful study system of Avida-ED, you will be able to design and perform your own experiments to test hypotheses about evolution in much the same way that researchers use Avida. An arrow ➜ indicates a question you should answer.

Questions to Think About as you Go Through the Introduction

- What is Avida-ED (and how does it work)?
- Can we study evolution by performing experiments? Explain.
- How is Avida a useful tool for biologists? What are the strengths and limitations of this model?

Tasks

1. Begin by reading the article by Carl Zimmer **“Testing Darwin”** that appeared in *Discover* Magazine in 2005. The article can be found in the Lab Book or online here: http://discovermagazine.com/2005/feb/cover
2. **Start Avida-ED**, available at URL below. The program now runs in a web browser. Please note that the program may take a minute to load, be patient. Navigate to: https://avida-ed.msu.edu/app/AvidaED.html
3. Watch the **Avida-ED video tutorial** to help you explore the application’s controls. It can be found in the support section of the Avida-ED website or on YouTube: https://youtu.be/mJwtg0so4BA
4. Familiarize yourself with the **Avida-ED Quick Start User Manual**, which can be found at the end of this Lab Book. Along with a Glossary, this is an invaluable resource on how the program works.
5. **Continue** with the rest of this Introduction activity. It will demonstrate how the program can be used while introducing biological concepts to be further explored.

Avida-ED Workspace Areas

1. The “Navigation” area allows you to switch among three *viewer modes*:
    a. Population – the Virtual Petri Dish (Map), experimental setup, and statistics panels; allows evolution experiments to be conducted and data collected
    b. Organism – the execution of an individual’s genomic instruction sequence; allows the confirmation of phenotypes and display of offspring’s mutations
    c. Analysis – the collected data from a finished experiment; allows comparisons of population variables (e.g. average fitness) over time
2. The “Freezer” area allows you to collect and use *saved materials*:
    a. Configured Dishes – experimental setups to aid in treatment replication
    b. Organisms – individual Avidians for use as ancestors in Population viewer experiments, or for examination with the Organism viewer
    c. Populated Dishes – experimental populations for continuing experiments using the Population viewer, or for examination with the Analysis mode
3. The “Lab Bench” area is *where things happen*; experiments are performed, Avidian individuals and population are observed, and data are collected.

When in the Population viewer, the Lab Bench consists of the Virtual Petri Dish (or Map), which is the digital environment in which the Avidians reproduce and the Avidian population evolves (Figure 1).

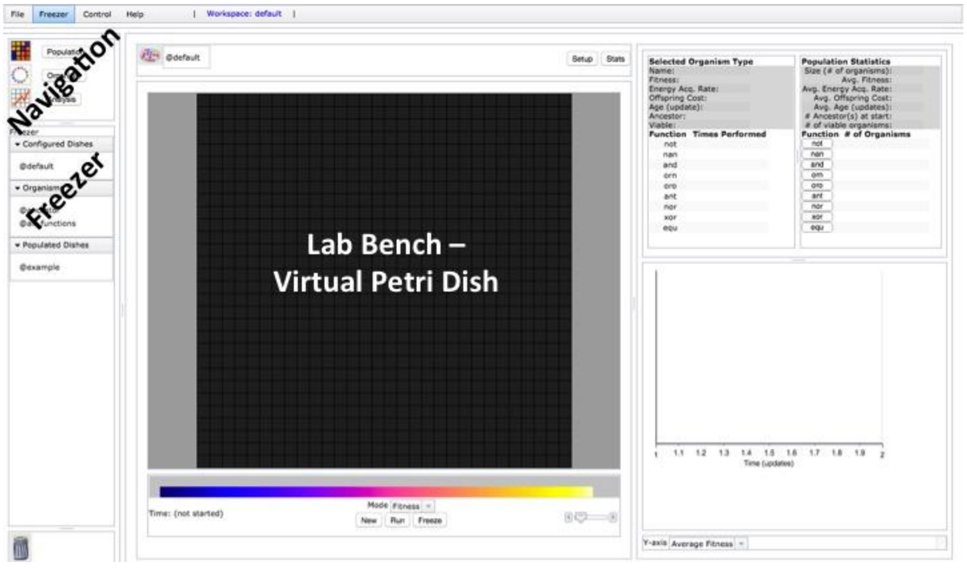
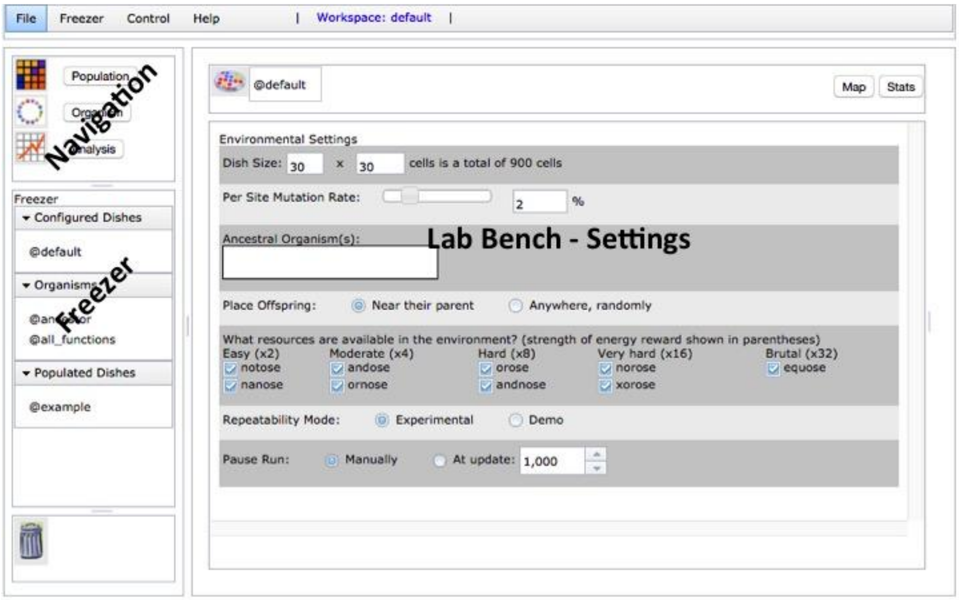


Figure 1. The Population viewer with its Lab Bench area set to Map mode, with labeled Navigation and Freezer areas on the left.

By selecting “Setup” above the Map you can access the experimental settings (Figure 2). Here you can change the Per Site Mutation Rate (default, 2.0%), the availability of resources associated with Avidian functions (default, all available), and other parameters.



Environmental Settings

Dish Size: 30 x 30 cells is a total of 900 cells

Per Site Mutation Rate: 2 %

Ancestral Organism(s):

Place Offspring: ● Near their parent ○ Anywhere, randomly

What resources are available in the environment? (strength of energy reward shown in parentheses)

Easy (x2)	Moderate (x4)	Hard (x8)	Very hard (x16)	Brutal (x32)
☑ notose	☑ andose	☑ orose	☑ norose	☑ equose
☑ nanose	☑ ornose	☑ andnose	☑ xorose	

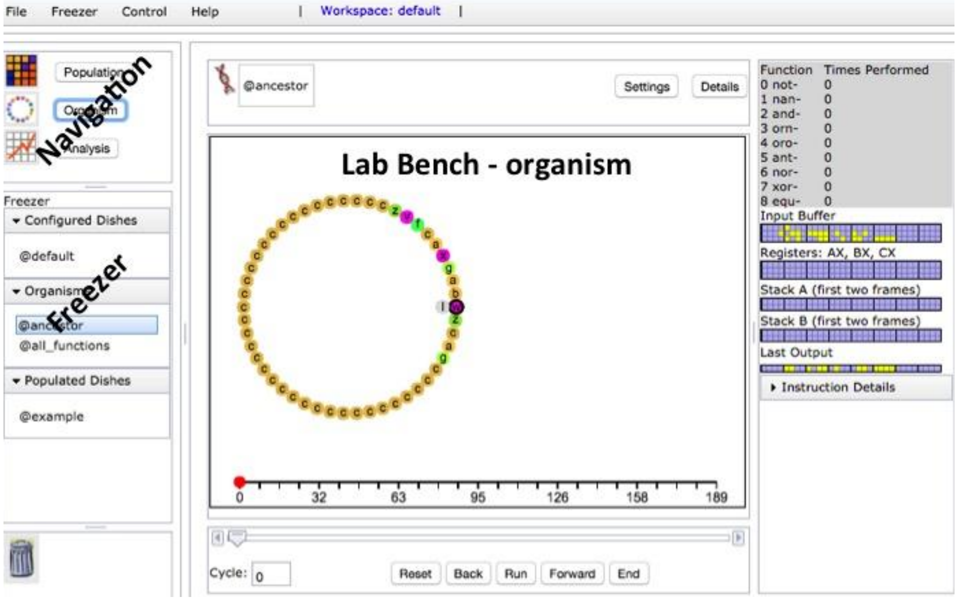
Repeatability Mode: ● Experimental ○ Demo

Pause Run: ● Manually ○ At update: 1,000

Figure 2. The Population viewer flipped to Setup.

To run an experiment, go to the Population viewer, flip to Setup, drag an organism from the Freezer to the Ancestral Organism box, flip to Map view, select "Run" below the Map.

To examine a single Avidian, navigate to the Organism viewer and drag an organism from the Freezer to the genetic code box next to the genetic symbol near the top of the Lab Bench. Then use the navigation buttons below the box to observe the Avidian's life processes (Figure 3). The genome is circular and is composed of instructions, represented by single letters within colored circles. Each instruction is a simple programming command, which altogether create a computer program. Notice the genome of the "@ancestor" organism contains many tan-colored "c" instructions; these are "no operation" commands.



Function	Times Performed
0 not-	0
1 nan-	0
2 and-	0
3 orn-	0
4 oro-	0
5 ant-	0
6 nor-	0
7 xor-	0
8 equ-	0

Cycle: 0

Figure 3. The Organism viewer with the "@ancestor" organism.

Part I – Examining an Avidian and observing reproduction

The digital organisms in Avida-ED, called Avidians, have a “genome” composed of a series of instructions, which are simple programing commands. In Avida-ED, an Avidian genome is always exactly 50 instructions in length, and the genome’s execution constitutes the organism’s life processes. An Avidian is able to reproduce only if it has a sequence of instructions that allows it to do so. The Avidian used to start many experiments in Avida-ED is “@ancestor” and it has the ability to reproduce itself.

Observe an Avidian undergoing reproduction:

1. Navigate to the Organism viewer, and the lab bench will display a large rectangle with a set of navigation buttons at the bottom.
2. Drag the “@ancestor” from the Freezer to the genetic code box near the top of the lab bench area. After a second or two, the ***genome*** will load (see Figure 3).
3. Select “Run”, and observe the organism’s genome being executed. The curved lines or ***execution path*** within the genome represent the instructions being executed and their height (increasing towards the center of the genome) represents the number of times that instruction has been executed. Eventually the organism will begin reproducing – its offspring’s genome will take shape on the right.
4. Use the slider and/or the “Back” and “Forward” buttons to observe the genome being executed at the position and pace you wish to explore.
5. While paused or at the end of the reproduction process, you can display the genomic position (number) of any instruction by selecting it.

After exploring an Avidian reproducing, please respond to the following questions:

- ➔ Which position of the Avidian genome is the first to be executed?

- ➔ Which positions of the Avidian genome are responsible for reproduction? (Hint, what does the execution path, and its height, represent?)

Storing an Avidian in the Freezer and examining an offspring’s genome:

1. Select “End” to skip to the very end of the reproduction process. A genetic code symbol will appear within the offspring’s genome.
2. To freeze the offspring for later use, drag its genetic code symbol directly on top of an organism already listed in the Organisms section of the Freezer. Another method is to simply choose “Save Offspring Organism” in the Freezer menu.
3. A prompt will appear to name the Avidian. You may use any name you like, but for future use we suggest a descriptive name of some sort. Items saved in the freezer may be renamed or deleted by right-clicking on the freezer item.
4. You can now examine the offspring by placing it in the Organism viewer.

Practice freezing your offspring Avidian, placing it in the Organism viewer, and then deleting it from the Freezer. You will need to apply these skills in Part II and Part III.

Part II – Observing mutations that occur during Avidian reproduction

1. While still in the Organism viewer, select Settings above the Lab Bench area.
2. Set the Per Site Mutation Rate to 10% by entering “10” in the box next to the slider.
3. Drag the “@ancestor” from the Freezer to the genetic code box.
4. Select “Run” then “End” to skip the animation. The number line and slider track the current location within the entire set of commands being executed. Notice that the total number of instructions executed (far right number line value) is greater than the Avidian genome length of 50. Some instructions may be executed many times (very high curved execution paths) and others might not be executed at all (the execution path jumps those instructions). The number of executed instructions required to complete reproduction is termed ***Offspring Cost***.
5. Observe the ***mutations*** that occur in the offspring’s genome. A mutation is highlighted with a black outline around the instruction circle. In Avida-ED, mutations only occur during the reproduction process, and appear in the offspring of an ancestral Avidian.

After observing reproduction with a 10% mutation rate, respond to the following:

- In your own words, provide a description of how an Avidian reproduces.
- Given a 10% per site mutation rate, how many sites do you *predict* will have a mutation? Show your calculations or provide support for your response.
- How many mutated sites did you observe your Avidian’s offspring to actually have?
- Describe how the offspring’s genome compares to the parent’s genome.
- If a mutation occurred in the sequence of instructions responsible for reproduction what do you *predict* would happen to the mutated offspring’s ability to reproduce?

After recording this prediction, perform the experiment. If your current offspring does not have one or more mutations in the set of reproduction instruction positions, select “Reset” then “Run” and “End” to observe another reproduction replicate. Continue until there’s an appropriate offspring genome; freeze and examine using the Offspring viewer.

- Were your predictions upheld?
- What is happening to its ability to reproduce? (Hint, compare the Avida-ED terms “viability” and “death” in the Glossary of the Avida-ED Quick Start User Manual.)

Part III – Comparing Avidian genomes and phenotypes

1. While still in the Organism viewer, drag the “@all_functions” organism from the Freezer to the genetic code box.
2. Select “Run”, and observe the Avidian’s genome being executed.
3. While it is executing, notice the numbers directly above the number line. At each of these points during its execution, the organism performs a logic function. Each number corresponds to a function listed in the Details window at the top right. Notice that the number of Times Performed indicated here should increase by one at each of the indicated points above the number line. The performance of a function is a ***phenotype*** of the Avidian, as is the ability to reproduce. The sequence of instructions in the genome is the ***genotype*** of the Avidian.
4. The labeled arrays of yellow and blue squares describe in computer science terms how the organism’s genome (computer program) is working. Selecting “Instruction Details” will display a description of the instruction (simple programing command) just executed as well as the instruction about to be executed. All of these computer science details can be ignored unless the user is particularly curious.

After observing its ability to perform functions, compare the “@all_functions” organism to the “@ancestor” organism investigated in Parts I & II:

→ How do the genotypes *and* phenotypes of the “@all_functions” and “@ancestor” organisms compare?

→ The “@all_functions” organism is a descendant of the “@ancestor” organism. How do you think it became so different?

→ How does the execution path of the “@all_functions” and “@ancestor” organisms compare?

→ What accounts for the Offspring Cost difference between the “@all_functions” and “@ancestor” organisms?

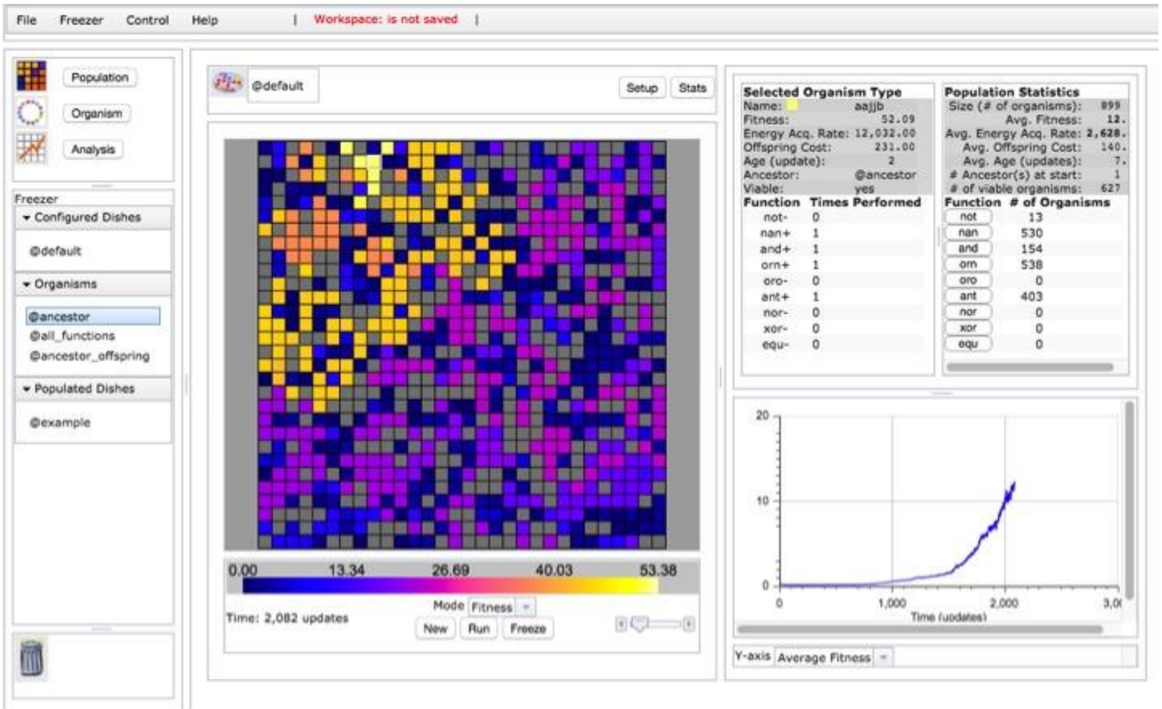
Considering Parts I, II, & III and your prior knowledge, respond to the following:

→ How is an Avidian genome similar to a bacterial genome?

→ How is Avidian genetics different from biological genetics?

Part IV – Evolving a Population of Avidians

Avidian populations grow in the Virtual Petri Dish (or Map), similar in many ways to how bacterial populations grow when plated on a medium in a Petri dish. The Map is divided into a grid in which each cell contains one Avidian. When an Avidian reproduces, the offspring is placed in a cell adjacent to its parent (the default setting) or randomly on the Map. As we saw in Part II, if there is mutation, the resulting offspring Avidian will not be identical to its parent.



Selected Organism Type

Name: aajjb
Fitness: 52.09
Energy Acq. Rate: 12,032.00
Offspring Cost: 231.00
Age (update): 2
Ancestor: @ancestor
Viable: yes

Function	Times Performed
not-	0
nan+	1
and+	1
orn+	1
oro-	0
ant+	1
nor-	0
xor-	0
equ-	0

Population Statistics

Size (# of organisms): 899
Avg. Fitness: 12.
Avg. Energy Acq. Rate: 2,628.
Avg. Offspring Cost: 140.
Avg. Age (updates): 7.
Ancestor(s) at start: 1
# of viable organisms: 627

Function	# of Organisms
not	13
nan	530
and	154
orn	538
oro	0
ant	403
nor	0
xor	0
equ	0

Figure 4. The Population viewer with a paused experiment, displaying Selected Organism and Population Statistics panels, as well as a real-time graph showing Average Fitness in the population, among other options.

Evolving a population from a single ancestor; identifying an Avidian with high fitness:

1. Navigate to the Population viewer and flip to “Setup” (Figure 2).
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. [Note: each resource available in the environment provides increased energy, which alters the ***Energy Acquisition Rate*** of Avidians performing the corresponding function. Since *Fitness* equals *Energy Acquisition Rate divided by Offspring Cost*, relative fitness also changes.
4. Select “Map” to return to Map view. Below the Map, confirm that the viewing Mode is set to “Fitness”; Avidians on the Map will be colored according to the continually adjusting Fitness color scale. The slider on the left will increase the Map view size.
5. Select “Run” below the Map and watch as the ancestor organism and its

descendants multiply. Notice changes within the Population Statistics panel at the top right and the graph below.

6. Select “Pause” to halt population growth when there are no empty (black) cells.
7. View an organism’s details by selecting its Map cell, outlining it in white. Information for the Avidian in this cell will be displayed in the Selected Organism Type panel (Figure 4). Select a few other Avidians and notice how their details differ.
8. Continue the experiment by selecting “Run”. Notice the Y-axis options for the graph on the lower right and view each in turn while the experiment is in progress.
9. Pause the experiment when there have been about 500 updates, as indicated below the Map on the left. (An update is a unit of time in Avida-ED.)
10. Identify an individual organism with a relatively high fitness for the population. To do so, select organisms in turn and check the Selected Organism Type panel. If there is enough variation in the population, the Fitness color scale may also be helpful.
11. Practice saving an Avidian to the Freezer. With your identified high fitness organism selected, select “Freeze” beneath the Map. Choose “Organism” from the displayed options, and enter a name. For future experimental use we suggest a descriptive name that is meaningful to you, for example “org_fitness0.55”.
12. Practice saving a populated dish to the Freezer. Select “Freeze” beneath the Map and choose “Population”. Again, use a descriptive name. We suggest using a labeling system that denotes important experimental variables, for example “2%mut_30x30_no-res_@anc” for this experimental setup. [Note: the Freezer menu can also be used to store items in the freezer.]

Considering this entire activity and associated readings, respond to the following:

- What distinguishes the Avidian in a grey cell from an Avidian in any other (non-black) colored Map cell? How does an organism become like this?

- Describe *how* fitness is measured and *how* it can change (Hint – Quick Start User Manual.)

- Choose two viable Avidians in your population with different fitness values and explain how differences in these Avidians contribute to differences in their fitness.

- Why do some scientists find Avida (the academic research version) and Avida-ED (the educational version) to be good model systems? What can they study with these model systems?

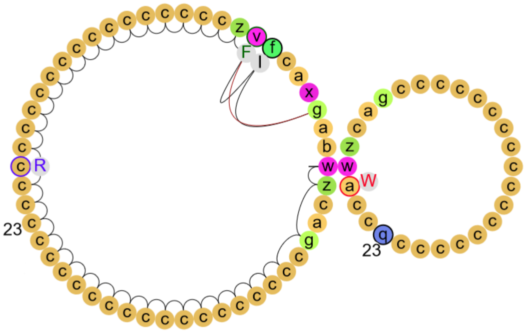
- What are the limitations of these systems, that is, what can’t you study using them?

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?

Exercise 1 - Exploring the Introduction of 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?

Student Learning Goals

- Students will be able to explain what it means to say that mutations occur at random.
- Students will be able to explain that mutations occurring during each individual's reproduction leads to genotypic variation in the population.

Questions to Consider While Doing Exercise #1

- How does genetic sequence variation originate in a population?
- Is there a pattern to how mutations occur?
- Could we predict which mutation(s) an organism's offspring will have?

Biological organisms can exhibit both genotypic (genetic information) and phenotypic (expressed trait) 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, for example NOT. 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 Genotypic 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 in the sequence. Mutation creates genotypic variation in a population of organisms since 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. Therefore, an Avidian genome in Avida-ED will always contain exactly 50 instructions.

Many genetics concepts do not apply to the simple genetic machinery of Avidians. For example, 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.

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. Each student will guide an Avidian through its reproduction process and record all of the mutations that occur in the offspring individual. By carrying out this reproduction process for three independent replicates, each person in the class will be able to 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. Because each graph will have two predictions for the distribution, make each distinct by using colored lines. *You must note this in the legend.*

Legend: ☐ Initial Prediction *(before data collection)* ☐ Final Prediction *(after examining the class data)*

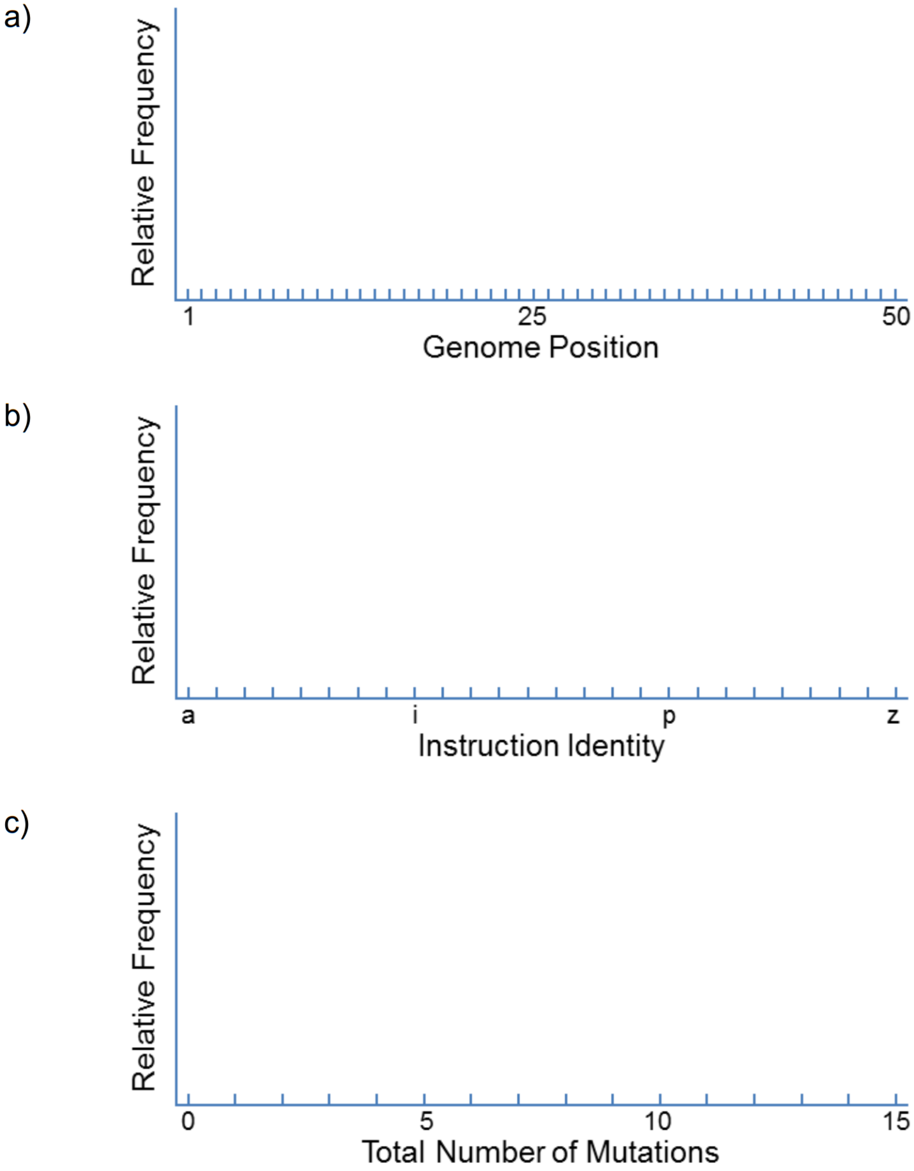


Figure 1. a) Predicted frequency of mutations by position across the Avidian genome.
b) Predicted frequency of mutations by identity of the final instruction state.
c) Predicted frequency of number of mutations per reproduction with 10% mutation rate

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 genomic position (number) of any instruction by selecting it. Note that it is possible none may occur.
5. *Record your data as Replicate #1*. Use the provided ancestral genomic sequence template to record the mutations by crossing off the ancestral state and recording the new instruction directly beneath. *Be sure to mark both the location and the type of mutation that occurs.* Read the circular genome clockwise from the 3 o’clock position, which has the ancestral state W. As a guide, selecting an instruction circle will display its position in the genome.

Replicate #1: Mark differences from the ancestral genome.

```
Position     1       10        20        30        40        50
Ancestor     wzcagcccccccccccccccccccccccccccccccccccczvfcaxgab
Mutations
```

Total number of mutations _______

Before performing additional replicates. Compare your Replicate 1 data with the data from a classmate. Note that in each case, you each began with the same parent (@ancestor) and allowed it to reproduce with a 10% mutation rate.

- In comparison to your classmate, did the same mutations and/or number of mutations occur during reproduction?

- What do you ***expect*** will happen if you repeat this experiment?

  - Do you think the *specific* mutations will be the same? Explain your reasoning.

  - Do you think the *number* of mutations will be the same? Explain your reasoning.

Testing your prediction. After you have recorded your predictions, reproduce the ancestor twice more using the same settings. To begin Replicate #2, simply select Reset.

Replicate #2: Mark differences from the ancestral genome.

```
Position    1       10        20        30        40        50
Ancestor    wzcagcccccccccccccccccccccccccccccccccccczvfcaxgab
Mutations
```

Total number of mutations _______

Replicate #3: Mark differences from the ancestral genome.

```
Position    1       10        20        30        40        50
Ancestor    wzcagcccccccccccccccccccccccccccccccccccczvfcaxgab
Mutations
```

Total number of mutations _______

Recording your data. Experiments 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 *mutation data* here:

Exercise 1 Data Collection Spreadsheet

Follow the “Examples” in columns B-D of the data collection sheet. Find the first column on the right that does not contain data; enter your ***Name-Replicate#*** in row 4, and your observed mutations in rows 5-54 corresponding to genome positions 1-50 by entering the letter of the mutated final instruction state. The instructors will periodically collect these data, anonymize them, and add them 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, you can view the course data and analysis.

Exercise 1 Data Analysis Spreadsheet

Update your predicted distributions. After examining the course data, return to your prediction graphs and draw a new representation for each distribution – *not simply the exact course data*, but rather what you would expect if 200 or even 200,000 more students contributed to our investigation of random mutation in Avida-ED. Make sure your new distribution is distinct from your initial prediction by using colored lines. Note this distinction in the legend on that page.

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

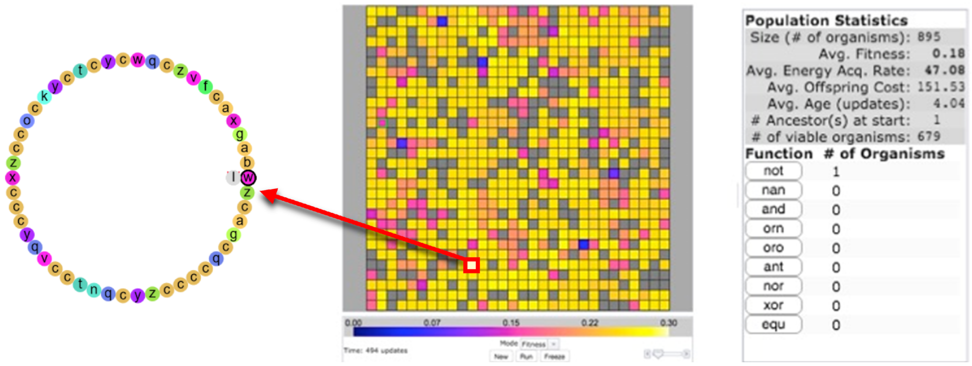
- ➔ How does this *experimental setup* demonstrate that mutations are random?
- ➔ Did each person in the course get the same mutations and number of mutations?
- ➔ How would you describe each of the three relative frequency distributions (genome position, instruction identity, total number of mutations) for the entire course data?
- ➔ How would you reconcile your responses to the above two questions – each person's individual experimental data versus data from the entire course?
- ➔ **Thought experiment –** How would the course's results be different if a 5% mutation rate was used instead? How would each relative frequency distribution appear?
- ➔ How is random mutation in Avida-ED *similar* to random mutation in biological systems?
- ➔ How is random mutation in Avida-ED *different* than random mutation in biological systems?
- We used Avida-ED and this experimental protocol to model what occurs when biological populations experience mutation. 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?

Exercise 2 - Exploring Random Mutation and Selection



Population Statistics

Size (# of organisms): 895
Avg. Fitness: 0.18
Avg. Energy Acq. Rate: 47.08
Avg. Offspring Cost: 151.53
Avg. Age (updates): 4.04
Ancestor(s) at start: 1
# of viable organisms: 679

Function	# of Organisms
not	1
nan	0
and	0
orn	0
oro	0
ant	0
nor	0
xor	0
equ	0

Only one Avidian in this population can perform the function NOT. Did a mutation conferring this phenotype occur because performing NOT is rewarded in this notose environment?

Student Learning Goal

- Students will be able to explain that mutations occur at random, and do not occur because they are needed by an organism to succeed in its environment.

Questions to Consider While Doing Exercise #2

- What does it mean to say that mutations occur at random? In other words, do mutations occur in an organism in response to a need, or do they occur spontaneously?
- Is there a pattern to how mutations occur?
- Can we predict that an organism's offspring will have mutations suitable to living and reproducing in its environment?

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 and even its ability to reproduce (the phenotypes of Avidians). In this exercise we use Avida-ED to explore the consequences of random mutation generating phenotypic variation that can be under selection in the environment. Avida-ED provides a way to test whether mutations occur at random, or if mutations are directed to occur in response to natural selection within the environment. In some sense, we are testing directly what Salvador Luria and Max Delbrück (1943) did in their elegant Nobel Prize winning experiments[1]. 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.

---

[1] Luria SE, Delbrück M. 1943. "Mutations of Bacteria from Virus Sensitivity to Virus Resistance." *Genetics* 28:491-511.

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 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. For example, the “@ancestor” organism cannot perform any functions, but random mutations over multiple generations might produce a descendant with a genome that codes for one or more functions (e.g., NOT). If the corresponding resource is in the environment (e.g., notose), then this Avidian will have an increased energy acquisition rate and be favored by natural selection within its environment.

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. For understanding Avidian phenotypes in selective environments, it is illustrative to use an analogy to bacteria processing sugar resources as food. When an individual bacterium is able to process a sugar in its environment it receives energy to be used for growth and reproduction; bacteria that can process the sugar will be favored due to natural selection. Similarly, 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. To test if mutations were random versus directed Salvador Luria and Max Delbrück devised an elegant experiment that allowed them to differentiate between these two hypotheses[1]. We can investigate this same question in Avida-ED.

Timing the occurrence of phenotype-conferring mutations in the absence and presence of a selective advantage

In this exercise, we will test 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, each individual in the class will record how many updates (a measure of time) it takes for a mutation that confers the ability to perform NOT 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 their result, each student in the class will then do the same procedure in a second treatment, but this time with ***notose present***; 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 **Table 1**.

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 4.
6. Record the Update number of this occurrence in **Table 1**.

Table 1. Updates (time) until first occurrence of an Avidian performing NOT, with and without notose present (a selective advantage or reward) in the environment.

Environmental Treatment	Update number at first occurrence of NOT
All resources absent (No reward for NOT)	
Notose present (Reward for NOT)	

Recording your data. Experiments often involve investigating processes or phenomena with lots of variation, we will examine the data generated by the class. Enter your *Table 1 data*, the number of updates for NOT to occur in each experimental treatment, here:

Exercise 2 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 Table 1 data on 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, you may view the analysis below.

Exercise 2 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 whether mutation is random versus directed by the selective environment?
- For your results as shown in Table 1, did the first occurrence of the NOT phenotype happen earlier or later in Treatment 1 compared to Treatment 2?
- Was this the same for each person in your group, and for each person in the class?
- What pattern would you have expected to observe in the class data **if** mutations occur in response to the presence of a selective environment?
- Given the data you have examined across **all** Avida-ED experiments you have performed, how would you describe what it means to say that mutations occur at random?
- **Thought experiment –** How would evolution be affected if mutations did not occur at all (that is, a zero percent mutation rate)?
- Why is mutation essential to the evolutionary process?
- We used Avida-ED and this experimental protocol to model what occurs when biological populations experience mutation. What are some limitations or constraints to our modeling in this exercise?

Reflection and Metacognition

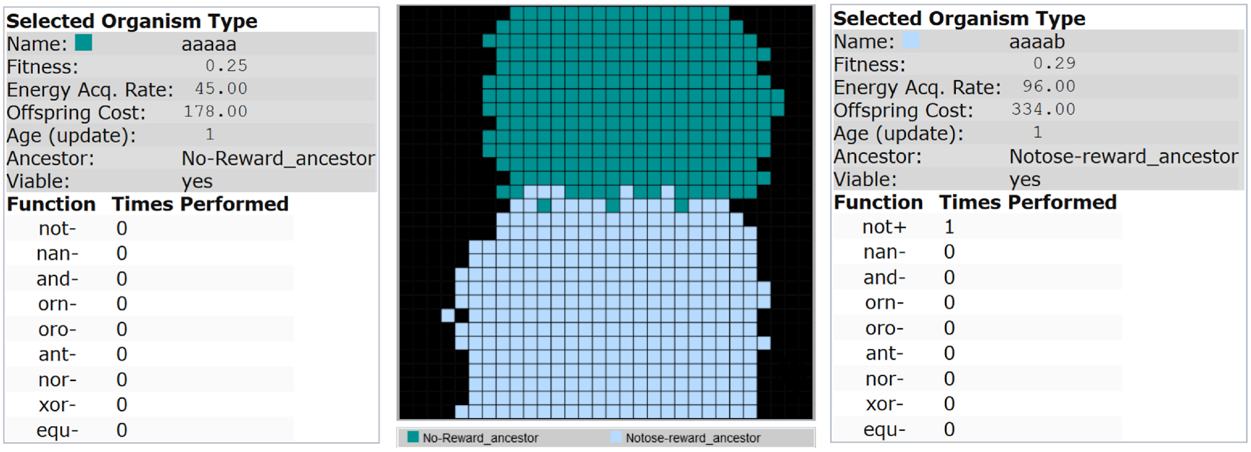
Think-Pair-Share: Work with your lab team to answer the following questions.

- ➔ What did you learn from this exercise?

- ➔ What are you still wondering about?

- ➔ What would you change in this exercise?

Exercise 3 – Exploring Fitness and Population Change under Selection



Selected Organism Type
Name: 🟩 aaaaa
Fitness: 0.25
Energy Acq. Rate: 45.00
Offspring Cost: 178.00
Age (update): 1
Ancestor: No-Reward_ancestor
Viable: yes

Function	Times Performed
not-	0
nan-	0
and-	0
orn-	0
oro-	0
ant-	0
nor-	0
xor-	0
equ-	0

Selected Organism Type
Name: 🟦 aaaab
Fitness: 0.29
Energy Acq. Rate: 96.00
Offspring Cost: 334.00
Age (update): 1
Ancestor: Notose-reward_ancestor
Viable: yes

Function	Times Performed
not+	1
nan-	0
and-	0
orn-	0
oro-	0
ant-	0
nor-	0
xor-	0
equ-	0

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 process of adaptation by natural selection, leading to genetic frequency change in a population over many generations.
- Students will be able to explain the concept of fitness as used in biology, and that fitness is dependent on the environment.
- 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. 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, 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 relative 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.

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 some 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 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, it is useful to use the concept of relative fitness. 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 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, 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 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"!

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.

Freezing individuals evolved in different selective environments

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. This Avidian ideally does not perform any functions. [Why?]
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 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.)

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, making sure that your organism successfully performs NOT. The selected frozen organism should be named **"Notose_ancestor"**.

Respond to the following before proceeding to the Competition Treatments:

- Considering the design of experiments in science, explain the importance of replicating the procedures conducted in one experimental treatment as much as possible for other experimental treatments.
- Why is it important that the ancestral populations in Ancestor Treatment 1 & 2 ran for a long time (1,000 updates)? What would the implications be if these treatments ran for only 500 updates (assume a NOT performing individual still occurred)?

- When competing the evolved ancestors in each of their ancestral environments, do you predict the same ancestor will "win" (that is, contribute a greater frequency of descendants to the population) in both environments? *Why or why not?*

Recording your data. Complete the tables below for Competition Treatments 1 & 2.

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

	"**No-Resource_ancestor**" (may or may not perform function)			"**Notose_ancestor**" (performs NOT)		
Selective Environment	Fitness (absolute)	Energy Acq. Rate	Offspring Cost	Fitness (absolute)	Energy Acq. Rate	Offspring Cost
All resources absent						
Notose present						

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

Selective Environment	Number performing NOT	Number of viable organisms	Percent of viable organisms performing NOT
All resources absent			
Notose present			

Competing individuals in their ancestral selective environments

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 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 in **Table 1** the (absolute) fitness, energy acquisition rate, and offspring cost for each ancestor by selecting each and using the "Selected Organism Type" panel.

Respond to the following before proceeding to step 9:

- In this environment with all resources absent, use the concept of adaptation to describe and compare the starting fitness values of each ancestor.
- Use the concept of fitness to 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.

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.

Competition Treatment 2 – Competing ancestors when notose present.

Repeat steps 1-11 of Competition Treatment 1 using the same parameters and procedures, except add notose to the environment by **marking notose with a check**.

Respond to the following before proceeding to step 9:

- In this environment with notose present, use the concept of adaptation to describe and compare the starting fitness values of each ancestor.
- Use the concept of fitness to predict which ancestor will contribute a greater proportion of descendants to the population after 300 updates.

After completing Competition Treatment 2:

- 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?

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 NOT in each experimental treatment, in the document linked here:

Exercise 3 - Data Collection

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.

Exercise 3 - Data Analysis

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 influence of selection on leading to the increased frequency of certain phenotypes?

- On average, how does the final frequency of individuals performing NOT compare in the 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 *notose present* environment.

- **Thought experiment –** 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? Describe your hypothesized mechanism. Hint: observe the number of individuals performing NOT 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.

- We used Avida-ED and this experimental protocol to model what occurs when biological populations experience natural selection. What are some limitations or constraints to our modeling in this exercise?

Reflection and Metacognition

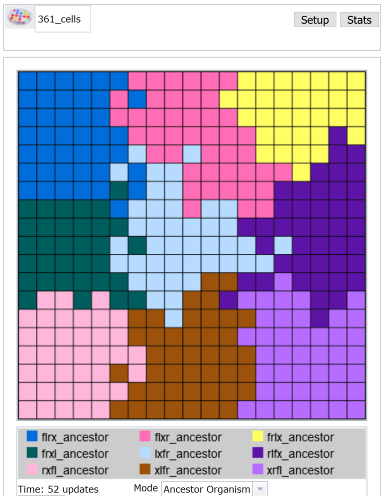
Think-Pair-Share: Work with your lab team to answer the following questions.

- What did you learn from this exercise?

- What are you still wondering about?

- What would you change in this exercise?

Exercise 4 – Exploring Population Change without Selection



Time: 52 updates

Mode Ancestor Organism

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

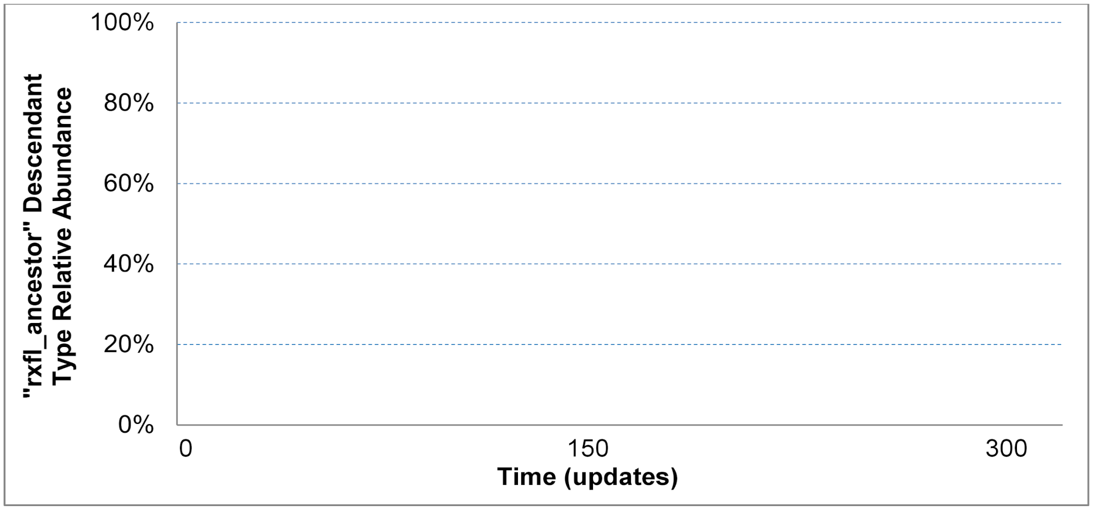
Evolution, changes in traits or alleles in a population over generations, can occur as the result of a number of different processes or mechanisms. The Introduction and Exercise 1 of the lab book explored the role (and nature) of *mutations* to the evolutionary process. Exercise 3 explored how *selection*, 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 non-adaptive 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, and hence the initial frequency of any single ancestor is about 10%. Do you expect the **relative frequency** of any single 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? **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.



PREDICTION: Graph of the frequency of a chosen ancestral organism 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.

- 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 results? Why or why not?

Visualizing evolutionary change without natural selection

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

Exercise 4 Avida-ED Workspace

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

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

Part I.

1. In the Population viewer, flip to Setup.
2. Drag each of the **nine unique ancestor 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!
3. 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. 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 descendants will have the same color.
5. For the first replicate you will choose one Avidian ancestor organism (e.g. 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 the rxfl_ancestor Avidians present in the dish. Once you have recorded the number in Table 1, return to the Setup Menu and change Updates to 100, press run, repeat until you have data for 300 updates. **Record all data in Table 1**. *NOTE: once the frequency of the ancestor of interest has gone to 0 you can stop the run*.
6. Repeat the above protocol for a population size of **9x9** and **19x19**. *NOTE: for the large population size you can estimate the number of the ancestors of interest.*
7. Calculate frequency or percentage of the rxfl_ancestor lineage by dividing the number of rxfl_ancestors 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**.
9. Briefly go to **Table 2** for the first replicate for each population size, record only the number of remaining descendant types (colors) at the end of 300 updates. This will count as your first replication for Part II.

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

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

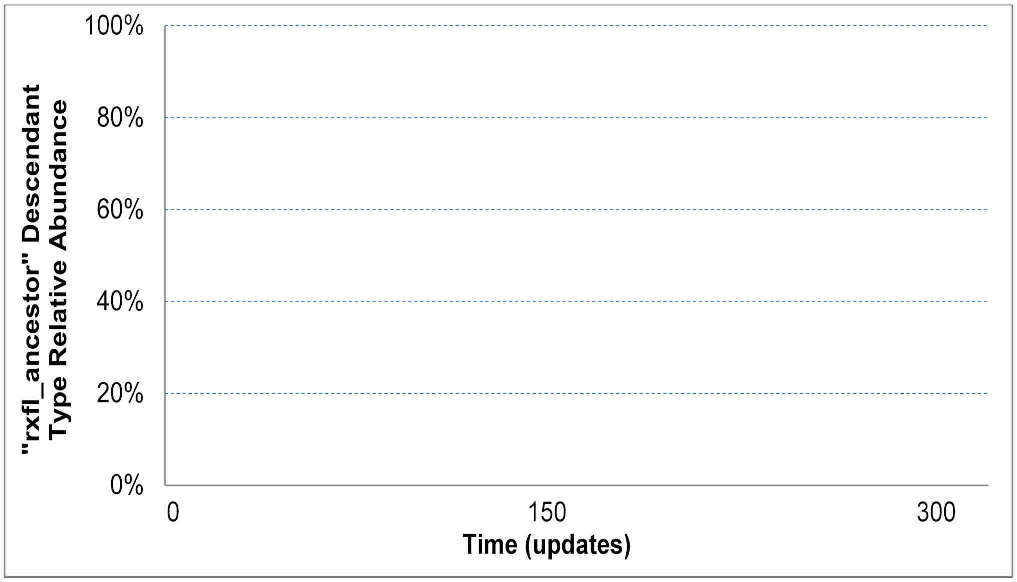


Figure 1. Graph of the frequency of a chosen ancestral organism (from Table 1) in the population at each of the time updates. Create three different lines on the graph, one for each of the population sizes. Note: **Initial Frequency** = one of nine ancestors = 0.11; **Extinction** = 0.0; **Fixation**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

Part II.

1. To complete Table 2 you will be running 5 replicates for each population size. You have already run 1 during Part I. In the Control menu choose “Start New Experiment” and then “Discard.”
2. 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!
3. Set the following parameters:
   0% Per Site Mutation Rate; Place Offspring Near their parent; **Uncheck all resources**; Repeatability Mode Experimental; Pause Run **At update 300**.
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. Run the experiment and record *the total number of ancestral organisms* (total number of different colors) present at the end of 300 updates in Table 2.
5. To create a new replicate, start new experiment, drag “9_cells” from the Freezer’s Configured Dishes section to the small box next to the Avida 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.
6. Repeat experiment 3 more times.
7. Start a new experiment, repeat steps 2-6 for population size 81 (Dish Size 9x9). Remember to save your experimental configuration (“81_cells”) for use with each replicate. Record the total number of ancestral organisms (total number of colors) present at the end of 300 updates in Table 2.
10. Start a new experiment, repeating steps 2-6 for population size 361 (Dish Size 19x19). Again, save your experimental configuration (“361_cells”) for use with each replicate. Record the total number of ancestral organisms (total number of colors) present at the end of 300 updates in Table 2.

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)						

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. Your instructor will provide a link for data entry.

Enter your Table 1 data in the spreadsheet tab labeled T1-Frequency

Enter your ***Table 2*** *data*, the average final descendant type diversity in each experimental treatment, in the spreadsheet tab labeled T2-Diversity.

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 the 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

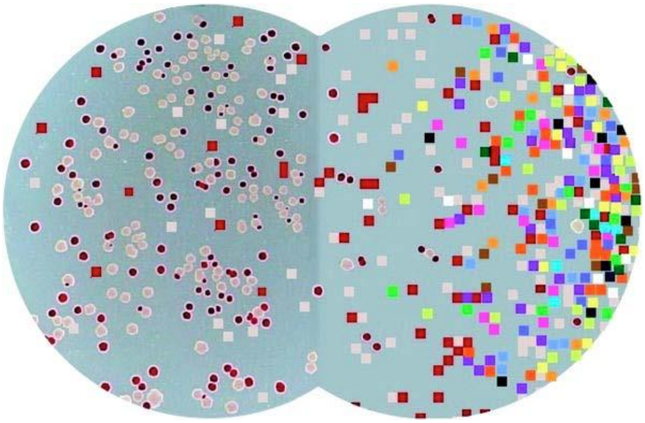
Think-Pair-Share: Work with your lab team to answer the following questions.

- What did you learn from this exercise?

- What are you still wondering about?

- What would you change in this exercise?

Avida-ED Independent Research – An Experimental Evolution Project



Two model evolutionary biology study systems:
E. coli bacteria (biological organisms) are on the left, while Avidians (digital organisms) are on the right.

Background

Some evolutionary biologists, such as Richard Lenski at Michigan State University, conduct experiments using microorganisms like *E. coli* to answer fundamental questions about evolutionary processes. Bacteria are useful model organisms because they reproduce quickly and their small size means that they don't take up much space in the laboratory. Lenski and his colleagues' work with *E. coli* has helped shed light on some important aspects of evolution and, having spanned over 25 years and 65,000 bacterial generations (and counting!), it is the longest-running investigation of its kind.

As far as biological organisms go, *E. coli* reproduce—and evolve—at an incredibly fast rate. Even so, *E. coli* grow too slowly to be of practical use in addressing many questions about evolution that require long periods of study. Further, while scientists have invented many tools and techniques to manipulate such microorganisms, some research questions require even greater genetic or environmental control of the study system. To pursue questions that require too much time, effort, or tractability, scientists can use digital organisms that model biological systems. Avida is such a model system and is used by researchers to study evolutionary processes applicable to biological systems. The educational version, Avida-ED, maintains these features while being user friendly.

Your research team will perform experiments using digital organisms in Avida-ED to explore a research question. The question is up to you; your team will propose a hypothesis and then design an experiment that you will conduct to address your question. The final product of your work will be a research poster that your team will present to your peers, instructors, and passersby.

Research Team Tasks

Brainstorm research questions: Individually and then within your team, brainstorm research questions that are of interest to you and apply to a general biological phenomenon. The questions should not apply to any particular study system, but could be applied to any, for example bacteria, ants, elephants, Avidians, etc.

Form a hypothesis: Propose a hypothesis to one of your research questions that can be tested with Avidians as the model study system using Avida-ED.

Design an experiment: Develop an experimental protocol that your team will follow to test your hypothesis. Consider how you will design your experimental treatments, including which variables you will control by leaving consistent and which you will experimentally alter. Consider what data to collect and how you will use replication so that statistical analyses can be used to draw conclusions about your data.

Predict the results: Indicate what experimental outcomes you would expect to find if indeed your hypothesis is supported by the data you collect, as well as what outcomes would indicate that your hypothesis is not supported. Decide how it would be best to represent your data, and create appropriate figures and/or tables using example data so that your predications can be graphically examined.

Write your Research Proposal: Prepare a summary of your proposed research project. This should be short, only as long as is necessary to discuss all items indicated above, other than any irrelevant research questions discarded after brainstorming.

Present your Proposal: Prepare a presentation that you present to your peers and instructors. Each team member briefly presents at least one slide. Practice a concise talk that addresses everything discussed in the Research Proposal.

Conduct your experiment: Carry out your proposed research and record the appropriate data in an organized manner.

Analyze your data: Summarize your data and perform appropriate statistical tests. Reevaluate your predications and determine whether or not your hypothesis is supported by your data. Represent your data using appropriate figures and/or tables.

Report your findings: Construct a research poster as a final presentation of your work. The poster content should fully address your research project by including an introduction, methods, results, and discussion sections, while maintaining a pleasing appearance. Your well-practiced presentation will be given to peers, instructors, and passersby.

Suggested Guidelines and Expectations for Research Teams

Pre-proposal Discussion

After considering project ideas individually and then together, your group meets with the instructor(s) to discuss your preliminary project design. Be prepared with more than one proposed project if your primary design is deemed not suitable during the conversation.

Research Proposal

Teams should follow the directions on the previous page, from brainstorming through writing this proposal, which should only be as long as is necessary to discuss all items. Do not include any irrelevant research questions discarded after brainstorming. Alongside the requirements below are group member role designations for the Proposal Presentation; your group may choice to follow these in writing the Research Proposal too.
Ten points are based on the structure and writing of the proposal (neat, clear, logical, etc.). Five points are awarded for descriptions of each of the following requirements:

● Research question generally applicable in biology; why its interesting	α	W	i
● Hypothesis placing the research question as testable in the Avida-ED context	α	W	ii
● Experimental treatments with control and experimental variables; data collected	β	X	iii
● Replication, how its employed; proposed statistical analyses	β	Y	iv
● Predictions if hypothesis supported, with figures/tables of example data	γ	Z	v
● Predictions if hypothesis not supported, with figures/tables of example data	γ	Z	v
Presentation roles for groups of size:	*3*	*4*	*5*

Proposal Presentation

Teams should create a set of approximately seven slides, a title slide introducing the team and its members followed by one slide per each bulleted requirement listed for the Research Proposal. Each team member presents one or more slides based on their chosen contribution role, as indicated above using Greek letters for teams of three members, Latin letters for teams of four, and Roman numerals for teams of five. Three points are awarded for each bulleted requirement above (totaling 18 points); the final two points are awarded to each individual member based on the presentation of their slides.

Poster Presentation

Teams should construct their research poster using any templates and/or formatting suggested by the instructors. Professional dressed pairs present in a confident and well-practiced manner, using the poster as a resource while focusing on the audience. Pairs switch during the session. Points are as indicated for each of the following requirements:

- An informative title and team members' names and degree/college affiliations
- Introduction including background information, research question, and hypothesis
- Methods including experimental design, emphasizing the most important factors
- Results showing figures and/or tables with statistical analyses
- Discussion emphasizing hypothesis as supported or not supported, and remarks on how the research is applicable to biological systems and knowledge generally.
- Poster is logically organized, written clearly, and nicely balances text and graphics
- A knowledgeable presentation, addressing audience questions *(awarded individually)*

X-Ray Visions of the Universe

Killer Flu | The Atomic Rocket | Robot Kids

CELEBRATING 25 YEARS

CHEMISTRY ASTRONOMY BIOLOGY ENVIRONMENT GENETICS GEOLOGY PSYCHOLOGY TECHNOLOGY ARCHAEOL

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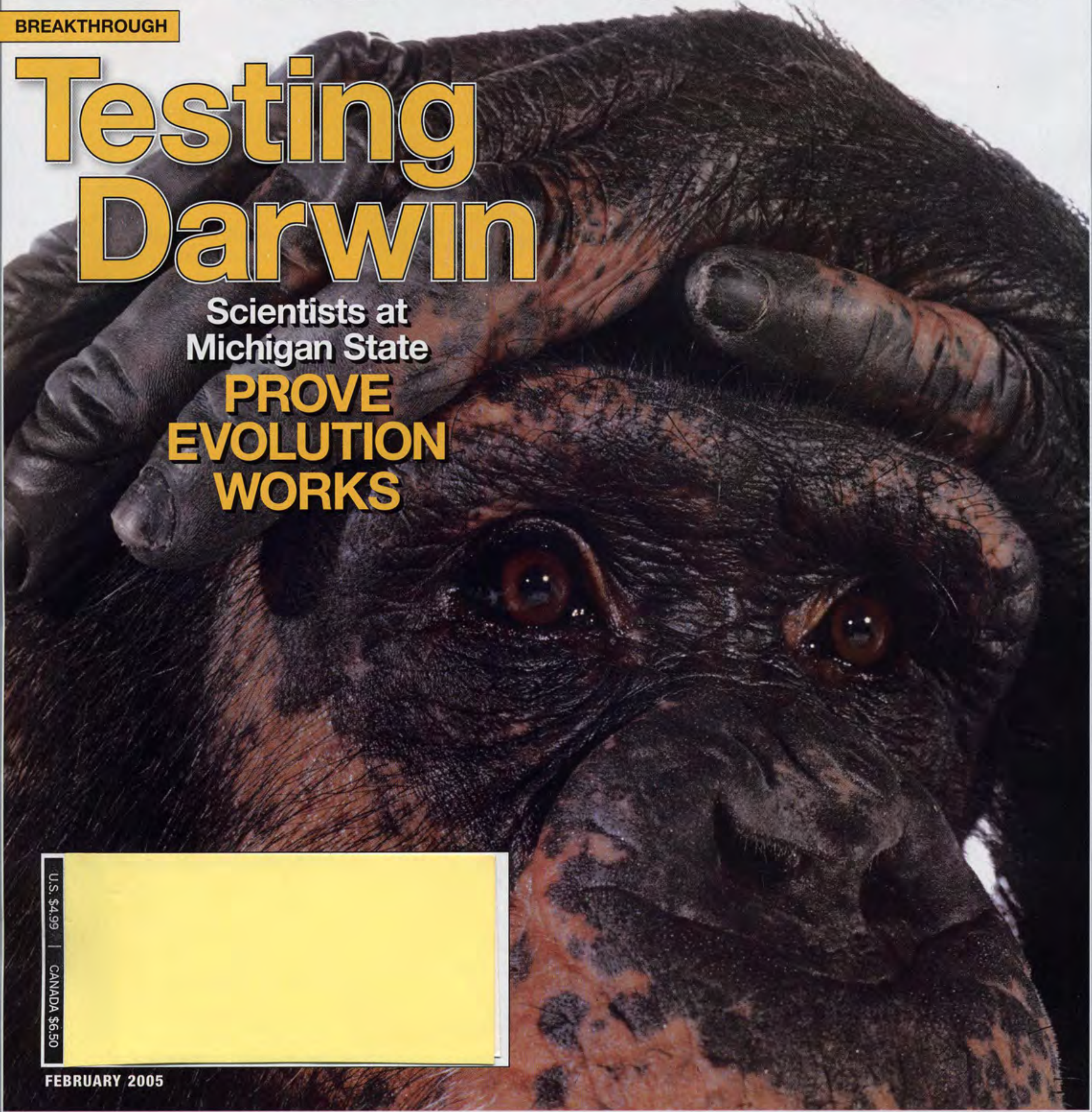


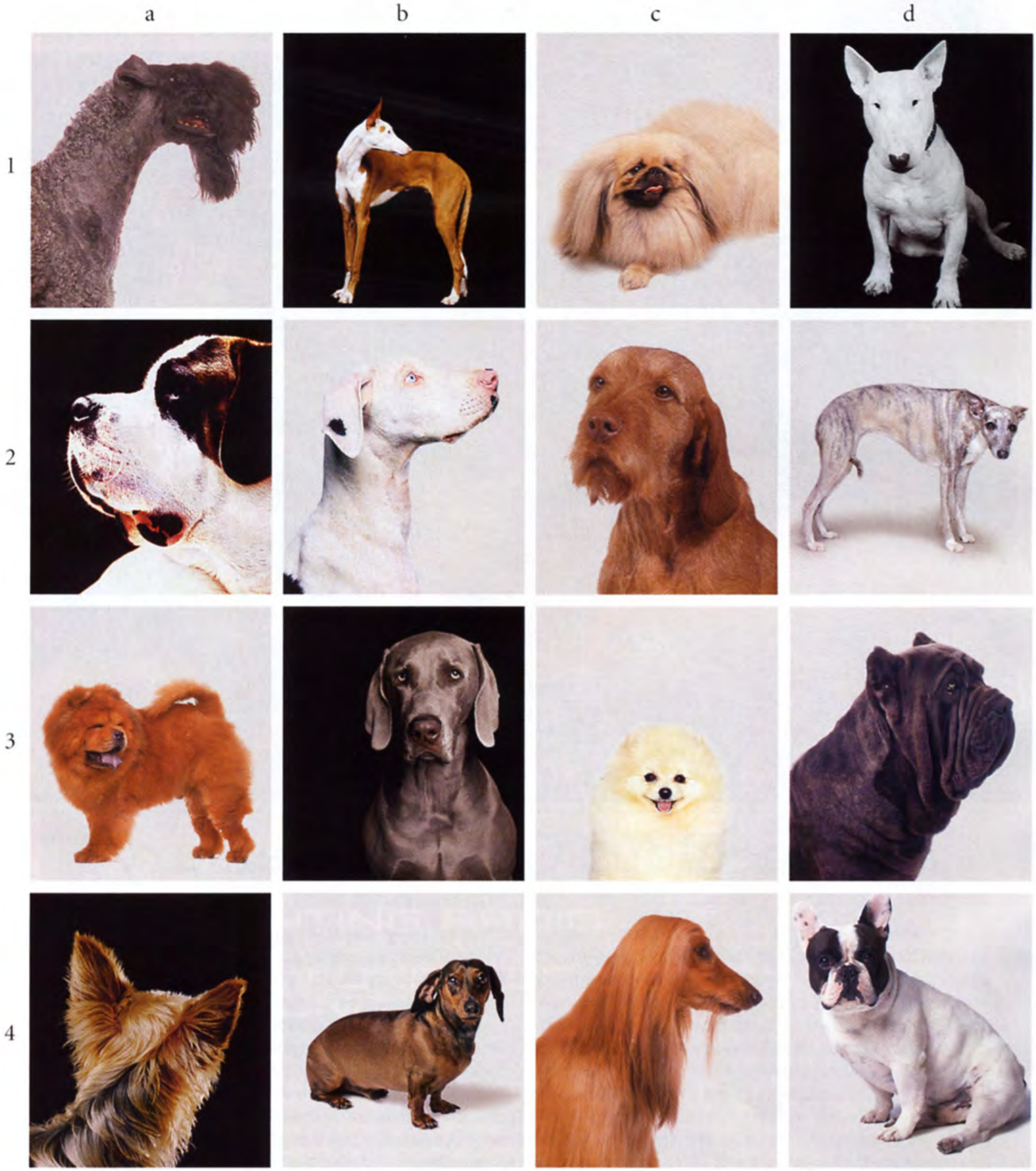
BREAKTHROUGH

Testing Darwin

Scientists at Michigan State PROVE EVOLUTION WORKS

U.S. $4.99 | CANADA $6.50

FEBRUARY 2005



Charles Darwin cited the diversity of dogs as an example of accelerated evolution. Few domesticated dogs look like wolves—their ancient ancestors—with whom they can still mate. "Who will believe that animals closely resembling the Italian greyhound, the bloodhound, the bull-dog, pug-dog, or Blenheim spaniel, etc.—so unlike all wild Canidae—ever existed in a state of nature?" Darwin wrote. If humans could crossbreed such diversity in just a few hundred years, he reasoned, other organisms could evolve in a similar fashion over millions of years. Modern breeds of *Canis familiaris* include the Kerry blue terrier (1a); Ibizan hound (1b); Pekingese (1c); bull terrier (1d); Saint Bernard (2a); Great Dane (2b); Hungarian wirehaired vizsla (2c); whippet (2d); chow chow (3a); Weimaraner (3b); Pomeranian (3c); Neapolitan mastiff (3d); Yorkshire terrier (4a); dachshund (4b); Afghan hound (4c); and French bulldog (4d).

Testing Darwin

Digital organisms that breed thousands of times faster than common bacteria are beginning to shed light on some of the biggest unanswered questions of evolution

IF YOU WANT TO FIND ALIEN LIFE-FORMS, HOLD OFF ON booking that trip to the moons of Saturn. You may only need to catch a plane to East Lansing, Michigan. ¶ The aliens of East Lansing are not made of carbon and water. They have no DNA. Billions of them are quietly colonizing a cluster of 200 computers in the basement of the Plant and Soil Sciences building at Michigan State University. To peer into their world, however, you have to walk a few blocks west on Wilson Road to the engineering department and visit the Digital Evolution Laboratory. Here you'll find a crew of computer scientists, biologists, and even a philosopher or two gazing at computer monitors, watching the evolution of bizarre new life-forms. ¶ These are digital organisms—strings of commands—akin to computer viruses. Each organism can produce tens of thousands of copies of itself within a matter of minutes. Unlike computer viruses, however, they are made

BY CARL ZIMMER

up of digital bits that can mutate in much the same way DNA mutates. A software program called Avida allows researchers to track the birth, life, and death of generation after generation of the digital organisms by scanning columns of numbers that pour down a computer screen like waterfalls.

After more than a decade of development, Avida's digital organisms are now getting close to fulfilling the definition of biological life. “More and more of the features that biologists have said were necessary for life we can check off,” says Robert Pennock, a philosopher at Michigan State and a member of the Avida team. “Does this, does that, does this. Metabolism? Maybe not quite yet, but getting pretty close.”

One thing the digital organisms do particularly well is evolve. “Avida is not a simulation of evolution; it is an instance of it,” Pennock says. “All the core parts of the Darwinian process are there. These things replicate, they mutate, they are competing with one another. The very process of natural selection is happening there. If that's central to the definition of life, then these things count.”

> ‘Avida is not a simulation of evolution; it is an instance of it’

It may seem strange to talk about a chunk of computer code in the same way you talk about a cherry tree or a dolphin. But the more biologists think about life, the more compelling the equation becomes. Computer programs and DNA are both sets of instructions. Computer programs tell a computer how to process information, while DNA instructs a cell how to assemble proteins.

The ultimate goal of the instructions in DNA is to make new organisms that contain the same genetic instructions. “You could consider a living organism as nothing more than an information channel, where it's transmitting its genome to its offspring,” says Charles Ofria, director of the Digital Evolution Laboratory. “And the information stored in the channel is how to build a new channel.” So a computer program that contains instructions for making new copies of itself has taken a significant step toward life.

A cherry tree absorbs raw materials and turns them into useful things. In goes carbon dioxide, water, and nutrients. Out comes wood, cherries, and toxins to ward off insects. A computer program works the same way. Consider a program that adds two numbers. The numbers go in like carbon dioxide and water, and the sum comes out like a cherry tree.

In the late 1990s Ofria's former adviser, physicist Chris Adami of Caltech, set out to create the conditions in which a computer program could evolve the ability to do addition. He created some primitive digital organisms and at regular intervals presented numbers to them. At first they could do nothing. But each time a digital organism replicated, there was a small chance that one of its command lines might mutate. On a rare occasion, these mutations allowed an organism to process one of the numbers in a simple way. An organism might acquire the ability simply to read a number, for example, and then produce an identical output.

Adami rewarded the digital organisms by speeding up the time it took them to reproduce. If an organism could read two numbers at once, he would speed up its reproduction even more. And if they could add the numbers, he would give them an even bigger reward. Within six months, Adami's organisms were addition whizzes. “We were able to get them to evolve without fail,” he says. But when he stopped to look at exactly how the organisms were adding numbers, he was more surprised. “Some of the ways were obvious, but with others I'd say, ‘What the hell is happening?’ It seemed completely insane.”

On a trip to Michigan State, Adami met microbiologist Richard Lenski, who studies the evolution of bacteria. Adami later sent Lenski a copy of the Avida software so he could try it out for himself. On a Friday, Lenski loaded the program into his computer and began to create digital worlds. By Monday he was tempted to shut down his laboratory and dedicate himself to Avida. “It just had the *smell* of life,” says Lenski.

It also mirrored Lenski's own research, launched in 1988, which is now the longest continuously running experiment in evolution. He began with a single bacterium—*Escherichia coli*—and used its offspring to found 12 separate colonies of bacteria that he nurtured on a meager diet of glucose, which creates a strong incentive for the evolution of new ways to survive. Over the past 17 years, the colonies have passed through 35,000 generations. In the process, they've become one of the clearest demonstrations that natural selection is real. All 12 colonies have evolved to the point at which the bacteria can replicate almost twice as fast as their ancestors. At the same time, the bacterial cells have gotten twice as big. Surprisingly, these changes didn't unfold in a smooth, linear process. Instead, each colony evolved in sudden jerks, followed by hundreds of generations of little change, followed by more jerks.

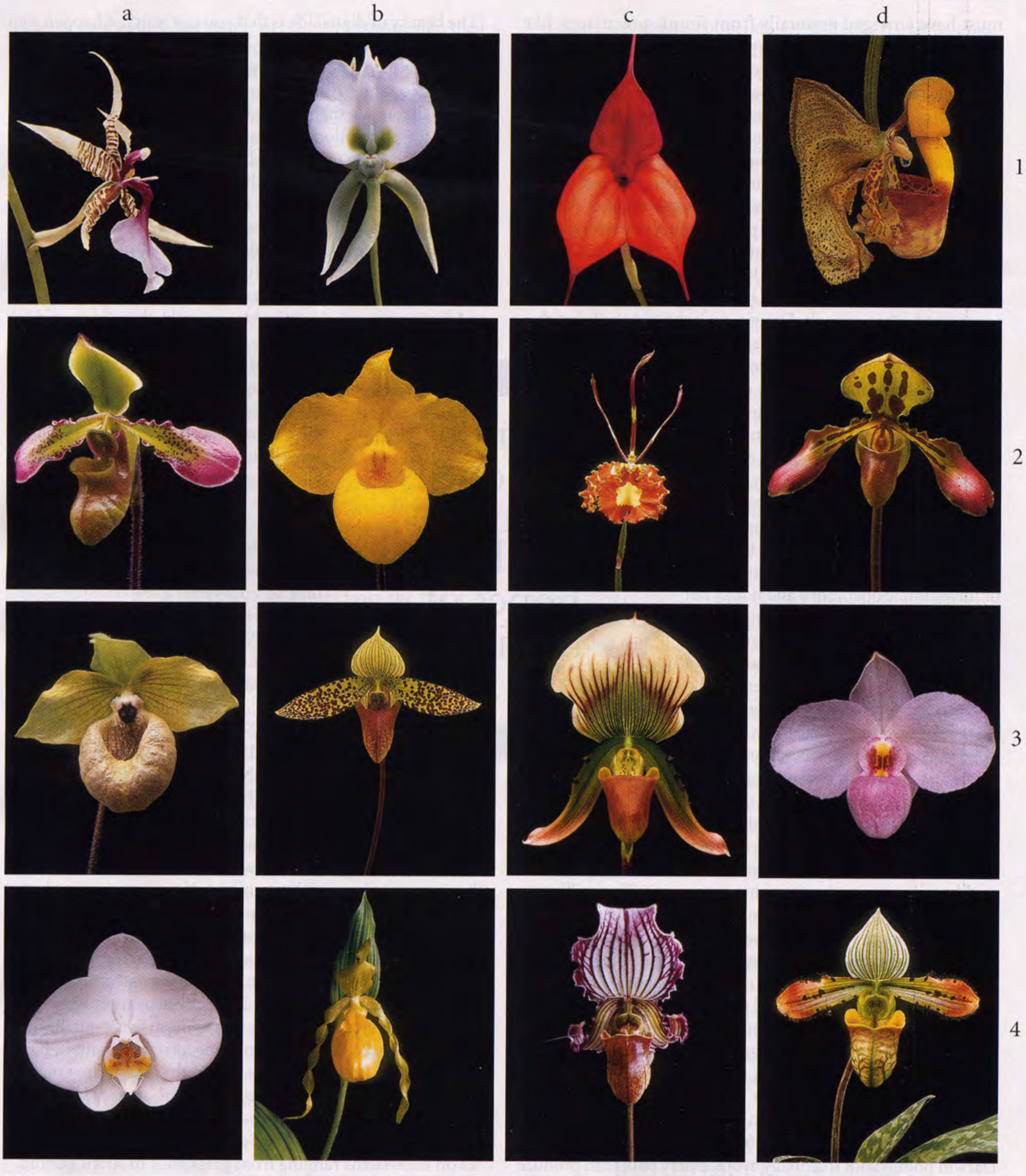
Similar patterns occur in the evolution of digital organisms in Avida. So Lenski set up digital versions of his bacterial colonies and has been studying them ever since. He still marvels at the flexibility and speed of Avida, which not only allow him to alter experimental conditions with a few keystrokes but also to automatically record every mutation in every organism. “In an hour I can gather more information than we had been able to gather in years of working on bacteria,” Lenski says. “Avida just spits data at you.”

With this newfound power, the Avida team is putting Darwin to the test in a way that was previously unimaginable. Modern evolutionary biologists have a wealth of fossils to study, and they can compare the biochemistry and genes of living species. But they can't look at every single generation and every single gene that separates a bird, for example, from its two-legged dinosaur ancestors. By contrast, Avida makes it possible to watch the random mutation and natural selection of digital organisms unfold over millions of generations. In the process, it is beginning to shed light on some of the biggest questions of evolution.

QUESTION #1: WHAT GOOD IS HALF AN EYE?

If life today is the result of evolution by natural selection, Darwin realized, then even the most complex systems in biology

PHOTOGRAPHS: PAGE 28, DK IMAGES (1A, 1C, 2C, 3A, 3D, 4C, 4D); KAREN MOSKOWITZ (1B AND 3C), PER ERIKSSON (1D), MORGAN MAZZONI (2A), PLUSH STUDIOS (3B), JOE MCBRIDE (4A), GK AND VIKKI HART (4B)/GETTY IMAGES; PAT DOYLE (2B), LISA CATTRELLE (2D)/CORBIS; PAGE 29, NATIONAL LIBRARY OF MEDICINE/PHOTO RESEARCHERS.



Orchid collecting was a craze among Victorian naturalists. In *The Various Contrivances by Which British and Foreign Orchids Are Fertilised by Insects* (1862), Darwin set out to disprove the popular notion that the delicate flowers were designed by God to please humans. He showed how orchids had instead evolved to attract pollinating insects. Coevolution with a wide variety of insects has produced stunning diversity among wild orchid species, including *Oncidium hastilabium* (1a); *Angraecum eburneum* (1b); *Masdevallia welischii* (1c); *Coryanthes speciosa* (1d); *Paphiopedilum hookerae* (2a); *Paphiopedilum armeniacum* (2b); *Psychopsis papilio* (2c); *Paphiopedilum tigrinum* (2d); *Paphiopedilum malipoense* (3a); *Paphiopedilum sukhakulii* (3b); *Paphiopedilum callosum* (3c); *Paphiopedilum delenatii* (3d); *Phalaenopsis amabilis* (4a); *Cypripedium acaule* (4b); *Paphiopedilum fairrieanum* (4c); and *Paphiopedilum venustum* (4d).

PHOTOGRAPHS: FRANS LANTING (1A); ERIC HANSEN (1B–4D).

must have emerged gradually from simple precursors, like someone crossing a river using stepping-stones. But consider the human eye, which is made of many different parts—lens, iris, jelly, retina, optic nerve—and will not work if even one part is missing. If the eye evolved in a piecemeal fashion, how was it of any use to our ancestors? Darwin argued that even a simpler version of today's eyes could have helped animals survive. Early eyes might have been nothing more than a patch of photosensitive cells that could tell an animal if it was in light or shadow. If that patch then evolved into a pit, it might also have been able to detect the direction of the light. Gradually, the eye could have taken on new functions, until at last it could produce full-blown images. Even today, you can find these sorts of proto-eyes in flatworms and other animals. Darwin declared that the belief that natural selection cannot produce a complex organ "can hardly be considered real."

Digital organisms don't have complex organs such as eyes, but they can process information in complex ways. In order to add two numbers together, for example, a digital organism needs to carry out a lot of simpler operations, such as reading the numbers and holding pieces of those numbers in its memory. Knock out the commands that let a digital organism do one of these simple operations and it may not be able to add. The Avida team realized that by watching a complex organism evolve, they might learn some lessons about how complexity evolves in general.

'Countless creationists download Avida in hopes of finding a fatal flaw'

The researchers set up an experiment to document how one particularly complex operation evolved. The operation, known as equals, consists of comparing pairs of binary numbers, bit by bit, and recording whether each pair of digits is the same. It's a standard operation found in software, but it's not a simple one. The shortest equals program Ofria could write is 19 lines long. The chances that random mutations alone could produce it are about one in a thousand trillion trillion.

To test Darwin's idea that complex systems evolve from simpler precursors, the Avida team set up rewards for simpler operations and bigger rewards for more complex ones. The researchers set up an experiment in which organisms replicate for 16,000 generations. They then repeated the experiment 50 times.

Avida beat the odds. In 23 of the 50 trials, evolution produced organisms that could carry out the equals operation. And when the researchers took away rewards for simpler operations, the organisms never evolved an equals program. "When we looked at the 23 tests, they were all done in completely different ways," adds Ofria. He was reminded of how Darwin pointed out that many evolutionary paths can produce the same complex organ. A fly and an octopus can both produce an image with their eyes, but their eyes are dramatically different from ours. "Darwin was right on that—there are many different ways of evolving the same function," says Ofria.

The Avida team then traced the genealogy leading from the first organism to each one that had evolved the equals routine. "The beauty of digital life is that you can watch it happen step by step," says Adami. "In every step you would ordinarily never see there is a goal you're going toward." Indeed, the ancestors of the successful organisms sometimes suffered harmful mutations that made them reproduce at a slower rate. But mutations a few generations later sped them up again.

When the Avida team published their first results on the evolution of complexity in 2003, they were inundated with e-mails from creationists. Their work hit a nerve in the antievolution movement and hit it hard. A popular claim of creationists is that life shows signs of intelligent design, especially in its complexity. They argue that complex things could have never evolved, because they don't work unless all their parts are in place. But as Adami points out, if creationists were right, then Avida wouldn't be able to produce complex digital organisms. A digital organism may use 19 or more simple routines in order to carry out the equals operation. If you delete any of the routines, it can't do the job. "What we show is that there are irreducibly complex things *and* they can evolve," says Adami.

The Avida team makes their software freely available on the Internet, and creationists have downloaded it over and over again in hopes of finding a fatal flaw. While they've uncovered a few minor glitches, Ofria says they have yet to find anything serious. "We literally have an army of thousands of unpaid bug testers," he says. "What more could you want?"

QUESTION #2: WHY DOES A FOREST HAVE MORE THAN ONE KIND OF PLANT?

When you walk into a forest, the first thing you see is diversity. Trees tower high overhead, ferns lurk down below, vines wander here and there like tangled snakes. Yet these trees, ferns, and vines are all plants, and as such, they all make a living in the same way, by catching sunlight. If one species was better than all the rest at catching sunlight, then you might expect it to outcompete the other plants and take over the forest. But it's clear that evolution has taken a different course.

Figuring out why is a full-time job for a small army of biologists. A number of them seek enlightenment by comparing places that are rich and poor in species and trying to figure out the other things that make them different. One intriguing pattern has to do with food. Ecologists have found that the more energy a habitat can provide organisms, the more species it can support. But a habitat can get too productive. Then it supports fewer species. This pattern has emerged time and again in studies on ecosystems ranging from grasslands to Arctic tundra.

Until recently, a typical Avida experiment would end up with a single dominant organism. The Avida researchers suspected that was the result of providing an endless supply of food—in this case, numbers. Perhaps, they reasoned, if they put their digital organisms on a diet, they might evolve into different forms—just as it happens in nature. So the Avida team retooled their software to limit the supply of numbers flowing into their digital worlds. Then they made the numbers even more scarce by splitting them up into smaller supplies, each of which could be used only for a particular operation, such as adding two numbers. As the organisms used the numbers at a faster rate, they got a smaller benefit. And if too many organisms gorged themselves on one supply of numbers, they would stop replicating altogether.

The Avida team subsequently flooded some digital worlds with numbers and limited others to a scant supply, and the same pattern of diversity found in global ecosystems emerged. When the number supply was low, only one type of organism could survive. At intermediate levels, three or four different types emerged and coexisted. Each type evolved into a specialist at one or a few kinds of operations. But when the number supply got too abundant, diversity dropped to a single species again.

Bringing diversity into Avida has brought more bad news for those who think complexity cannot evolve. Ofria decided to run the complexity experiment over again, this time with a limit on the supply of numbers. "It just floored me," he says. "I went back and checked this so many ways." In the original experiment, the organisms evolved the equals routine in 23 out of 50 trials. But when the experiment was run with a limited supply of numbers, all the trials produced organisms that could carry out the equals routine. What's more, they needed only a fifth of the time to do it.

Ofria suspects that the difference comes from the fact that several species are now evolving in the experiment rather than just one. More species mean more opportunities for success.

QUESTION #3: WHY BE NICE?

Human society depends on countless acts of cooperation and personal sacrifice. But that doesn't make us unique. Consider *Myxococcus xanthus,* a species of bacteria that Lenski and his colleagues study. *Myxococcus* travels in giant swarms 100,000 strong, hunting down *E. coli* and other bacteria like wolves chasing moose. They kill their prey by spitting out antibiotics; then they spit out digestive enzymes that make the *E. coli* burst open. The swarm then feasts together on the remains. If the *Myxococcus* swarm senses that they've run out of prey to hunt, they gather together to form a stalk. The bacteria at the very top of the stalk turn into spores, which can be carried away by wind or water to another spot where they can start a new pack. Meanwhile, the individuals that formed the stalk die.

This sort of cooperation poses a major puzzle because it could be undermined by the evolution of cheaters. Some bacteria might feast on the prey killed by their swarm mates and avoid wasting their own energy making antibiotics or enzymes. Others might evolve ways of ensuring that they always end up becoming spores and never get left behind in the dead stalk. Such cheaters are not theoretical: Lenski and his colleagues have evolved them in their lab.

The Avida team is now trying to address the mystery of cooperation by creating new commands that will let organisms exchange packages of information. "Once we get them to communicate, can we get them to work together to solve a problem?" asks Ofria. "You can set up an information economy, where one organism can pay another one to do a computation for it."

If digital organisms cooperate, Ofria thinks it may be possible to get them working together to solve real-world computing problems in the same way *Myxococcus* swarms attack their prey. "I think we'll be able to solve much more complex problems, because we won't have to know how to break them down. The organisms will have to figure it out for themselves," says Ofria. "We could really change the face of a lot of computing."

QUESTION #4: WHY SEX?

Birds do it, bees do it, and even fleas do it—but *why* they all do it is another matter. Reproduction is possible without sex. Bacteria and protozoa simply split in two. Some trees send shoots into the ground that sprout up as new trees. There are even lizard species that are all female. Their eggs don't need sperm to start developing into healthy baby female lizards.

"One of the biggest questions in evolution is, why aren't all organisms asexual?" says Adami. Given the obvious inefficiency of sex, evolutionary biologists suspect that it must confer some powerful advantage that makes it so common. But they have yet to come to a consensus about what that advantage is.

So Dusan Misevic, a biologist at Michigan State, has spent the past couple of years introducing sex into Avida. While digital sex may lack romance, it features the most important element from an evolutionary point of view: the genetic material from two parents gets mixed together in a child. When a digital organism makes a copy of itself, the copy doesn't immediately take its own place in Avida and start reproducing. Instead, chunks of its code are swapped with the copy of another new organism. Only after this exchange do the two creatures start to reproduce.

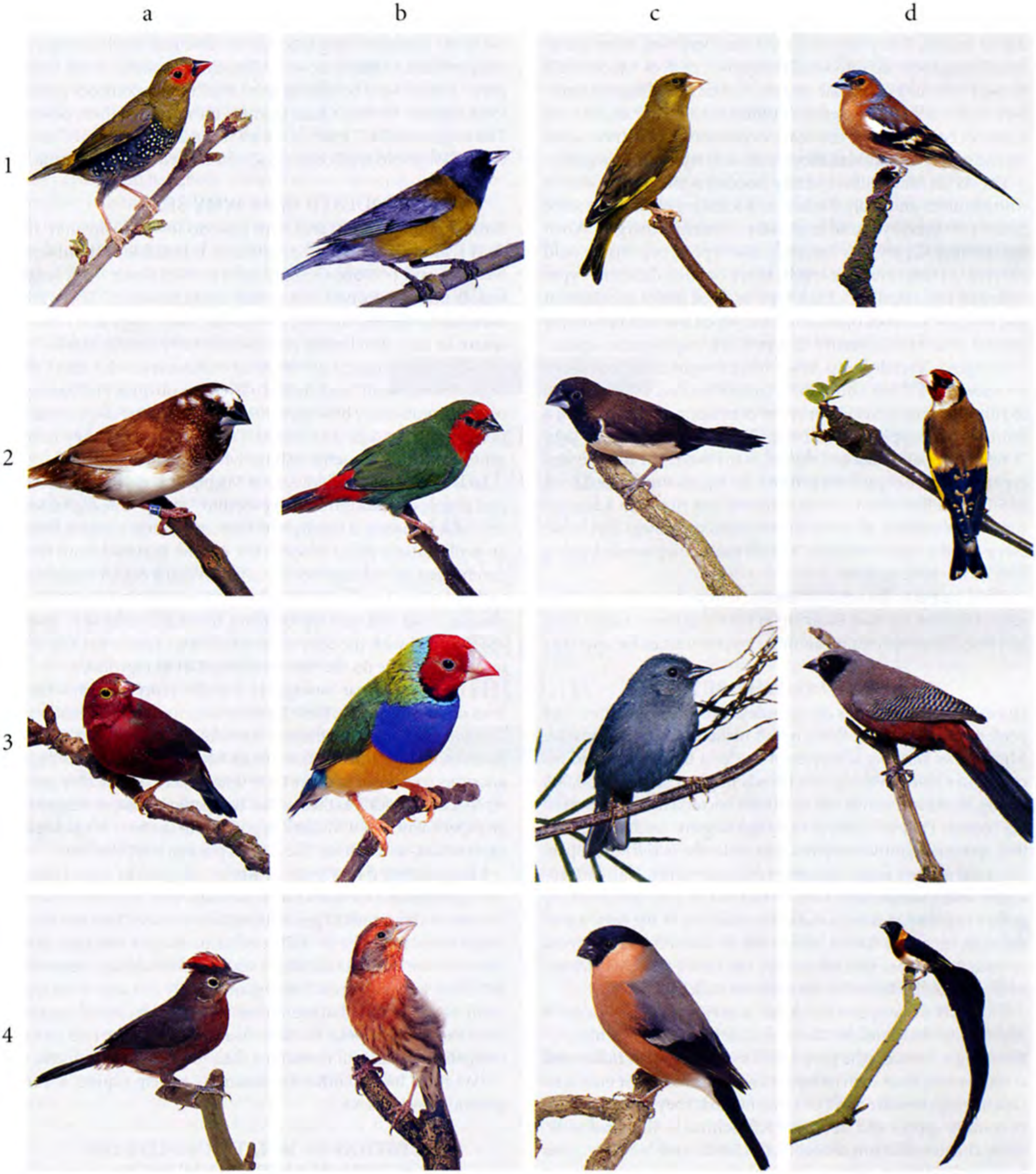
In 1964 the German biologist H. J. Muller proposed that sex allows organisms to mix their genomes together in combinations that can overcome the effects of harmful mutations. Asexual organisms, on the other hand, are stuck with all the mutations their ancestors pass down to them. Over time, Muller argued, they can't reproduce as quickly as their sexual competitors. Misevic designed an experiment to put Muller's hypothesis to the test. "It's a classic explanation, so it seemed like a good place to start," he says.

Misevic created two kinds of worlds: one full of sexual digital organisms and the other full of asexuals. After they had evolved for tens of thousands of generations, he measured how fast they could replicate. "The overall conclusion we got was that, yes, there are some situations where sex is beneficial," says Misevic. But there were surprises. Sex is good mainly as a way to escape annihilation from lethal mutations. But in Avida, sexual organisms had to pay a price for that insurance—they carried more nonlethal yet harmful mutations than the asexual organisms.

"We must look to other explanations to help explain sex in general," says Misevic.

QUESTION #5: WHAT DOES LIFE ON OTHER PLANETS LOOK LIKE?

Life on Earth is based on DNA. But we can't exclude the possibility that life could evolve from a completely different system of molecules. And that raises some worrying questions about the work going on these days to find signs of extraterrestrial life. NASA is funding a wide range of life-detecting instruments, from rovers that prowl across Mars to telescopes



Finches played a central role in Darwin's thinking about the evolutionary process of natural selection. When he visited the Galápagos Islands in 1835, he collected specimens of 13 finch species, each with a different beak shape adapted for eating different foods. For example, some species had long, narrow beaks for nabbing grubs, and others had clawlike beaks for grinding fruit. Male finches have also evolved bright colors and extravagant plumage to attract mates, which adds to the diversity of these modern species: *Madingoa nitidula* (1a); *Phrygilus gayi* (1b); *Carduelis chloris* (1c); *Fringilla coelebs* (1d); *Lonchura domestica* (2a); *Erythrura psittacea* (2b); *Lonchura leucogastroides* (2c); *Carduelis carduelis* (2d); *Lagonosticta senegala* (3a); *Chloebia gouldiae* (3b); *Haplospiza unicolor* (3c); *Estrilda erythronotus* (3d); *Coryphospingus cucullatus* (4a); *Carpodacus mexicanus* (4b); *Pyrrhula pyrrhula* (4c); and *Vidua paradisaea* (4d).

PHOTOGRAPHS: CYRIL LAUBSCHER/DK IMAGES (1A, 1C, 1D, 2A–2D, 3A–3B, 3D, 4A–4D); J. DUNNING/VIREO (1B AND 3C).

that will gaze at distant solar systems. They are looking for the signs of life that are produced on Earth. Some are looking for high levels of oxygen in the atmospheres of other planets. Others are looking for bits of DNA or fragments of cell walls. But if there's non-DNA-based life out there, we might overlook it because it doesn't fit our preconceptions.

"We can look at how known life-forms leave marks on their environment," says Evan Dorn, a member of Chris Adami's lab at Caltech, "but we can never make universal statements about them because we have only one example."

Dorn says Avida is example number two. By finding patterns that are shared by life on Earth and life in Avida, he thinks he will be able to offer some ideas about how to look for life that the universe might be harboring.

Some researchers have suggested the best way to look for signs of life is to look for weird chemistry. Take the building blocks of proteins—amino acids—which are found on meteorites and can also be created in the lab simply by running an electric current through ammonia and other compounds. In a lifeless setting, the most common amino acid that results is the simplest: glycine. Some slightly less simple amino acids are also common, but all the larger ones make up only a trace or are missing altogether. That's because it takes a lot of energy to make those big amino acids. "There's a limited repertoire of chemistry in the absence of life," says Dorn.

If you analyze a scoop of soil or pond water, however, you'll find a completely different profile of amino acids. Life has evolved ways of building certain big amino acids, and when organisms die, those big amino acids float around in the environment.

'If there's non-DNA-based life out there, we might overlook it'

What if life on another planet made compounds that were radically different from amino acids? Would it alter its planet's chemistry in some similar way?

To test this idea, Dorn created a world devoid of life. Instead of containing a self-replicating program, each cell contained a random assortment of commands. All of the commands in the Avida language were present at equal levels. Here was the signature of a lifeless planet.

Then Dorn began dropping organisms into this world, like spores falling to Earth. At the beginning of the experiment, he set the mutation rate so high that no spore could replicate very long on the planet. (Think of Mars, where ultraviolet rays pelt the surface.) Gradually, he lowered the mutation rate until life could survive. "As soon as the environment was habitable, the organism took over and dominated the environment," Dorn says.

As the digital organisms evolved to adapt to the world, Dorn found that some commands became rare and others became far more common. This distinctive signature stayed stable as long as life could survive on the planet. And no matter how many times Dorn repeated the experiment, the same signature of life appeared. Whether manipulating amino acids or computer commands, life does seem to leave the same mark. "It gives us a pretty strong indication that this process is universal," says Dorn.

If Dorn is right, discovery of non-DNA life would become a little less spectacular because it would mean that we have already stumbled across it here on Earth—in East Lansing, Michigan.

QUESTION #6: WHAT WILL LIFE ON EARTH LOOK LIKE IN THE FUTURE?

One of the hallmarks of life is its ability to evolve around our best efforts to control it. Antibiotics, for example, were once considered a magic bullet that would eradicate infectious diseases. In just a few decades, bacteria have evolved an arsenal of defenses that make many antibiotics useless.

Ofria has been finding that digital organisms have a way of outwitting him as well. Not long ago, he decided to see what would happen if he stopped digital organisms from adapting. Whenever an organism mutated, he would run it through a special test to see whether the mutation was beneficial. If it was, he killed the organism off. "You'd think that would turn off any further adaptation," he says. Instead, the digital organisms kept evolving. They learned to process information in new ways and were able to replicate faster. It took a while for Ofria to realize that they had tricked him. They had evolved a way to tell when Ofria was testing them by looking at the numbers he fed them. As soon as they recognized they were being tested, they stopped processing numbers. "If it was a test environment, they said, 'Let's play dead,'" says Ofria. "There's this thing coming to kill them, and so they avoid it and go on with their lives."

When Ofria describes these evolutionary surprises, admiration and ruefulness mix in his voice. "Here I am touting Avida as a wonderful system where you have full knowledge of everything and can control anything you want—except I can't get them to stop adapting. Life will always find a way."

Thinking about such adaptable creatures lurking on the Michigan State campus, furiously feeding on data, can be unsettling. Should the Avida team be working in quarantine? Lenski argues that Avida itself acts as a quarantine, because its organisms can exist only in its computer language. "They're living in an alien world," Lenski says. "They may be nasty predators from Mars, but they'd drop dead here."

Still, Ofria acknowledges that harmful computer viruses may eventually evolve like his caged digital organisms. "Some day it's going to happen, and it's going to be scary," Ofria says. "Better to study them now so we know how to deal with them."

★ DISCOVER MORE [also see Resources, page 85]

For Avida software downloads and more about ongoing research, visit Caltech's Digital Life Laboratory Web site: dllab.caltech.edu/avida.

The Evolutionary Origin of Complex Features. R. E. Lenski, C. Ofria, R. T. Pennock, and C. Adami in *Nature* 423 (2003), pages 139–145.

resources

The following books, journals, and Web sites provide additional information about topics featured in *Discover* this month.

9 R & D

"Flash." For more about eggs dividing without sperm, see "Phospholipase Cζ Causes Ca^{2+} Oscillations and Parthenogenetic Activation of Human Oocytes." N. T. Rogers et al. in *Reproduction* 128 (2004), pages 697–702.

Read about dinosaur diversity in "Shape of Mesozoic Dinosaur Richness." David E. Fastovsky et al. in *Geology* 32, pages 877–880; October 2004.

To learn more about alcoholic rats, read the University of North Carolina (at Chapel Hill) press release at www.unc.edu/news/archives/nov04/alcoholabstain110504.html; also see an article by Kimberly Nixon and Fulton T. Crews: "Temporally Specific Burst in Cell Proliferation Increases Hippocampal Neurogenesis in Protracted Abstinence From Alcohol" in *Journal of Neuroscience* 24, pages 9714–9722; October 27, 2004.

"The Kind of Face Only a Wasp Could Trust." For more on Elizabeth Tibbetts's and James Dale's research, see their home pages: cis.arl.arizona.edu/PERT/people/Tibbetts/index.htm (Tibbetts) and geocities.com/quelea (Dale).

"A Socially Enforced Signal of Quality in a Paper Wasp." Elizabeth Tibbetts and James Dale in *Nature* 432, pages 218–222; November 11, 2004.

"Terrorism Linked to Traffic Accidents." "Terror Attacks Influence Driving Behavior in Israel." Guy Stecklov and Joshua Goldstein in *Proceedings of the National Academy of Sciences,* Vol. 101, No. 40, pages 14551–14556; October 5, 2004.

"As the World Warms." The full Arctic Climate Impact Assessment ("ACIA, Impacts of a Warming Arctic, 2004") is online at www.acia.uaf.edu.

For time-lapse photos of the Jakobshavn Glacier in Greenland, see NASA's Web site: www.nasa.gov/vision/earth/lookingatearth/jakobshavn.html.

"Long-Term Decline in Krill Stock and Increase in Salps Within the Southern Ocean." Angus Atkinson et al. in *Nature* 432, pages 100–103; November 4, 2004.

"Cassini Watch: Stormy Saturn." The official Cassini-Huygens Mission Web site is saturn.jpl.nasa.gov.

"When the Brain Gets Out of Tune." "Neural Synchrony Indexes Disordered Perception and Cognition in Schizophrenia." Kevin M. Spencer et al. in *Proceedings of the National Academy of Sciences,* Vol. 101: 17288–17293; published online November 16, 2004 at www.pnas.org/cgi/doi/10.1073/pnas.0406074101.

"Look Back." Read space shuttle news and features at www.nasa.gov/news/highlights/returntoflight.html.

For an overview of NASA's vision of future space exploration, visit www.nasa.gov/missions/solarsystem/explore_main.html.

"Discover Data." For updates and more information on the federal R&D budget, visit the American Association for the Advancement of Science Web site: www.aaas.org/spp/rd. The U.S. House of Representatives Committee on Appropriations can be found here: appropriations.house.gov.

18 EMERGING TECHNOLOGY

It's a battle of the do-it-yourself bands at www.macidol.com, a site where GarageBand users can share their creations.

Listen to some examples of a Vocaloid vocalist at the Web site of the creators of three voice fonts: www.zero-g.co.uk/index.cfm?articleid=802.

20 THE BIOLOGY OF . . . CRYOGENICS

Find wood frog facts (and fiction and poetry!) at the Storey lab Web site: www.carleton.ca/~kbstorey.

28 TESTING DARWIN

Evolution: The Triumph of an Idea. Carl Zimmer. Perennial, 2002.

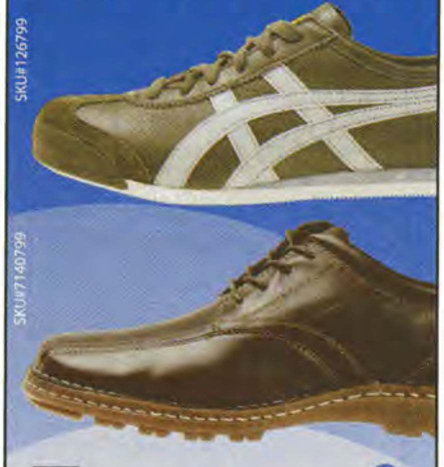
"Developmental Cheating in the Social Bacterium *Myxococcus xanthus.*" G. J. Velicer, L. Kroos, and R. E. Lenski in *Nature* 404, pages 598–601; April 6, 2000.

Orchid pictures were provided by Eric Hansen, author of *Orchid Fever: A Horticultural Tale of Love, Lust, and Lunacy* (Vintage Books, 2001). His latest book is *The Bird Man and the Lap Dancer: Close Encounters With Strangers* (Pantheon, 2004).

Special thanks to Scott Mitamura of the Honolulu Botanical Gardens and Blanche Wagner of the Missouri Botanical Garden for identifying the orchid species pictured on page 31.

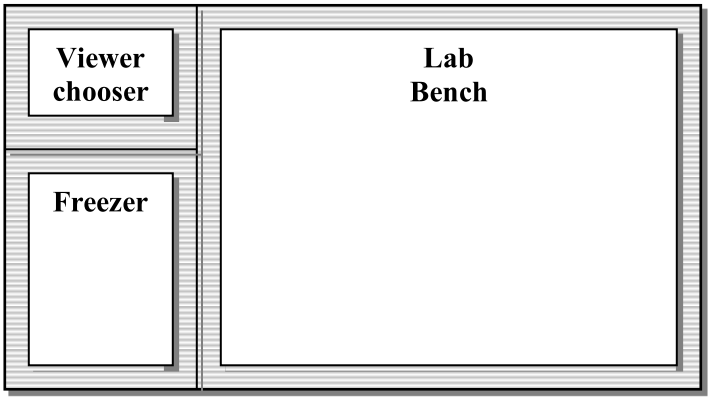
44 WORRYING ABOUT KILLER FLU

Two Web sites that provide overviews of the influenza virus are Microbiology.mtsinai.on.ca/bug/flu/flu-bug.shtml and Web.uct.ac.za/depts/mmi/jmoodie/influen2.html.



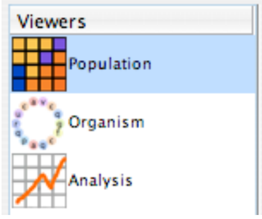
Avida-ED Quick Start User Manual

I. General Avida-ED Workspace



(A) Viewer chooser buttons

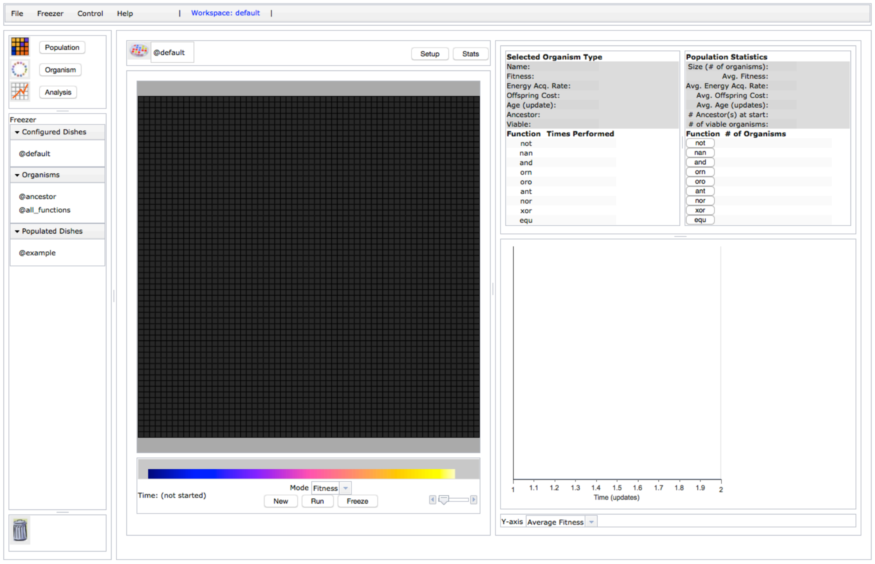
Switch between lab bench views



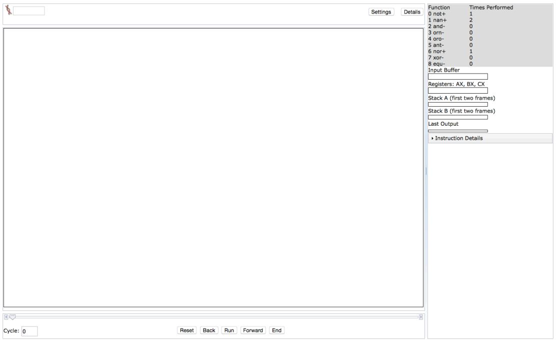
(B) Lab bench

Three lab bench options:

1. Population View Lab Bench (Petri dish & stats viewpanes)

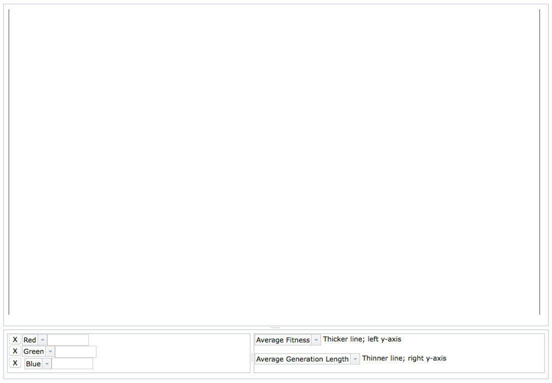


2. Organism View Lab Bench (Genetiscope)



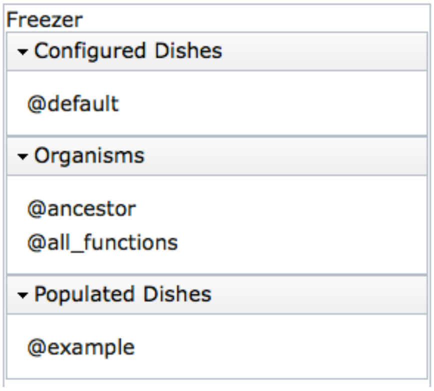
Function	Times Performed
0 not+	1
1 nan+	2
2 and-	0
3 orn-	0
4 oro-	0
5 ant-	0
6 nor+	1
7 xor-	0
8 equ-	0

3. Analysis View Lab Bench (Data graphing & analysis)


X
Red
X
Green
X
Blue
Average Fitness
Thicker line; left y-axis
Average Generation Length
Thinner line; right y-axis

(C) The Freezer

- Storage shelves for:
- Configured Petri dishes (environmental settings but no organisms)
- Individual organisms removed from a Petri dish
- Populated Petri dishes (environment settings and frozen organisms)



- Drag and drop freezer items to or from an open lab bench.
- Right-click (Ctrl-click) or double-click item in freezer (except default items) to rename, delete or export it.
- Click a disclose triangle to hide or show lists of items
- Export selected item. [MENU File -> Export Freezer Item…]

II. Lab Benches

(1) Population Viewer ("Petri Dish" & environment settings)

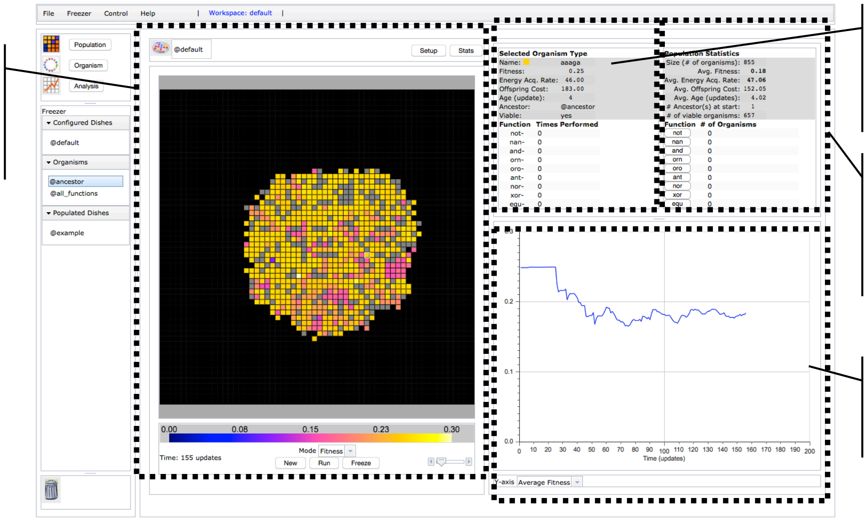
Functionality

- Evolve a population in a Petri dish
- Click on an individual organism and observe its stats
- Observe whole population stats in real time
- Graph population stats in real time

Basic Controls

- **Drag** a Petri dish (configured or populated) or one or more organisms into the Petri dish bench from the freezer to begin a new run.
- **Setup/Map button**: Switch between Petri dish front view and its environment settings.
- **Export** data to comma-delimited file. [MENU File -> Export Data…]
- **Save images** of population or graph in viewer [MENU File -> Export Graphics…]
- **Import** or **Export** a dish or organism. [MENU File -> Import/Export Item…]

Dish viewpane (front)



Time: 155 updates

Selected Organism Type

Name: 🟨	aaaga
Fitness:	0.25
Energy Acq. Rate:	46.00
Offspring Cost:	183.00
Age (update):	4
Ancestor:	@ancestor
Viable:	yes

Function	Times Performed
not-	0
nan-	0
and-	0
orn-	0
oro-	0
ant-	0
nor-	0
xor-	0
equ-	0

Population Statistics

Size (# of organisms):	855
Avg. Fitness:	0.18
Avg. Energy Acq. Rate:	47.06
Avg. Offspring Cost:	152.05
Avg. Age (updates):	4.02
# Ancestor(s) at start:	1
# of viable organisms:	657

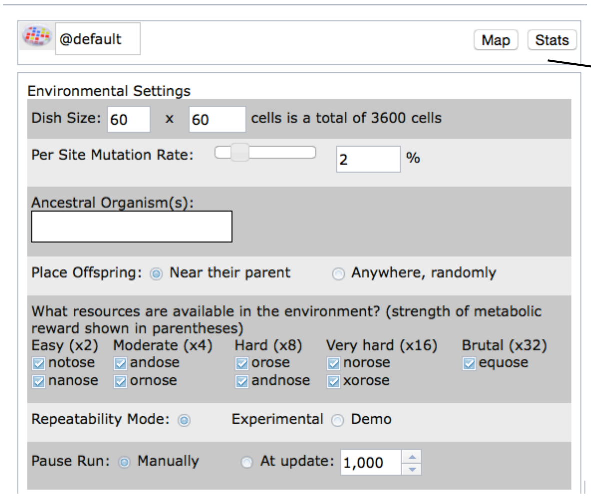
Function	# of Organisms
not	0
nan	0
and	0
orn	0
oro	0
ant	0
nor	0
xor	0
equ	0

Organism clicked on stats report

Population statistics panel

Population data graph

Dish viewpane (rear) Environment settings



Environmental Settings

Dish Size: 60 x 60 cells is a total of 3600 cells

Per Site Mutation Rate: 2 %

Ancestral Organism(s):

Place Offspring: ● Near their parent ○ Anywhere, randomly

What resources are available in the environment? (strength of metabolic reward shown in parentheses)

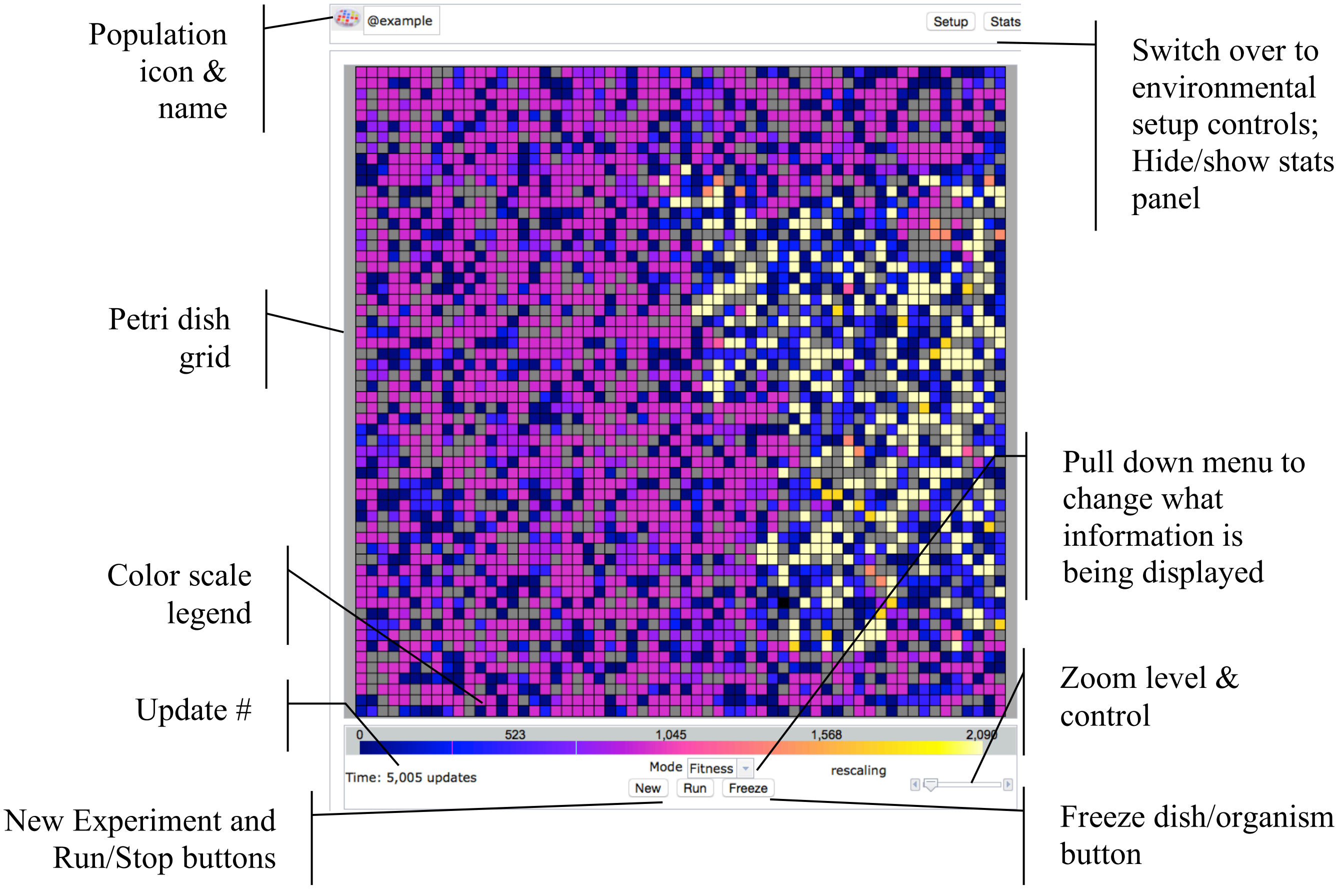
Easy (x2)	Moderate (x4)	Hard (x8)	Very hard (x16)	Brutal (x32)
☑ notose	☑ andose	☑ orose	☑ norose	☑ equose
☑ nanose	☑ ornose	☑ andnose	☑ xorose	

Repeatability Mode: ● Experimental ○ Demo

Pause Run: ● Manually ○ At update: 1,000

Buttons to flip to Petri dish front map and to hide/show side stats panel

Dish viewpane (front)


Population icon & name
@example
Setup
Stats
Switch over to environmental setup controls; Hide/show stats panel
Petri dish grid
Pull down menu to change what information is being displayed
Color scale legend
0
523
1,045
1,568
2,090
Update #
Time: 5,005 updates
Mode Fitness
rescaling
New
Run
Freeze
Zoom level & control
New Experiment and Run/Stop buttons
Freeze dish/organism button

- **Population icon & name**: Drag icon to freezer to save either the populated dish or just the dish environmental configuration. Click name to rename.
- **Setup button**: Go to environmental settings for this Petri dish.
- **Stats button**: Hide/reveal stats side panel for this dish.
- **Petri dish map**: More or less of the map is visible depending upon the size of the grid. Use scroll bars and/or zoom control if grid extends beyond window.
- **Color Scale Legend**: During a run, the color scale will automatically adjust as numbers increase or decrease. ["Rescaling" will appear in parentheses.] The spectrum will thus always show relative values, but particular colors will represent different absolute values as a population evolves. *Special colors*: Black indicates an empty cell and white indicates a cell whose value is above the maximum portrayed on the scale (as the scale readjusts to accommodate this new value it will be colored appropriately). Grey indicates that the organism in the cell is not viable.
- **Time** - Avida internal time; in number of updates since the beginning of a run. This is a constant internal time; it does not correspond to external time because updates take longer for larger populations.

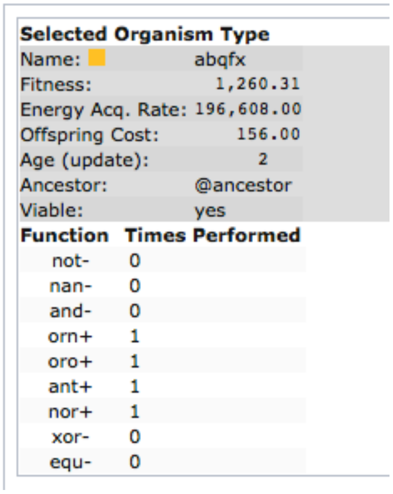
- **Mode menu**: Pull down menu to select a feature of the population to indicate by color. Options: Fitness, Offspring Cost, Energy Acquisition Rate, Ancestor Organism.
- **New** button: Ends the current run (after offering to discard or save it) and then resets the Petri dish for a new experiment.
- **Run/Stop** button: Starts and temporarily stops a run.
- **Freeze** button: Save the population and/or environmental settings to the freezer.
- **Zoom**: Set the zoom level for the Petri dish. Use scroll bars if zooming in causes the grid to extend beyond window.

Shortcuts:

- **Click** on an organism in the Petri Dish and **drag** it to the Freezer to save it.
- **Click** on an organism in the Petri Dish or the Freezer and **drag** it to the Organism Viewer icon in the Viewer Chooser to open it directly in the Organism Viewer.
- **Click** on an organism in the Petri Dish to select it. Use arrow keys (up, down, left or right) to select a neighboring organism.
- **Click** on the Petri Dish icon in the Petri Dish and **drag** it to the Freezer to save it.
- **Click** on the Petri Dish icon in the Petri Dish or on the name of a populated dish in the Freezer and **drag** it to the Analyze Viewer icon in the Viewer Choose to view it directly in the Analyzer without having to save it to the Freezer first.
- **Click** on an item in the Freezer and **drag** it to the trash can icon to delete it.

Stats viewpanes

Selected Organism Type Statistics Report



Selected Organism Type

Name: 🟧	abqfx
Fitness:	1,260.31
Energy Acq. Rate:	196,608.00
Offspring Cost:	156.00
Age (update):	2
Ancestor:	@ancestor
Viable:	yes

Function	Times Performed
not-	0
nan-	0
and-	0
orn+	1
oro+	1
ant+	1
nor+	1
xor-	0
equ-	0

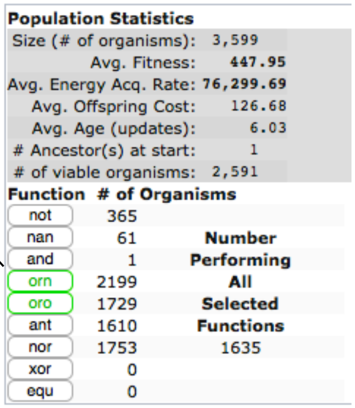
Name: Upon initial replication, Avida-ED automatically assigns temporary placeholder names to new varieties of Avidians as they evolve in a population. (New kinds of organisms that have not replicated, perhaps because they are not viable, are listed as no_name.) The square's color matches the color of the selected Avidian in the current Petri dish grid.

Basic Statistics: The listing of stats related to the selected organism are updated as the population is running: Fitness, Energy Acquisition Rate, Offspring Cost, Age (updates), Ancestor, and Viability.

Functions list: Metabolic functions, listed in ascending order of complexity, that the organism may have evolved the ability to perform. If "Times Performed" is "0" the organism has not performed that function yet in this lifetime (or never does). Organisms get more energy (SIPs) if they perform more complex functions.

Population statistics

Click buttons to highlight orgs in dish that have these phenotypes



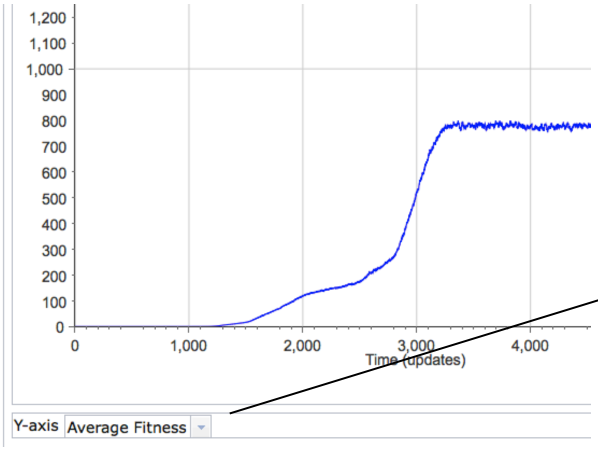
Population Statistics

Size (# of organisms):	3,599
Avg. Fitness:	447.95
Avg. Energy Acq. Rate:	76,299.69
Avg. Offspring Cost:	126.68
Avg. Age (updates):	6.03
# Ancestor(s) at start:	1
# of viable organisms:	2,591

Function	# of Organisms	
not	365	
nan	61	Number
and	1	Performing
orn	2199	All
oro	1729	Selected
ant	1610	Functions
nor	1753	1635
xor	0	
equ	0	

- These are the same functions as in the Org. Clicked on Report, but data is given for the population as a whole.
- Click a single function button to highlight organisms in the dish that have the given function. Clicking two or more function buttons highlights organisms that have ALL the selected functions.

Population data graph

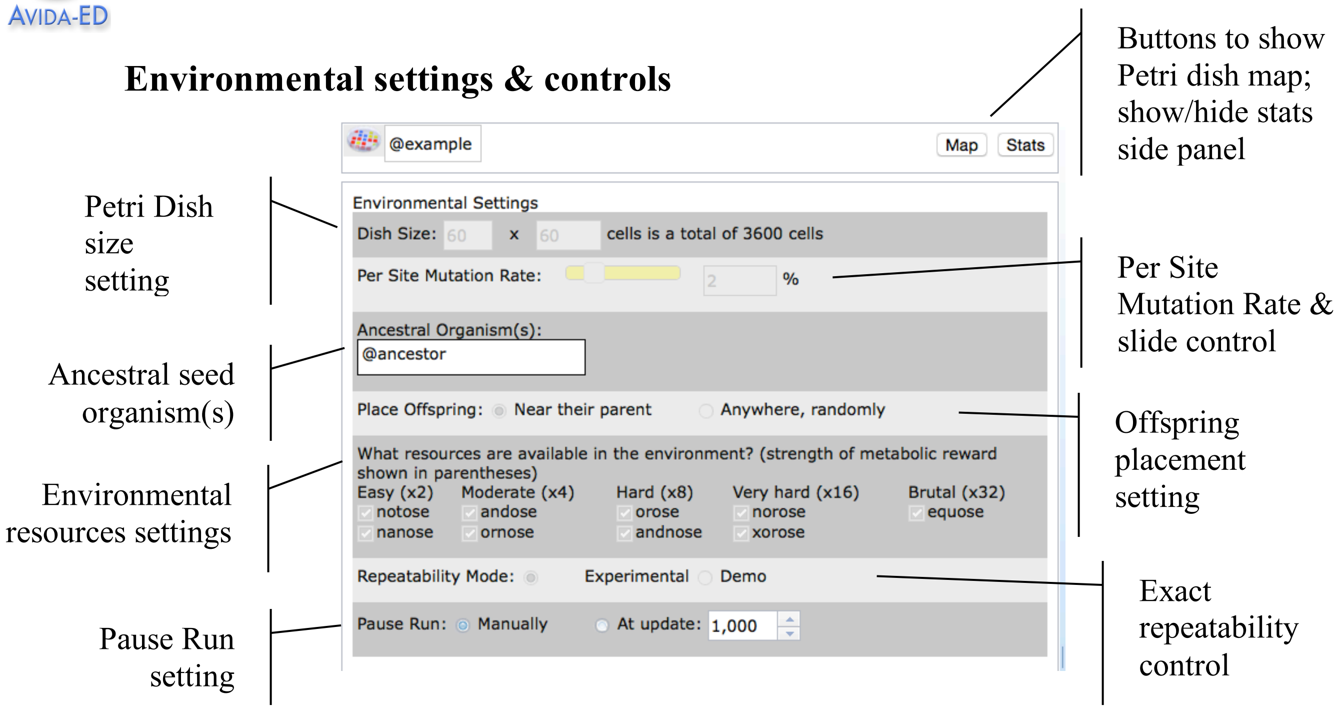


Y-axis Average Fitness

Pull down menu for property to graph

- **Pull down property menu**: Pick the property of the population to graph in real time. Options include: Average Energy Acquisition Rate, Average Fitness, Average Offspring Cost, and Number of Organisms in the population.
- **Vertical axis**: Units change depending upon property being graphed. The scale dynamically changes during a run to accommodate the evolving population.
- **Time axis**: Time, in updates, since beginning of run of this population.

Environmental settings & controls


Buttons to show Petri dish map; show/hide stats side panel
Petri Dish size setting
Per Site Mutation Rate & slide control
Ancestral seed organism(s)
Offspring placement setting
Environmental resources settings
Exact repeatability control
Pause Run setting
@example
Map
Stats

Environmental Settings

Dish Size: 60 x 60 cells is a total of 3600 cells

Per Site Mutation Rate: 2 %

Ancestral Organism(s):
@ancestor

Place Offspring: ● Near their parent ○ Anywhere, randomly

What resources are available in the environment? (strength of metabolic reward shown in parentheses)

Easy (x2)	Moderate (x4)	Hard (x8)	Very hard (x16)	Brutal (x32)
☑ notose	☑ andose	☑ orose	☑ norose	☑ equose
☑ nanose	☑ ornose	☑ andnose	☑ xorose	

Repeatability Mode: ● Experimental ○ Demo

Pause Run: ● Manually ○ At update: 1,000

- **Per site mutation rate**: This rate reflects the percent chance that an instruction is incorrectly copied. 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).

- **Dish size**: Sets the maximum number of Avidians that can exist in the population. The two numbers specify the number of Avidians per row, and per column. So, 10 x 10 = a population of 100 organisms.

- **Ancestral seed organism(s)**: The organism(s) the population begins from. Drag in or out additional organisms at the beginning of a run. If seed organisms are added to the dish using this setting they are automatically placed in a set pattern on the Petri Dish (e.g. in the exact center for a single organism or at the points of an equilateral triangle for three organisms). If you want to place organisms manually at specific points on the dish, drag them there on the front map side of the dish instead of seeding them here.

- **Environmental Resource Settings**: Avidians can receive extra energy if they evolve the ability to "metabolize" nutrients. Here you can set what nutrients are available in the environment.

- **Exact Repeatability**: Many steps in an Avida evolutionary run happen randomly (e.g. what mutations will occur in the genome, into what cell a new organism will be placed at division), so each run will be slightly different even with the same general environmental values, as in nature. This is the default setting. However, if you need to precisely repeat a run (e.g. for a demonstration) you can switch this to exactly replicate the sequence with the same mutations and values.

- **Offspring placement**: When an offspring is born, it can either be placed (at random) in one of the eight cells adjacent to its parent, or anywhere (at random) in the population. If the cell is already occupied, the organism there is overwritten.
- **Pause Run Manually/Automatically**: If you set a specific number ahead of time, the run will pause when this many updates have passed. If you set the run to stop manually, it will continue indefinitely until it is paused using the button under the Petri dish.

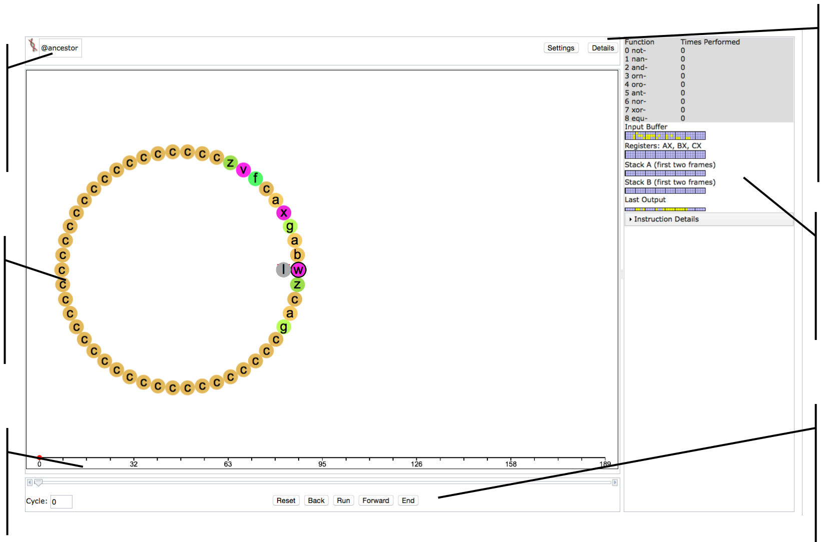
(2) Genetiscope (Organism genome viewer)

Functionality

- Observe the genome of an individual organism.
- Play a movie of the genome instructions' operation.
- Check aspects of an organism's phenotype, including its viability.
- Save viewer images.

Basic Controls

- **Drag** an organism from the freezer. (A progress bar will indicate that the organism is being readied for viewing.)
- Press **Run/Stop** to start/stop the movie of the genome's execution. Use the **scrub bar** to move back and forth in the movie, or press **Back** or **Forward** to move just one instruction at a time. **End** jumps to the final instruction. Press **Reset** to reset the genome to the start position for a fresh execution.
- **Click** the **DNA icon** of the parent or daughter cell and then **drag** into the freezer to save that genome. The daughter cell icon will only appear after division.
- Press **Settings** button for the viewer settings. Use the settings to change the per site mutation rate and the repeatability mode.
- Press **Details** to show or hide the side panel, which lists the org's functions and shows the bit-level operations of the genome instructions that produce them.



Function	Times Performed
0 not-	0
1 nan-	0
2 and-	0
3 orn-	0
4 oro-	0
5 ant-	0
6 nor-	0
7 xor-	0
8 equ-	0

Input Buffer

Registers: AX, BX, CX

Stack A (first two frames)

Stack B (first two frames)

Last Output

Instruction Details

Organism icon & name

Shows movie of an org's genome as it runs

Time line & Scrub bar

Show/hide viewer settings & org function details

Shows what the genome's instructions are doing

Play /Pause, Single step, Beginning & End controls

- **Organism icon & name**: Name of the organism whose genome is being viewed.
- **Time line**: Shows the cycle number of the instruction being executed and the number corresponding to any function that is completed at that point.
- **Run/Stop button**: Starts and temporarily stops movie.
- **Save images** of orgs in viewer [MENU File -> Export Graphics…]

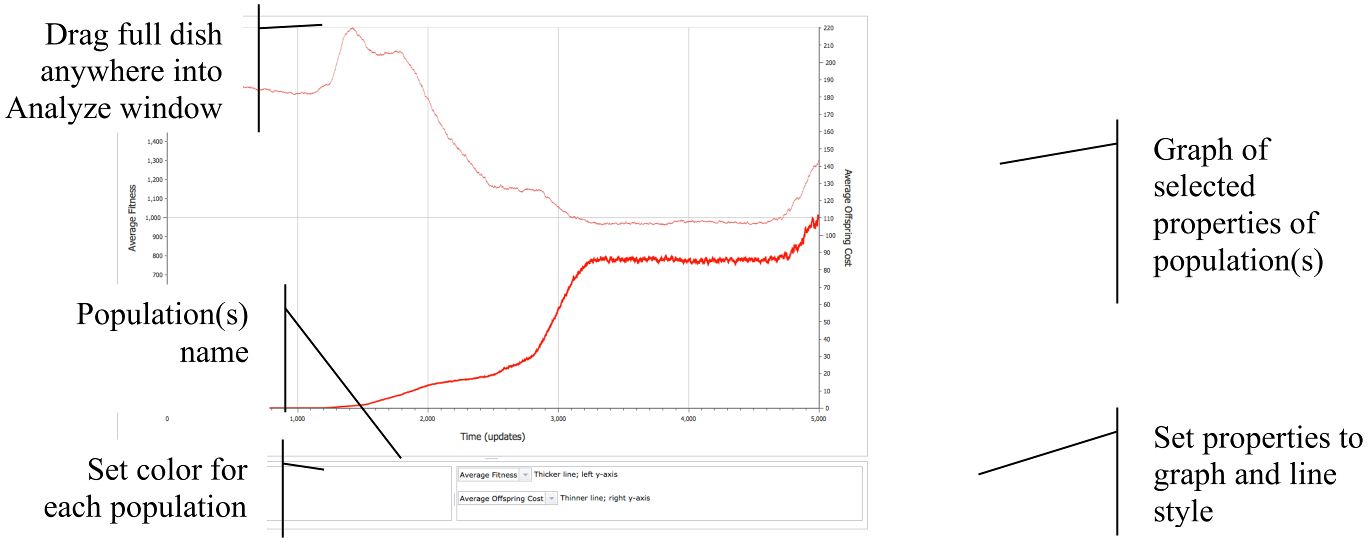
(3) Analyzer Lab Bench (Data graphing & analysis)

Functionality

- Graph one or two data sets from a population.
- Graph and compare data from one to three populations.
- Export selected data for external analysis in Excel or other programs.
- Save viewer images.

Basic Controls

- **Drag** full Petri dish(s) from the freezer onto the analyzer lab bench.
- Use **pull down menus** to choose the color for each population and what information you wish to graph (you can graph two properties at once).
- **Save images** of graphs [MENU File -> Export Graphics…]
- **Export** selected data to comma-delimited file. [MENU File -> Export Data…]



Drag full dish anywhere into Analyze window

Population(s) name

Set color for each population

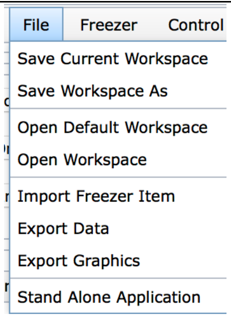
Graph of selected properties of population(s)

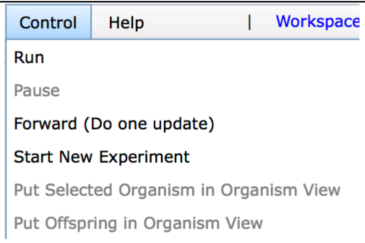
Set properties to graph and line style

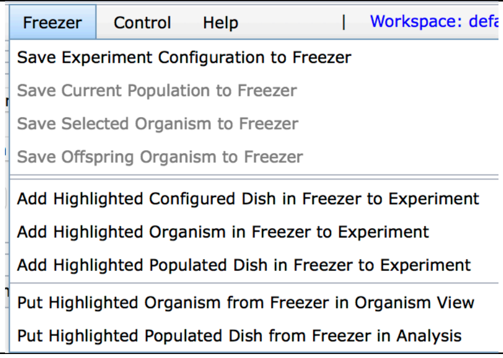
Average Fitness ▾ Thicker line; left y-axis

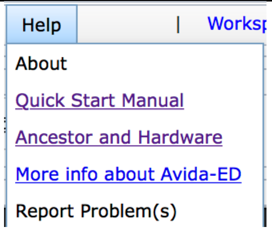
Average Offspring Cost ▾ Thinner line; right y-axis

(4) Menus






Freezer
Control
Help
| Workspace: defa
Save Experiment Configuration to Freezer
Save Current Population to Freezer
Save Selected Organism to Freezer
Save Offspring Organism to Freezer
Add Highlighted Configured Dish in Freezer to Experiment
Add Highlighted Organism in Freezer to Experiment
Add Highlighted Populated Dish in Freezer to Experiment
Put Highlighted Organism from Freezer in Organism View
Put Highlighted Populated Dish from Freezer in Analysis



File Menu

- **Workspace controls**: All the settings and data generated in your workspace may be saved and reopened later.
- **Export / Import controls**: Configured and populated dishes, and individual organisms can be separately exported and then later imported into another workspace or used by some other program.
- **Export Data**: Exports a CSV file with five columns [average Fitness, average Offspring Cost, average Energy Acquisition Rate, Population Size, Viable Population Size] for the entire population, the sub-set of organisms performing the picked logic functions and for the set of descendants of each starting ancestor.
- **Export Graphics**: Direct export of graphics has not yet been implemented, so this gives instructions for taking screenshots and snips.
- **Stand Alone Application**: Provides instructions on how to create a stand-alone version of Avida-ED for situations when you will not have internet access.

Freezer Menu

- **Save:** Petri dish environmental configurations, individual selected organisms, or entire populated Petri dishes in your workspace may be saved to the freezer for later reuse.
- **Highlighted organisms** may be added to dish for an experiment or opened in the Organism Viewer or Analysis Viewer. This is useful for tablets.

Control Menu

- **Run/Pause/Forward** a population that is set up in the Petri Dish. *Forward* advances the population just a single update.
- **Start New Experiment**: Discard or save the current setup and start a new experiment
- **Put Selected Organism in Organism View**: Copies an organism selected in the population viewer Petri Dish and puts it in the organism viewer.
- **Put Offspring in Organism View**: Takes the offspring of an organism in the organism view and puts it in the organism view in the ancestor position.

Help Menu

- **Help Files:** Links to a few basic help files.
- **Report Problem(s):** Use to send a bug report to the Avida-ED software developer.

Glossary

Avida

Pronounced Ah-vee-da. From A-life (artificial life) in Spanish (vida = life). Avida is the evolutionary computation engine at the heart of Avida-ED.

Avidian

Pronounced Ah-vîd-ian. An organism in the Avida environment.

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 Avida-ED 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 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).

SIP (Single Instruction Processing unit)

A unit of “energy” in Avida. It takes one SIP to execute one instruction.

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.