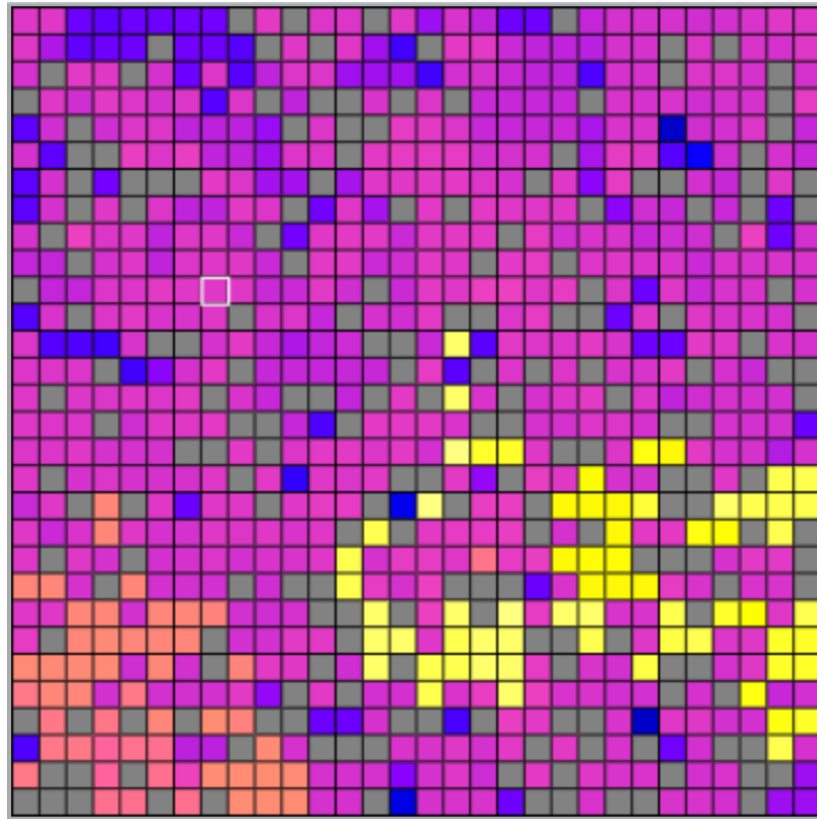


Avida-ED Lab Book

Fall 2016



Avidians in Action

Avida-ED Project Curriculum Development Team

Wendy Johnson, Cory Kohn, Amy Lark, Louise Mead,
Robert T. Pennock, Jim Smith, Michael Wiser

<http://avida-ed.msu.edu>

<http://bit.do/avida-ed>

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Preface

This is the fall 2016 revised edition of the Avida-ED Lab Book, which contains a set of Avida-ED exercises that serve as the basis for a series of conference presentations and workshops presented by Michigan State University's Avida-ED Project Curriculum Development Team. Based on feedback from our Active LENS faculty development workshop participants, this edition reverses the order of exercises 2 and 3, and incorporates a change of terminology 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 what was previously called "gestation time" is now called "offspring cost." This change highlights the energy flow in the system, specifically the energy that is acquired by performing metabolic functions and the energy that is expended to execute instructions to reproduce. Fitness is now understood as the *energy acquisition rate divided by the offspring cost*, which equals the rate of production of offspring. All the exercises have been updated to reflect this terminological change.

This new edition also has updated screenshots and instructions for the public beta of the new web-based version of Avida-ED (vers. 3.0). This new 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 new version and we want to especially call out her amazing efforts in making this major software update come to fruition. Earlier versions of Avida-ED will remain available but will no longer be maintained and we recommend that instructors now use the new version.

The Lab Book is divided into two sections. The first section contains materials used as a part of the Laboratory Curriculum in the Introductory Cell and Molecular Biology course (LB145) taught by Jim Smith at Michigan State University in the fall of 2014. Several of these are modifications of exercises developed earlier for other courses and one "Exploring Mutations and Selection: Pre-adaptive or Post-adaptive?" was developed for this course. The second section in the Lab Book consists of materials from three additional Avida-ED exercises previously developed by members of the Avida-ED Curriculum Development Group, "Exploring the Effects of Mutation Rate on Individuals", "From Genotype to Phenotype: Understanding the Introduction of Phenotypic Variations", and "Evolving TCE Biodegraders". Student handouts are provided for each of these exercises, as are web links to more complete curricular materials. All model exercises in the Lab Book and Avida-ED web site have been peer-reviewed and classroom tested.





Getting the Avida-ED software

Avida-ED 3.0 beta is the current version of the software and its use remains free of charge. The public beta was released on June 18, 2016. Avida-ED 3 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 or Chrome*. It will remain in beta status through the end of 2016, and will be regularly updated as bugs are uncovered and squashed.

- Access the latest software, curriculum and news from the Avida-ED home page:
<http://avida-ed.msu.edu/>

The direct URL for Avida-ED 3.0 is:

<https://avida-ed.beacon-center.org/app/AvidaED.html>

We also have two mirror locations:

<http://avida-ed-mirror1.beacon-center.org/AvidaED.html>

or

<http://bit.do/avida-ed>

- Once Avida-ED has loaded in your browser, it runs entirely on your own computer's CPU, not on any remote server. That means it requires an internet connection initially to 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.

- Go to <http://avida-ed.msu.edu/>.
- Click on "App Download".
- Select the radio button for the version you want to download.
- Click on "Download Avida-ED now".
- Open the file and drag the Avida-ED icon to your desktop. Open the application.



Part I: Avida-ED Exercises

The first part of the Avida-ED Lab Book consists of Avida-ED Exercises used in slightly modified form, originally developed for various undergraduate biology curricula.

Introduction - Avida-ED and Digital Evolution

This introductory exercise is a modification of the Introduction to Digital Evolution Handout & Tutorial by Wendy Johnson, Robert T. Pennock and Louise Mead contained in the Hands-on Activity: Studying Evolution with Digital Organisms available via the TeachEngineering.Org website at

https://www.teachengineering.org/view_activity.php?url=collection/mis_/activities/mis_avid_a/mis_avida_lesson01_activity1.xml.

Exercise 1. Understanding the Introduction of Genetic Variation by Random Mutation

This exercise is a modification of the exercise of the same name originally produced by Robert T. Pennock and Amy Lark. Complete materials for this original lesson are available under the CURRICULUM link at <http://avida-ed.msu.edu>.

Exercise 2. Exploring Mutations and Selection: Pre-adaptive or Post-adaptive?

This exercise was newly developed for LB145 F14 by Jim Smith.

Exercise 3. Exploring Fitness, Functions, and Selection

This exercise is a modification of the exercise titled, "Exploring Selection and Fitness" written by Amy Lark and Robert T. Pennock. Complete materials for this original lesson are available under the CURRICULUM link at <http://avida-ed.msu.edu>.

Independent Research – Experimental Evolution Project with Evolving Digital Organisms

This exercise is a modification of the exercise by the same name written by Robert T. Pennock and Amy Lark. Complete materials for this original lesson are available under the CURRICULUM link at <http://avida-ed.msu.edu>.





Introduction - Avida-ED and Digital Evolution

Avida-ED is adapted from 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 programs 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 mutation, replication, and selection. Scientists can study how digital organisms evolve, and examine questions related to the evolution of complex features, sex, intelligence, 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 this powerful tool, 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.

Driving Questions

- What is Avida-ED (how does it work)?
- What do biologists mean when they say the word “evolution”?
- Can we observe evolution? How?
- Can we study evolution by doing experiments? What kinds?
- How is Avida a useful tool for biologists? What are the strengths and limitations of such an approach?

Tasks

1. Begin by reading the article by Carl Zimmer “Testing Darwin” that appeared in *Discover* Magazine in 2005. The article can be found immediately following this Introduction or will be made available by your instructor.
2. Start Avida-ED. The program now runs in a web browser. Navigate to <https://avida-ed.beacon-center.org/app/AvidaED.html>. Please note that the program may take a minute or two to load, be patient.
3. Watch the Avida-ED video tutorial found in the support section of the Avida-ED website or on YouTube: <https://www.youtube.com/watch?v=mJwtg0so4BA&feature=youtu.be> Use it to help you explore the application’s controls.



Using Avida-ED

The Avida-ED workspace includes:

1. The “Navigation” area (*view mode buttons*) allows you to switch among three modes:
 - a. Population – the organisms evolving in the virtual Petri dish and the experimental set-up;
 - b. Organism – displays the “genome” of any single individual; and
 - c. Analysis – allows comparisons of population variables (e.g. average fitness) over time.
2. The “Freezer” (*saved materials*)
 - a. Configured dishes – settings, no organisms;
 - b. Organisms – individual organisms, including the “@ancestor”; can be saved by dragging or saving to the freezer; and
 - c. Populated Dishes – settings and organisms saved by freezing populations.
3. The “Lab Bench” (*where things happen*)

When in “Population” mode, the Lab Bench contains a “Virtual Petri Dish”, which is the place where your Avidians will grow and multiply (Figure 1). You can access the settings by clicking on the “Set-Up” button (Figure 2). There, you can change the dish size (30x30 is default), mutation rate (2.0% is default), whether or not functions are rewarded (default is all nine rewarded), and other options.

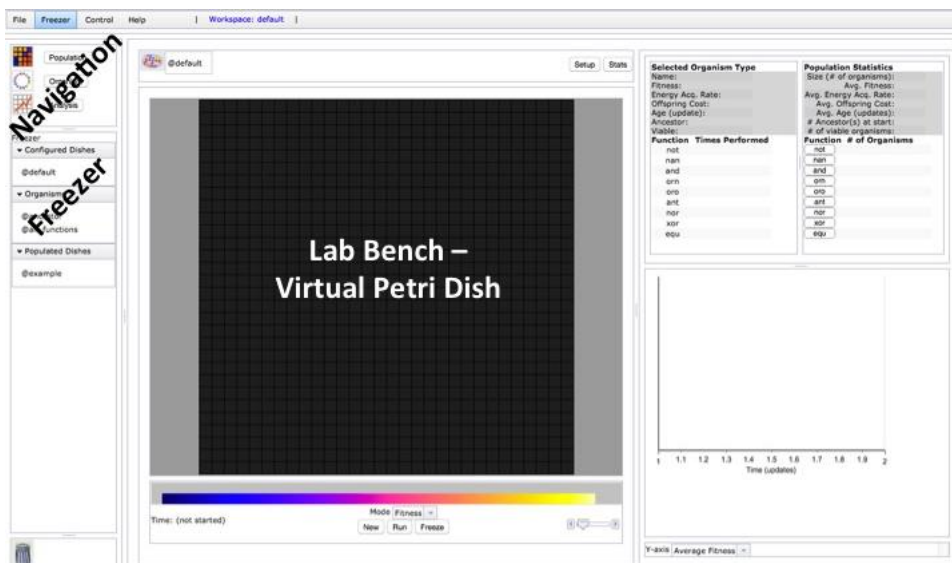


Figure 1. Screen shot of the Avida-ED Workspace in the “Population” map view. The virtual Petri Dish is where Avidians will grow and divide. The “Navigation” and “Freezer” areas are on the left.

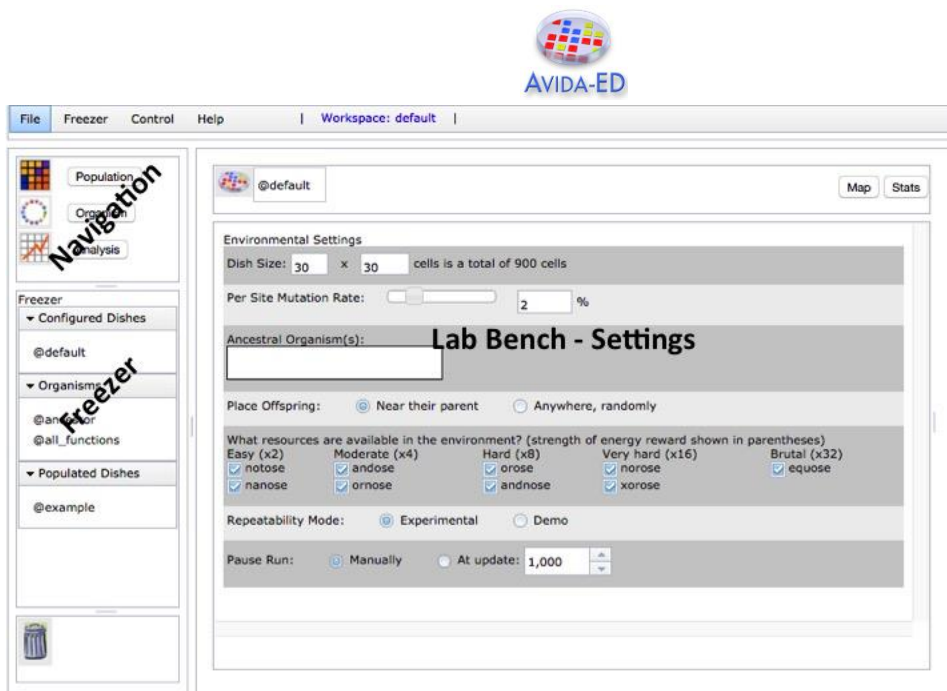


Figure 2. Screen shot of the Avida-ED Lab Bench Setting in the “Population” view.
Several parameters of the experimental set-up can be manipulated.

To run an Avida-ED experiment, drag an organism from the freezer to the virtual Petri dish (if in Map view) or to the Ancestral Organism box (if in Setup view) and click “Run” in Map view or choose “Run” under the Control pull down menu. NOTE: Loading the organism into the “Ancestral Organism Box” in “Setup” assures that the individual will be placed in the center of the virtual Petri dish. To examine a single Avidian, click on “Organism” in the Navigation panel, drag an organism from the freezer (e.g. “@ancestor”) to the lab bench area (Figure 3).

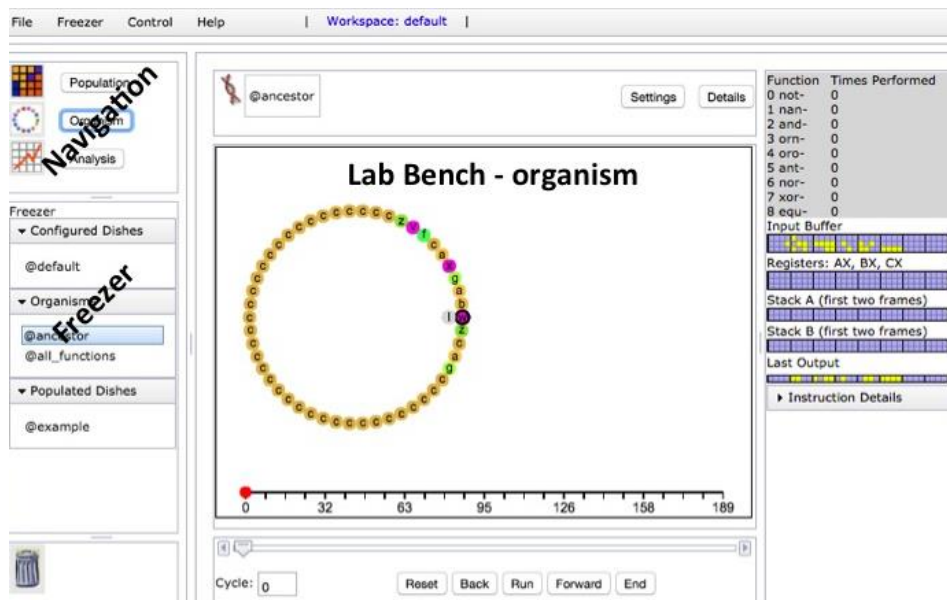


Figure 3. Screen shot of the Avidian “@ancestor” in the “Organism” view. The genome is circular and represented by colored letters. Each letter is a specific command. Notice that most of the instructions in “@ancestor” are tan-colored C’s (these are “no operation” commands and here essentially serve as placeholders).



Part I: Examining an Avidian Individual and Observing Replication

The digital organisms in Avida are referred to as Avidians, and are defined by a series of commands, which are simple computer instructions (Figure 3). During an experiment, the Avida-ED application reads the “genome” of an organism and carries out the commands, which are symbolized by letters. The default organism (“@ancestor”) has a circular genome of 50 letters, which includes a sequence of instructions for replication.

Follow the steps below to observe Avidian replication.

1. Click on “Organism” in the Navigation panel. The lab bench becomes an empty rectangle with a set of buttons at the bottom.
2. Drag the default organism (“@ancestor”) from the freezer panel to the lab bench area. A set of circles with letters inside them appears (see Figure 3).
3. Click the “Run” button and observe as the organism’s code is read by Avida-ED. At a certain point, you will notice that the organism replicates. Click the “Reset” button and repeat this step a number of times. You can observe the code being read and replicated more slowly by clicking on the “Forward” button, which moves the read head forward one instruction at a time. When paused, you can get the instruction number by clicking on an instruction.

Once you have observed a number of “Runs”, please respond to the following questions by entering your responses in the space provided.

- At which position of the Avidian “genome” does the program begin reading the instructions?
- At which positions of the “genome” are the instructions for replication?

Mutations in the offspring appear as an instruction with a black circle. Record the mutations for a single round of replication.

Position	1	10	20	30	40	50									
Ancestor	w	z	c	a	g	cc	z	v	f	c	a	x	g	a	b
Mutations															

Total # of mutated sites _____

Locations of mutated sites _____

- If a mutation occurred within the sequence of replication instructions what do you think would happen to that mutated offspring’s ability to replicate?



- If you wanted to determine the function of each letter (command) of the code, where would you find that information?
- How does the offspring Avidian compare to its parent? In other words, how many differences are there in the set of 50 commands, and where are the differences located in the “genome”?
- How is the instruction set (“genome”) for an Avidian similar to a bacterial genome?

Part II: Observing the Frequency and Location of Mutations that Occur During Replication

1. Under Settings (still in Organism view), find the mutation rate that you used above, and record it.
2. Next, if it isn’t already, set the per site mutation rate to 2% by moving the slider or typing “2%” in the box. ***If you use the slider to change the mutation rate, look carefully at the placement of the decimal to verify you have set it to (approximately) 2% and not 0.20%.*** You can either press the enter key, or click elsewhere on the screen, and the mutation rate will update. Then click on the x in the upper right corner of this box.
3. Then click play or drag the slider to watch the organism run through its code.

Please respond to the following questions by entering your responses in the spaces provided.

- There are 50 commands. How many sites do you expect will have a mutation given a 2% per site mutation rate?
- How does your replicated offspring compare to the parent?
- How did your offspring (replicated with the 2% mutation rate) compare to your neighbor’s offspring (also replicated with a 2% mutation rate)? Did they have the same number and/or type of mutations?

Storing an Avidian Individual in the Freezer

1. Click and drag the offspring genome into the Freezer Panel.
2. Then you will be prompted to “enter name of organism to freeze.” You may use any name you like, but we suggest something descriptive, perhaps indicating the mutation rate, or what tasks it can perform.

Part III: Evolving a Population

Avidians replicate in the virtual Petri dish, much the way bacteria replicate when plated on a medium. The virtual dish is divided into a grid in which each box holds one Avidian. When an Avidian replicates, the offspring are placed in a box adjacent to the parent (the default setting) or randomly on the grid. As we have seen above, if there is mutation, offspring will not be exactly like the parent.

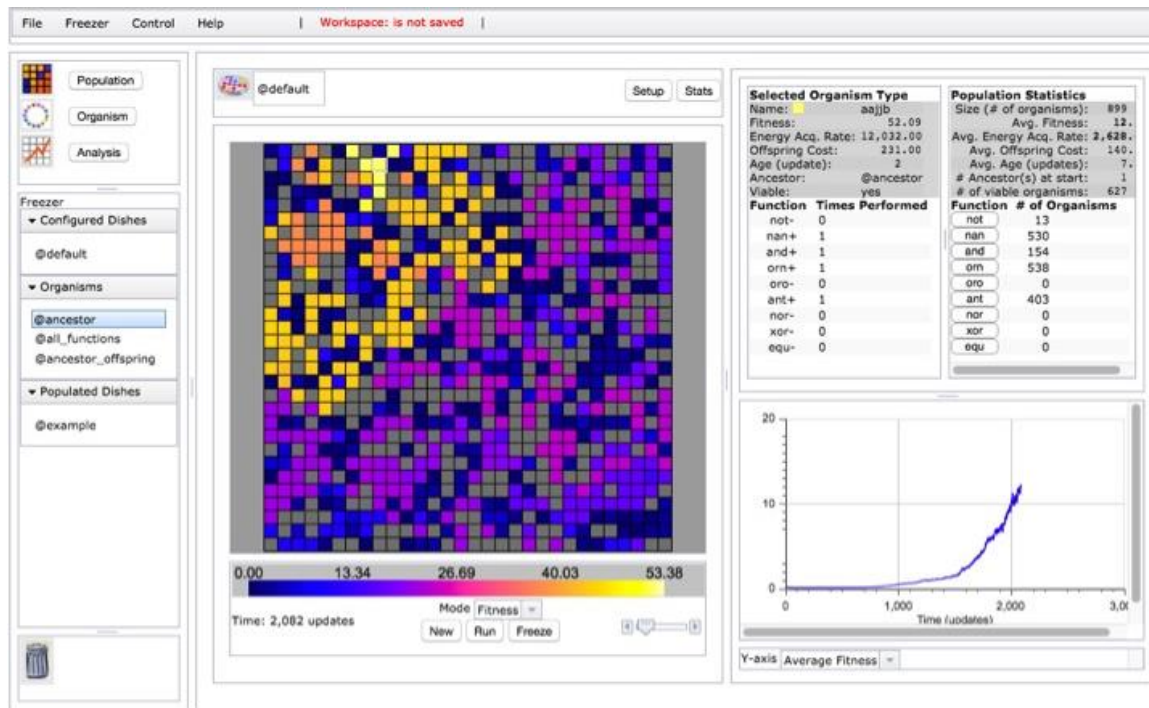


Figure 4. Screen shot of the panels displaying population and individual statistics. The upper right panel shows basic population statistics, plus how many individuals in the population perform each function. The panel just to its left does the same for a selected individual. The lower panel graphs a number of population parameters as the run progresses.

Carrying out the set of numbered tasks below will result in the growth of an Avidian population, with each individual in that population having descended from a single ancestral Avidian. At the end of the run, you will save the Petri plate containing your Avidians to the Freezer, as well as saving a single Avidian with relatively high fitness.



1. Click on "Population" in the navigation panel. The lab bench changes back to the Petri dish.
2. Click on "Setup". Drag the default organism ("@ancestor") from the freezer panel into "Ancestral Organism" box. Set the world size to 30 x 30 cells and the per site mutation rate to 2.0%. Make sure the "Near their parent" option is checked in the Place Offspring panel (should be the default). Turn off all resources (i.e., notose, nanose, etc.) by clicking in the box so that it is not checked. All other default settings should remain unchanged. [Note: resources, when made available, provide additional energy to Avidians that evolve the ability to use these resources.]
3. Click "Map" to return to the Petri dish view. Choose "Fitness" from the "Mode" drop down menu below the Petri dish. Use the slider below the Petri dish to increase the view size.
4. Push the "Run" button below the Petri dish and watch as the ancestor and subsequent descendants start multiplying. Each grid square represents an organism.
5. As you watch the Avidians multiply, notice that the information in the Population Statistics box and the graph change. When the dish looks full, click "Pause" to stop the growth in the Petri dish.
6. Click on an organism (a grid square). The information for the Avidian in this grid square appears in the Selected Organism Type panel (Figure 4). Click on a few other organisms and notice how their information differs. You may click on different individuals during the run to observe their characteristics in the organism information box.
7. Information on the population is displayed in the Population Statistics panel, and in the graph below this panel. Click the play button again and observe the dish and the population statistics boxes as the run proceeds.
8. Pause the run when there have been about 1,000 updates (unit of time for Avida-ED). The update number can be found under the bottom left corner of the Petri dish. Before proceeding with the next step, save the entire plate by clicking the "Freeze" button at the bottom and saving the population to the Freezer. You will be prompted whether you wish to save the Configuration, Organism, or Population; here you should save the Population. We recommend using a labeling system that keeps track of the mutation rate and world size (i.e., m2-w30x30-number of updates, but you can use any naming system that makes sense to you).
9. Click on individual organisms, one at a time, to find an individual with a high fitness. To do this, use the fitness scale below the Petri dish, as well as looking in the organism info panel for each organism.
10. Drag an organism with a relatively high fitness to the freezer panel. In the box, type a name for this organism followed by the fitness value.

Please respond to the following questions by entering your responses in the spaces provided.

- What do biologists mean when they use the word "fitness"? How is fitness measured in Avida-ED?



- Choose two Avidians in your population with different fitness and explain how differences in these Avidians contribute to differences in their fitness.
- Based on what you observed in the Population Statistics and the Organism Type boxes during the run, what do you think accounts for changes in individual fitness and changes in the average fitness in the population?

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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."

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 evolu-

tion. 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 hun-

dreds 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

'Avida is not
a simulation
of evolution;
it is an
instance of it'

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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 sesquipedale* (1b); *Masdevallia wellichii* (1c); *Coryanthes speciosa* (1d); *Paphiopedilum hookerianum* (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).

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.

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?"

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

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



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

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: *Matingoa 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).

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.

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." ☒

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

* DISCOVER MORE

[also see Resources, page 85]

For Avida software downloads and more about ongoing research, visit Caltech's Digital Life Laboratory Web site: dllib.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 C γ 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.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.

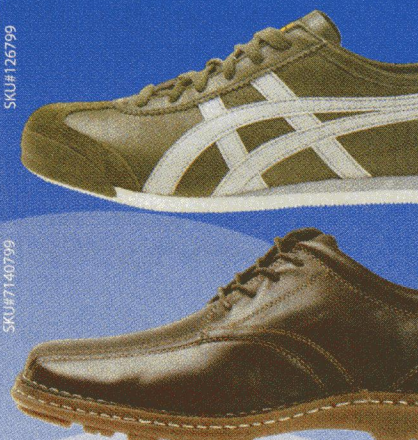
Orchid pictures were provided by Eric Hansen, author of *Orchid Fever: A Horticultural Tale of Love, Lust, and Lunacy* (Vintage Books, 2001). His latest book is *The Bird Man and the Lap Dancer: Close Encounters With Strangers* (Pantheon, 2004).

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.

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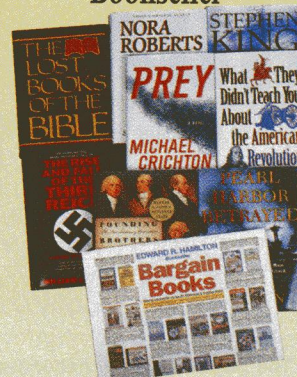


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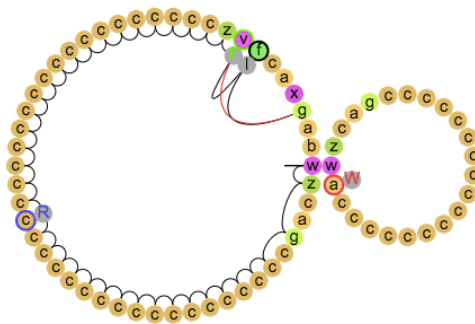
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Exercise 1 - Understanding the Introduction of Genetic Variation by Random Mutation



An Avidian in the process of replication

Student Learning Goal

- Students will be able to explain what it means to say that mutations occur at random.

Background

The basic components of Darwinian evolution are variation (V), inheritance (I), natural selection (S) and time (T). This exercise focuses on variation and one basic way it can be introduced into populations.

Natural selection acts upon phenotypic variations in a population of organisms. Variations can arise in a population in several different ways. Here we will look only at variations that may be introduced by genetic *mutations*—random changes in an organism’s genome—and not at other processes such as recombination, horizontal transfer, etc. Genetic mutations may be substitutions (changes from one instruction to a different one), insertions (additions of an instruction into the genome), or deletions (deletions of an instruction from a genome). [Note: For simplicity, this version of Avida-ED allows only substitutions, not insertions or deletions.]

As in nature, a population of Avidians—the model organisms in Avida-ED—can vary both phenotypically (in terms of the functions they can carry out) and genotypically (in terms of the instructions of code). Like bacteria, Avidians have a circular genome composed of simple genetic instructions. Different genetic sequences can produce different phenotypic functions. Each instruction (command) in the sequence is represented by a colored circle with a letter inside of it. During Avidian replication, mutations may occur at random in the sequence.



Making Mutant Avidians

Your goal in this exercise is to understand *how* mutations produce variation and, specifically, what it means to say that mutations in Avidians occur at “random”. We will then explore how these random mutations play a role in evolutionary processes.

Use the Organism Viewer to observe how substitution mutations change the genomes of organisms.

1. In the Organism viewer, go to settings.
2. Set the per site mutation rate to 10%. Keep repeatability mode set to Experimental.
3. Drag the “@ancestor” from the freezer.
4. Click “Run” to observe replication.
5. Record your data as Run #1.
 - a. Use the sequence template below to record the position, type of change, and total # of changes in the offspring’s genome.
 - i. Do this in clockwise order starting from the 3 o’clock position on the circular genome (W in the ancestor), which is the origin of replication.

Run #1: _____ % Mutation Rate. Mark differences from the ancestral genome. They will be highlighted in the organism viewer by a black outline.

Position	1	10	20	30	40	50
Ancestor	wzcagccccccccccccccccccccccccccccccccczvfcaxgab					
Mutations						

Total # of mutated sites _____

Location(s) of mutated sites _____

6. Compare your offspring organism (replicated with the 10% mutation rate) to your neighbor’s offspring (also replicated with a 10% mutation rate).

As an individual, please respond to the following questions by entering your responses in the spaces provided.

- Does your neighbor have the same number and/or type of mutations? How can you account for any differences?



- What do you **expect** to see if you repeat this using the same ancestor with the same mutation rate?
 - Do you think the *specific* mutations will be the same? Explain your reasoning.
 - Do you think the *number* of mutations always be the same? Explain your reasoning.

Testing your prediction. AFTER you have written down your predictions above, repeat the process of replicating the ancestor twice more using the same settings. For each run, record your results below. To begin Run #2, simply drag “@ancestor” into the Organism Viewer field.

Run #2: _____ % Mutation Rate. Mark differences from the ancestral genome.

Position	1	10	20	30	40	50
Ancestor	wzcagccccccccccccccccccccccccccccccccccccczvfcaxgab					
Mutations						

Total # of mutated sites _____

Location(s) of mutated sites _____

Run #3: _____ % Mutation Rate. Mark differences from the ancestral genome.

Position	1	10	20	30	40	50
Ancestor	wzcagccccccccccccccccccccccccccccccccccccczvfcaxgab					
Mutations						

Total # of mutated sites _____

Location(s) of mutated sites _____

Recording your data. We will be exploring the data generated by the entire group in all of their runs. Your instructor will provide additional instructions for entering your data in a class data sheet.



Discussion Questions and Wrap-up. After you've explored the class data, please work with your lab team to respond to the following questions. Be sure to write out responses below.

- Did everyone in the class get the same number and type of mutations?
- What does the distribution of everyone's data look like?
- How do you explain this?
- Given your observations, what can you say about the role of mutation in creating variation in a population?

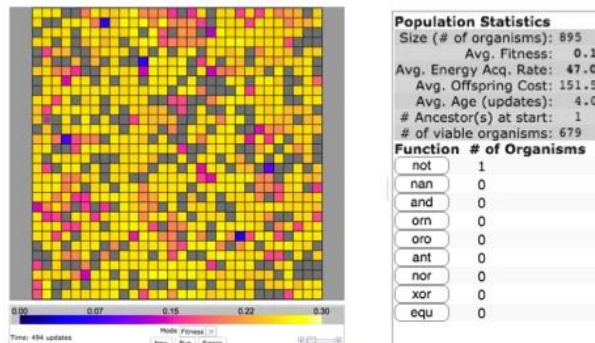
Reflection and Metacognition

Think-Pair-Share; work with another person or two and answer the following questions

- What did you learn from this exercise?
- What are you still wondering about?
- What would you change in the Exercise?



Exercise 2 - Exploring Mutations and Selection: Pre-adaptive or Post-adaptive?



Only one individual in this Avidian population can perform NOT. Did NOT evolve because it was needed?

Driving Question

Do mutations arise as a result of the presence of a selective agent (that is, a “need” to occur) or randomly regardless of the environment and potential for natural selection?

Student Learning Goal

- Students will be able to explain why we think that mutations do not occur because they are needed.

Luria, Delbrück and Directed Mutations

Before scientists understood the role of DNA, scientists hypothesized that bacteria could develop heritable genetic mutations depending on the circumstances or environment in which the bacteria resided – in other words, mutations were thought to be *directed*, or post-adaptive – when exposed to a particular selective pressure, bacteria were thought to be able to generate mutations that would allow them to respond to these environmental pressures and changes.

To test if mutations were random (pre-adaptive) or directed (post-adaptive), two scientists (Salvador Luria and Max Delbrück) devised an elegant experiment that allowed them to differentiate between these two scenarios. We can investigate this same question in Avida-ED.

In this exercise you will perform an experiment with two runs to explore the relationship between the time of appearance of a mutation in a population and the presence or absence of a selective agent (advantage). In the first run, you will monitor your run to determine at what update the first individual to perform NOT appears in the population. Next, you'll do the same thing in a run where NOT is rewarded. Do you think the individual performing NOT will appear earlier or later in the second run? Why?



Experiment 1. Does a mutation occur in response to a reward for that mutation?

Run 1 – First appearance of NOT with all resources turned off.

1. In the Population viewer, flip to Setup.
2. Set the following parameters: World size 30x30; 2% Mutation Rate; Drag “@ancestor” into the Ancestral Organism box; Turn off all resources. Place offspring near parent; Repeatability Mode Experimental; Pause manually.
3. Return to Map view and click on Run.
4. Pause your Run at the first occurrence of an organism that can perform the function, NOT. (Note: you’ll need to watch closely and be very quick on the Pause button!!)
5. Record the Update number of this occurrence in Table 3 (below).

Run 2 - First appearance of NOT with notose turned on.

1. Under the Control Menu, choose “Start a new experiment.”
2. In the Population viewer, flip to Setup.
3. Set the following parameters: World size 30x30; 2% Mutation Rate; Drag “@ancestor” into Ancestral Organism box; Turn on notose; Place offspring near parent; Repeatability Mode Experimental; Pause manually.
4. Return to Map view and click on Run.
5. Pause your Run at the first occurrence of an organism that can perform the function, NOT. (Note: you’ll need to watch closely and be very quick on the Pause button!!)
6. Record the Update number of this occurrence in Table 3.

Table 3. Update number for first appearance of an Avidian performing NOT, with and without a reward (notose) in the environment.

Environmental Condition	Update Number
No reward for NOT (no notose)	
Reward for NOT (notose present)	

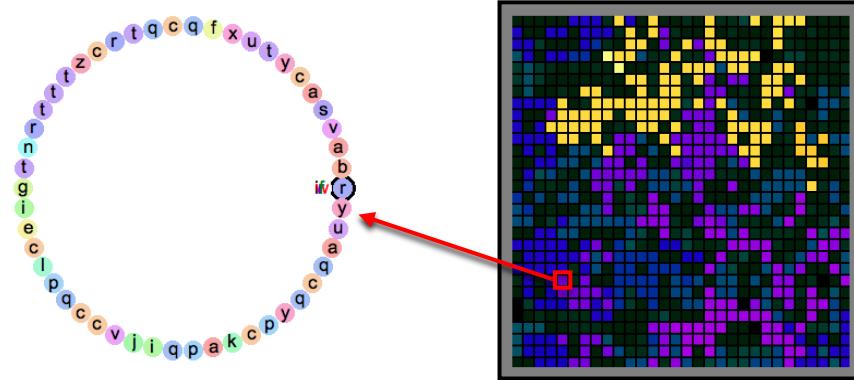
Recording your data. We will be exploring the data generated by the entire group in all of their runs. Your instructor will provide additional instructions for entering your data in a class data sheet.



Discussion Questions and Wrap-up. After you've explored the class data, please respond to the following questions by entering your responses in the spaces provided.

- What was the point of this exercise?
- Did the first occurrence of your mutation happen earlier, later, or at the same time in Run 1 compared to Run 2?
- Is this also true of the class as a whole?
- What would you have expected to see happen if mutations occur in response to the presence of a selective environment?
- Given the data you've seen, when do you think mutations arise that can confer a selective advantage relative to the presence of a selective agent?

Exercise 3 – Exploring Fitness, Functions, and Selection



Selection acts on individual variation within a population, resulting in differential fitness.

Driving Question

How does selection act on individual variation to produce differential fitness in a particular environment?

Student Learning Goals

- Students will be able to explain what it means to say that a trait has gone to fixation in a population, and why traits that are or are not subject to a selective advantage would be expected to go to fixation at different rates.
- Students will also be able to explain that while mutation is random, natural selection is not.

Fitness, Functions, and Selection

Individual variation is the result of random genetic mutations during genome replication. Variants that are better suited in a particular environment tend to have greater reproductive success (produce more offspring). Advantageous traits are thereby selected by the environment and become more common as individuals possessing those traits tend to leave behind more offspring. These individuals have greater fitness than organisms lacking the advantageous traits. If selection is strong enough, these advantageous traits will go to fixation, meaning that they will be present in every individual in the population. An individual's fitness is therefore dependent upon the interaction between its traits and the environment, and is relative to other individuals in the population.

As you have seen, Avidians are capable of carrying out different instructions, which in turn can allow an Avidian to perform a specific function. For our purposes here, it is probably simplest to keep these abstract, knowing simply that these are logic functions. There are nine possible functions that an Avidian can carry out: NOT, NAN, AND, ORN, ORO, ANT, NOR, XOR, EQU. The “@ancestor” is not capable of carrying out any of these functions, but mutations can change the genome and offspring can evolve a sequence of instructions that code for these functions. The ability to perform one of these functions can increase the fitness of an individual. As you have seen, there are resources that correspond to each of these functions: notose, nanose, andose, ornose, orose, antose, norose, xorose, equose. If an Avidian has evolved the ability to carry out one of these functions, and the corresponding resource is in the environment, the individual gains some processing speed



(which is reflected by an increase in the measured energy acquisition rate), thereby becoming more fit.

In comparing evolution in Avida to evolution in bacteria, the Function/Resource pairing is analogous to putting a particular food source in bacterial media so that it increases growth and reproduction. Alternatively, if drawing the analogy to antibiotic resistance, bacteria are able to survive in the presence of an antibiotic because they have some form of resistance to the effects of the antibiotic in much the same way that an Avidian is better able to outcompete its neighbors if it has evolved a function (assuming the corresponding resource for that function is present in the environment).

In this exercise you will perform a set of two experiments to explore relationships between variation, selection, and fitness. In the first experiment, you will evolve and freeze individual Avidians that have evolved for 1000 updates in the absence and presence of a reward for the ability to perform the logic function NOT. These organisms will then be competed with each other in environments both without and with rewards for NOT. Which organism do you think will win in each case? Why?

In the second experiment, we will explore the concept of an allele, genotype, or phenotype becoming more frequent in a population as a function of its environment. Will NOT become more frequent in a population if it is rewarded? Why? What does it mean to say that NOT has gone to fixation in a population?

Experiment 1. Competing individuals evolved in the presence or absence of a reward for NOT. The following experiment consists of four Avida-ED runs. At the end of Runs 3 & 4, you will be asked to answer a set of questions, which you should respond to in the spaces provided.

Run 1 - Evolve a population from the “@ancestor” with all resources turned off (No-Reward environment). Select an individual with high fitness.

1. In the Population viewer, flip to Setup.
2. Set the following parameters: World size 30x30; 2% Mutation Rate; Drag “@ancestor” into Ancestral Organism box; Place offspring near parent; Turn off all resources; Repeatability Mode Experimental; Pause Run after 1000 updates.
3. Return to Map view and Run.
4. Once 1000 updates have completed, use the Fitness Scale and the Organism Box to identify an individual with a relatively high fitness.
5. Freeze this individual (refer to Introduction for procedure).
6. Freeze the plate (refer to Introduction for procedure).
7. Verify that the individual you saved is capable of reproducing.
8. Save the workspace (refer to Introduction for procedure).

Question for thought: How does one individual end up having higher fitness than another if all resources are turned off?



Run 2 - Evolve a population from the “@ancestor” with only NOT turned on (Notose-Only environment). Select an individual with high fitness.

1. Under the Control Menu, “Start a new experiment”.
2. In the Population viewer, flip to Setup.
3. Set the following parameters: World size 30x30; 2% Mutation Rate; Drag “@ancestor” into Ancestral Organism box; Place offspring near parent; Turn on notose, leave all other resources off; Repeatability Mode Experimental; Pause Run after 1000 updates.
4. Return to Map view and Run.
5. Once 1000 updates have completed, use the Fitness Scale and the Organism Box to identify an individual with relatively high fitness **that can perform NOT**.
6. Freeze this individual (refer to Introduction for procedure).
7. Freeze the plate (refer to Introduction for procedure).
8. Verify that the individual you saved is capable of reproducing.
9. Save the workspace (refer to Introduction for procedure).

Run 3 - Competing Evolved Individuals in the Absence of Reward.

1. Under the Control Menu, “Start a new experiment”.
2. In Population viewer, go to Setup and set up experiment with the following: Dish size 60x60; 2% mutation rate; Place both the Avidians evolved in No-Reward and Notose-Only environments in the Ancestral Organism window; Turn off all rewards; Repeatability Mode is Experimental. Pause run at update 300.
3. Return to Map.
4. Select “Ancestor Organism” in the “Mode” Drop down menu.
5. Record in Table 1 (below) the starting fitness of the Avidian evolved in the No-Reward environment, and the starting fitness of the Avidian evolved in the Notose-Only environment.
 - a. This can be accomplished by pausing the run (Step 6) after exactly one update, and then selecting each Avidian to see its statistics.
6. Press Run.
7. Pause after 300 updates.
8. Estimate and record the relative frequencies of the No-Reward and Notose-Only descendants.

Please respond to the following questions by entering your responses in the spaces provided.

- In this run, what was the starting fitness of the Avidian evolved in the No-Reward environment? What was the starting fitness of the Avidian evolved in the Notose-Only environment? Do these numbers make sense? Explain.



Table 1. Starting fitness of two Avidians in two different environments.

	Avidian evolved in presence of notose (performs NOT)			Avidian evolved with no rewards (may or may not perform function)		
New Environment	Fitness	Energy Acq. Rate	Offspring Cost	Fitness	Energy Acq. Rate	Offspring Cost
With notose						
Without notose						

- Given the individual with the higher fitness is better at surviving and reproducing, what would you expect the frequency of descendants from these two individuals to be after running the experiment for 300 updates (with notose present)?
- What was the relative frequency of No-Reward descendants at 300 updates? Did everyone in the class get the same result?
- What conclusions can you draw from these observations?

Run 4 - Competing Evolved Individuals in the Presence of notose.

*Repeat Run 3 with the same set of initial settings, with the sole exception that in this trial you will **turn on** the notose resource.*

Please respond to the following questions by entering your responses in the spaces provided.

- In this run, what was the starting fitness of the Avidian evolved in the No-Reward environment? What was the starting fitness of the Avidian evolved in the Notose-Only environment?
- Given the individual with the higher fitness is better at surviving and reproducing, what would you expect the frequency of descendants from these two individuals to be after running the experiment for 300 updates?
- What were the relative frequencies of No-Reward descendants at 300 updates? Did everyone in the class get the same result?
- What conclusions can you draw from these observations?



Experiment 2. Exploring the concept of alleles or genotypes going to fixation. The following experiment consists of two Avida-ED runs. At the end of the two runs, you will be asked to answer a set of questions, which you should respond to in the spaces provided.

Run 1. Frequency of NOT in the absence of reward.

1. Under the Control Menu, “Start a new experiment”.
2. In “Setup”, choose the following options: Dish size 30x30; 0.2% mutation rate
[NOTE: MUTATION RATE SHOULD NOW BE 0.2% INSTEAD OF 2% (Why?)];
Drag your Notose-Only Avidian from the Freezer to the Ancestral Organism window;
Turn off all rewards; Experimental setup; Pause Run at 300 updates.
3. Return to Map.
4. Set Mode to Ancestor Organism. Click on the “not” button to see the individuals who are performing NOT.
5. Press Run. Observe the number of individuals in the dish performing NOT.
6. After 300 updates, record in Table 2 (below) the population size, the number of individuals that are performing NOT, and the percentage of the population that is performing NOT.

Run 2. Frequency of NOT in the presence of reward.

*Repeat Run 1 with the same set of initial settings, with the sole exception that in this trial you will **turn on** the notose resource.*

1. Again, watch how the number of individuals in the dish performing NOT changes.
2. Record after 300 updates the population size and number of individuals in the population that are performing NOT.

Record your data. We will be exploring the data generated by the entire group in each of their two runs. Record your data in the Table below, as well as in the class data collection instrument. Your instructor will provide instructions for entering data into class spreadsheet.

Table 2. Frequency of NOT in populations with and without notose reward

	w/ notose turned OFF	w/ notose turned ON
Population Size		
# performing NOT		
% performing NOT		

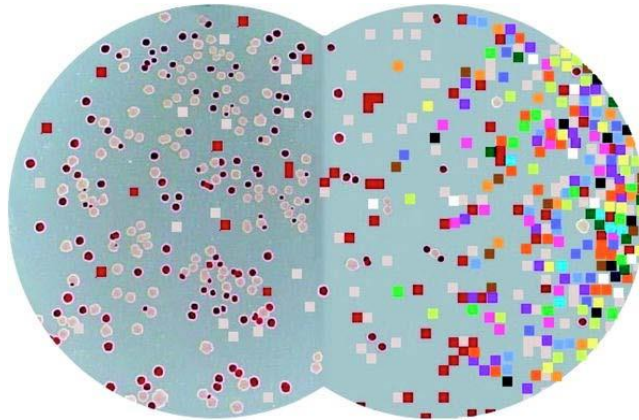


Discussion Questions and Wrap-up. After you've explored the class data, please respond to the following questions by entering your response into the spaces provided.

- How does the frequency of individuals performing NOT compare in the absence of reward and in the presence of reward?
- Did your classmates obtain similar results?
- Describe a mechanism that could provide an explanation for the pattern you observed.



Independent Research - Experimental Evolution Project with Evolving Digital Organisms



Two model systems: *E. coli* is 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 replicate quickly and their small size means that they don't take up much space in the laboratory. Lenski's work with *E. coli* has helped shed light on some important aspects of evolution and, having spanned 25 years and over 60,000 bacterial generations, it is the longest-running investigation of its kind.

As far as biological organisms go, *E. coli* replicates—and evolves—at an incredibly fast rate. Even so, *E. coli* grows too slowly to be of practical use in addressing many questions about evolution. To pursue such questions, scientists can use digital organisms that model biological systems. Avida is such a model system, and is used by researchers to study evolutionary processes and even to find solutions to engineering problems.

Your research team will perform experiments using digital organisms in Avida-ED to explore an evolutionary question. The question is up to you; your team will propose a hypothesis and then design an experiment that you will carry out to answer your question. The final product of your work will be a poster that your team will present to your peers for your final presentation.



Research Tasks

Hypothesize: Propose a hypothesis to an evolutionary question that can be tested using Avida-ED. Parameters to explore include mutation rate, world size, the presence and absence of rewards in the digital environment, and other settings.

Design your experiment: Develop an experimental plan that your team will follow to test your hypothesis. You should consider relevant variables, what data to collect, and how many replicates you will need.

Predict: Indicate what experimental outcomes you would expect to find if indeed your hypothesis is supported by the data you collect.

Write up a Proposal Brief: Write up a “brief” summarizing your team’s proposed research project. This should be 1-2 pages long and emphasize those items listed above. (In addition, tell the reader why the experiment is cool!)

Present your Proposal: As a team, prepare a PowerPoint, Keynote, or Prezi presentation that you present to the group. This should consist of six slides, with each team member presenting one or two of the slides.

Conduct your experiment: Carry out your proposed research and record the appropriate data.

Analyze your data: Summarize your data and perform appropriate statistical tests (chosen in consultation with the teaching team). Decide how best to represent your data and create appropriate tables and/or graphs.

Report your findings: Put together a poster for the final presentation. The poster should include: (i) introductory background information and a statement of your hypothesis; (ii) methods; (iii) results and conclusions; and (iv) future directions.



Avida-ED Independent Research Project Proposal and Proposal Presentation - Guidelines and Expectations (20 points Total)

Proposal (15 points)

Write up a *proposal brief* summarizing your team's proposed research project. The following sections must be included:

Question. Describe the evolutionary question that your team has. Why is it interesting and cool?

Hypothesis. Frame and present a hypothesis that will allow you to test your evolutionary question using Avida-ED.

Experimental Design. Develop an experimental plan that your team will follow to test your hypothesis. Describe relevant variables, what data you intend to collect, and how many replicates you will carry out.

Predict: Indicate what experimental outcomes you would expect to find if indeed your hypothesis is true.

Rubric for Grading the Proposal

Three points each awarded for:

1. The question your team is asking and why your team chose it.
2. Your team's hypothesis, stated formally.
3. The description of the experimental methods to be employed in your study.
4. How you will analyze and interpret your results, depending upon what they are. In other words, what conclusions will be drawn, depending on the outcomes of your experiments?
5. Overall structure and presentation of the written document. Is it logical and clear, etc.

Points will be awarded in each area based on the quality and the completeness of the write-up.



Avida-ED Proposal Presentation (5 points)

Teams should create a set of 6 pptx slides.

1. Title slide (Student “A”)

- Introduces the team and its members; shows project title

2. Introduction (Student “A”)

- Describes the team’s original question and their study system
- Tells audience why it’s interesting (why do I care?)
- Provides some of background (from the literature?)

3. Hypothesis (Student “B”)

- Shows and explains the team’s Experimental Hypothesis and why it is a high quality hypothesis

4. Experimental Methods (Student “C”)

- Describes the experimental methods to be employed in the study

5. Predictions (Student “B”)

- Describes what you predict you will see if your hypothesis is correct

6. Data Analysis (Student “D”)

- Shows what data resulting from the experiment might look like, and how the team will interpret them in the light of their hypothesis





Part II: Additional Avida-ED Exercises

The second part of the lab manual contains three additional exercises for possible classroom use with Avida-ED. These are:

A. Exploring the Effects of Mutation Rate on Individuals

Student handout from Amy Lark's exercise based on the Fukushima nuclear reactor disaster in the wake of the 2011 Japanese earthquake and tsunami. This natural disaster provided scientists with a natural experiment to explore the effects of mutation.

More complete lesson materials are available under the CURRICULUM link at <http://avida-ed.msu.edu>. See Lark, A., Richmond, G. & Pennock R. T. "Modeling Evolution in the Classroom: The Case of the Fukushima Butterflies" *American Biology Teacher* (2014, 76(7):450-454) for detailed information about this lesson.
<http://www.nabt.org/websites/institution/File/pdfs/american_biology_teacher/2014/ABT_Online_September_2014.pdf>

B. Artificial Selection: Evolution in Practice

Student handout from Wendy Johnson et al.'s activity in which student teams try to evolve a bacterium capable of degrading an environmental contaminant, TCE.

More complete lesson materials are available via the TeachEngineering.Org website at https://www.teachengineering.org/view_activity.php?url=collection/mis_/activities/mis_aida/mis_aida_lesson01_activity2.xml.

C. From Genotype to Phenotype: Understanding the Introduction of Phenotypic Variations by Random Mutation

Student handout from Robert T. Pennock's exercise helping students to understand that there are may be multiple independent pathways leading to a particular complex phenotype.

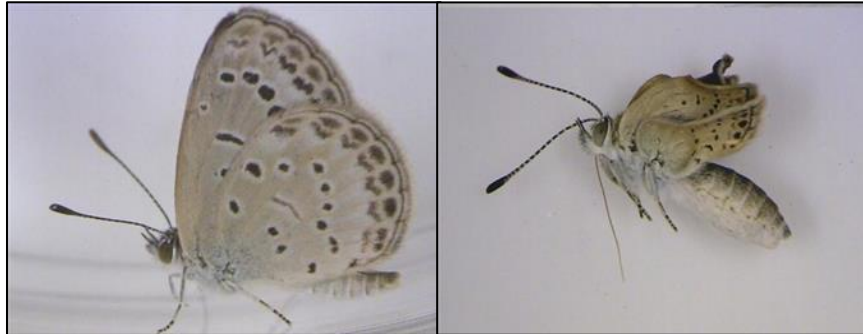
More complete lesson materials are available under the CURRICULUM link at <http://avida-ed.msu.edu>.

D. Avida-ED Quick Start Manual

This simple user manual explains the basic Avida-ED controls and also includes a glossary of terms.



Exploring the Effects of Mutation Rate on Individuals



Left: Pale grass blue butterfly (*Z. maha*) with normal fore and hind wings.
Right: Mutated butterfly with stunted wings.

Background

On March 11, 2011 the largest earthquake ever to hit Japan, and one of the five largest in recorded history, occurred 40 miles (70 km) off the coast of Tōhoku. The quake triggered a tsunami that produced waves with heights up to 133 feet (40.5 m) that traveled as much as 6 miles (10 km) inland, causing extensive damage to property and significant loss of human life. Among the infrastructure casualties was the Fukushima Daiichi Nuclear Power Plant complex. Three reactors sustained heavy damage, resulting in the worst nuclear accident since Chernobyl in 1986. The area within a 20-mile (~30 km) radius of the Fukushima plant was determined to have dangerously high levels of radiation, with the highest levels up to 2 miles (3 km) from the plant. The Japanese government prohibited access to this area and ordered the evacuation of anyone living between 2 and 12 miles (3 and 20 km) of the plant. People living between 12 and 20 miles (20 and 30 km) away were put on high alert and also encouraged to evacuate.

The disaster at Fukushima has provided scientists with an opportunity to investigate the biological impact of radiation on organisms. One species in particular, the pale grass blue butterfly (*Zizeeria maha*), is helping researchers pursue questions about the immediate and long-term effects of radiation at various doses (Hiyama et al., *Scientific Reports* **2** Article 570, 2012). Butterflies collected closer to the power plant experienced larger doses of radiation than those farther away, and the scientists found that larger doses were associated with increased infertility, mortality, and incidence of physiological abnormalities. Many of these abnormalities were inherited and amplified in offspring of butterflies that had been exposed to the radiation initially as overwintering larvae. The researchers concluded that “[I]t is most likely that the abnormal phenotypes observed are produced by random mutations caused by the exposure to radiation” (p. 8).

Using digital organisms as a model, your goal in this exercise is to test the scientists’ claim: assuming that higher doses of radiation are associated with increased mutation rates, can exposure to radiation account for the adverse biological effects reported in the study?



Assignment Tasks

Predict: What will happen to an individual Avidian's genome as it replicates at different mutation rates (low, medium and high)?

Test: Follow the instructions carefully. Use the table provided to record your data.

- 1.) In the Organism viewer, drag an ancestral organism (“@all_functions”) into the viewing pane. Under the Settings, set the per site mutation rate to 1%.
- 2.) Click on the Run button and allow the ancestor to replicate. *Note: After clicking Run you can skip visualizing the replication process by clicking End.*
- 3.) On your data sheet, record the number of mutations in the offspring (circled in black).
- 4.) Drag the offspring to the freezer. Name it in a way that will allow you to match it to your data (e.g., “1%no01”). Click the Reset button.
- 5.) Repeat steps 2 – 4 ten times, completely filling out the first “Mutations (n)” column of your data sheet.
- 6.) Drag your first saved offspring from the freezer into the Organism viewing pane. Click Run and record the number of functions (9 metabolic functions and the ability to replicate) it has **lost**. Repeat this for all remaining offspring until you have completely filled out the first “Abnormalities (n)” column of your data sheet. *Note: After clicking Run you can skip visualizing the replication process by clicking End.*
- 7.) Repeat steps 2 – 6 at 5%, 10%, and 15% mutation rates, recording data in the appropriate spaces on your data table.
- 8.) Use your data to calculate the average number of mutations and percentage of abnormalities for each of the four mutation rates.

Results: Were your predictions confirmed or disconfirmed by the data you collected?

Discussion: What do your tests reveal about the relationships between mutation rate, frequency of mutations, and physiological abnormalities?

Does the evidence from your study support the researchers' claim that “it is most likely that the abnormal phenotypes observed are produced by random mutations caused by the exposure to radiation”? How?



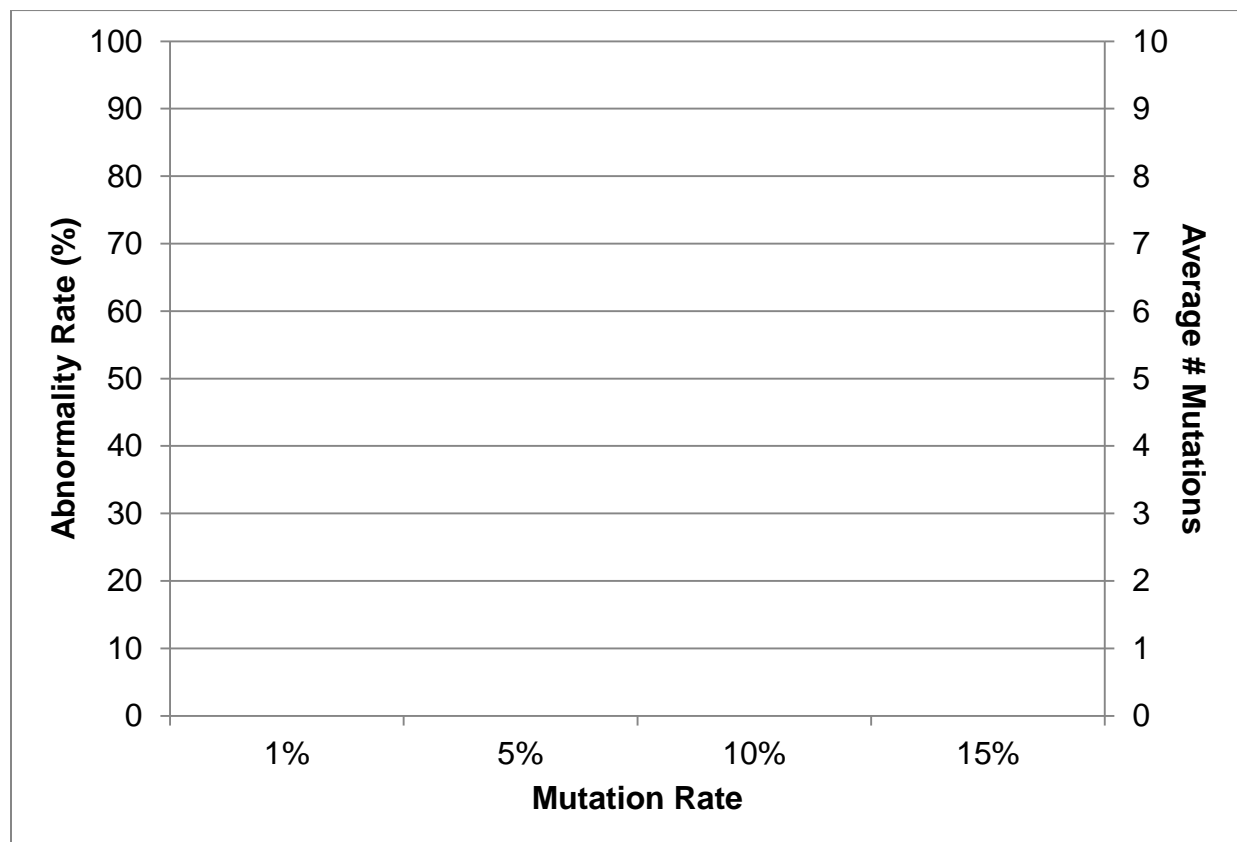


Data Table. Keep track of your experimental observations in the table provided.

	Mutation Rate							
	1%		5%		10%		15%	
Offspring	Mutations (n)	Abnormalities (n)	Mutations (n)	Abnormalities (n)	Mutations (n)	Abnormalities (n)	Mutations (n)	Abnormalities (n)
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
	Average number of mutations	Abnormality rate	Average number of mutations	Abnormality rate	Average number of mutations	Abnormality rate	Average number of mutations	Abnormality rate



Data Chart. Plot your data to reveal patterns.





Artificial Selection: Evolution in Practice

TCE Biodegraders Exercise

Design Challenge Scenario

Your school administrators want to buy an adjacent piece of property to build new athletic facilities. The property includes a large warehouse that has been used for various industrial purposes over the last 50 years. During the site inspection it was learned that the soil and water around the warehouse are contaminated by trichloroethylene (TCE), a hazardous chemical used as a spot remover in dry cleaning and as a degreaser for metal parts.

The school board has asked our class to team up with an environmental consulting company to help clean up the TCE so that our school can move ahead with purchasing and using the land. The environmental consulting company has informed us that current methods to remove TCE are expensive and require the contaminated soil be removed and disposed of in hazardous waste dumps. The company is interested in spiking the soil with bacteria that biodegrade (break down) TCE. The class's goal is to use a model system (Avida-ED) to evolve a bacterial strain that can biodegrade TCE and present a protocol to the company. Your goal is to convince the company (using data) that your protocol is likely to lead to an efficient means of evolving TCE degrading bacteria. Then they can use your recommendations to produce the bacteria to clean the soil on-site instead of dumping it into a hazardous waste landfill.



Before going any further, discuss the following questions:

1. What do you know, or think you know, about this problem?
2. What things do you not know, or need to know, about this problem?

Background

Researchers use models to test hypotheses when experiments would take too long to perform, be difficult to manage, or be too expensive to conduct. For this project, we are using a model of bacterial organisms in a Petri dish (Avida-ED). In real life, bacterial populations can double as fast as every 30 minutes. In Avida-ED, doubling times are about one second.

Imagine that the ORO function of Avida-ED degrades TCE (ORO is an enzyme). Your job is to evolve organisms that make ORO. Begin with the default organism ("@ancestor"), but change any of the environmental settings and use multiple different environments over the course of your experiment. Your goal is to determine the best procedure for evolving a population that most effectively metabolizes the orose resource in an environment with only orose.

Experimental Design

Design the experiment to answer the following question: **What conditions will lead to the evolution of the most efficient TCE degrading bacteria?**

Use the following questions to guide you in designing your experiment. Make sure that you answer each question thoroughly (on paper, word processor, or in your lab notebook) before beginning your investigation.



Artificial Selection: Evolution in Practice

1. What conditions (settings) might you change in order to increase the likelihood of the ORO function evolving and maximizing the effectiveness at which the organisms degrade TCE? List as many possibilities as you can (these are potential hypotheses).
2. What conditions do you think have the best potential for solving the problem? Explain your reasoning.
3. A hypothesis is a proposed explanation for an observation. You just wrote your hypothesis in answering the preceding question. Now state it concisely in a sentence or phrase.
4. Hypotheses lead to predictions. Based on your hypothesis about the best conditions for evolving efficient TCE degrading bacteria, your prediction is that the organisms will have a high fitness value in an environment with only the orose resource. Now state your hypothesis and your prediction together in an “if... then...” statement in which the “if” refers to the hypothesis and the “then” refers to the results that you predict to observe. Make this a single sentence that concisely links your hypothesis (the “why”) to the prediction (the “what”) that follows the “if... then...” format.
5. What data do you need to collect in order to test your hypothesis?
6. How many data points (number of replications, variables, etc.) do you need to be able to confidently support or reject your hypothesis? Avida-ED is an instance of evolution in a model environment, which means that every run is unique. To draw any conclusions, you must include at least five runs for each treatment.
7. Describe your experimental design. Make this a concise description of your methods (including settings, replications, data collection, etc.) that is clear enough for another group to replicate.
8. What are the dependent and independent variables and the controls? When determining what variables need to be controlled for, it is often helpful to go back to the alternative possible explanations to your original question and think of how to specifically rule those out in your procedure. Another type of control that is often essential is a point of comparison (a “no treatment” group).
9. How will you organize your data as you collect it? Make a data table to fill in during the investigation.
10. How will you present the data? Usually this is a graph or series of graphs. What type of graph will best display the pattern that you expect to show? What are the x (independent variable) and y (dependent variable) axes of the graph? It may be helpful to sketch a quick version of the graph that you expect to see if your prediction is correct (later, you can compare your actual data to this to see if it is consistent).

Data Collection

For each run, write down the parameters you changed, the number of updates it took to evolve the ORO function, what proportion of the population performed the ORO function, and the maximum fitness values. During and after each run, record observations that you think might be important, but that are not included in the experimental design. Use these observations to help you explain the results.

Data Analysis

Display your results as graphs. Make sure to title each graph and label the axes.





Conclusion

Write a description of how your results support or refute the hypothesis.

Proposal

Based on the results of your investigation propose a protocol for evolving bacteria to degrade TCE. Prepare a brief presentation to the class to explain your proposal.

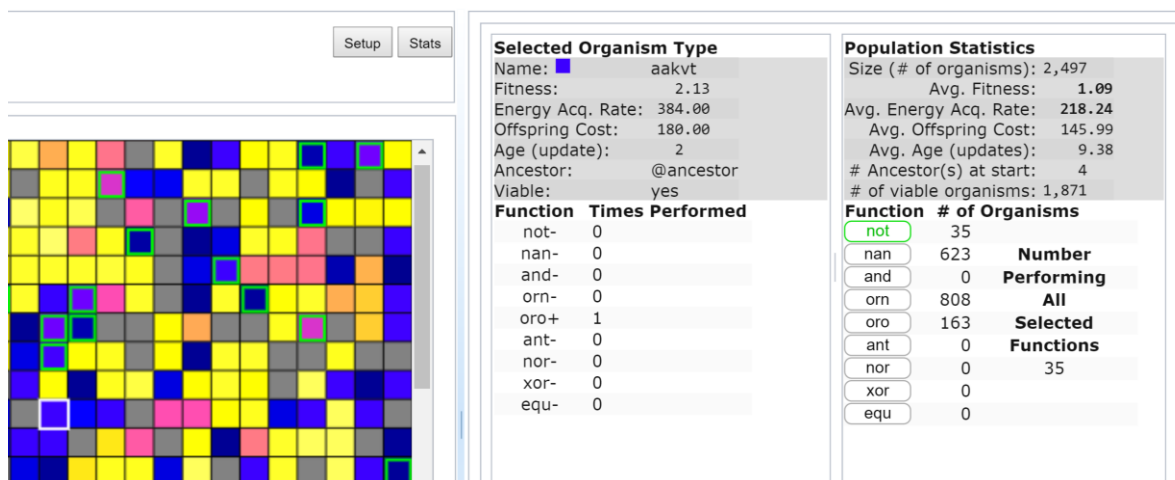
Discussion

As a class, evaluate the proposals and determine which protocol most effectively evolves efficient TCE degrading bacteria. Are there any unanswered questions? Your class may find that you have a protocol that you feel confident submitting to the environmental consulting company or you may decide that you need to run more experiments before doing so. It is important that you present data in support of your protocol in order to convince them that your protocol is likely to lead to a feasible plan for evolving TCE degrading bacteria.





From Genotype to Phenotype: Understanding the Introduction of Phenotypic Variations by Random Mutation



Background

In biology, the term *phenotype* refers to the observable traits of individuals that arise from the causal interaction of their genotype with the environment. “Observable” is meant in the scientific sense and so is not limited to traits that are directly visible. Any testable trait, whether physical or behavioral, is part of an organism’s phenotype so long as it has a genetic basis.

For instance, a scientist may be interested in whether a strain of bacteria can metabolize some sugar, say glucose. One organism that can perform this testable function—*ability to metabolize glucose*—is positive for the trait (Glu+) and a second that cannot is negative for it (Glu-). Some difference between these organisms’ genotypes determines which phenotypes they exhibit; perhaps a gene in the first produces an enzyme that makes glucose metabolism possible or some genetic sequence in the second changes the timing of a metabolic mechanism. One may, of course, think of these possibilities as phenotypic traits themselves—again, any observable trait with a genetic basis will count as part of the organism’s phenotype.

Similarly, in Avida-ED, phenotypic variation depends upon genotypic variations. As in nature, different sequences of instructions in an Avidian’s genome produce whatever different traits it can exhibit, from the performance (or non-performance) of the possible functions of its virtual metabolism to its offspring cost, viability, and so on.

Your goal in this exercise is to look at how changes in genetic sequence can lead to variation of phenotypic functions. At this point don’t worry about the particular mechanisms by which sequences of genetic instructions produce specific functions. Focus just on how genotype and phenotype relate to each other in general.



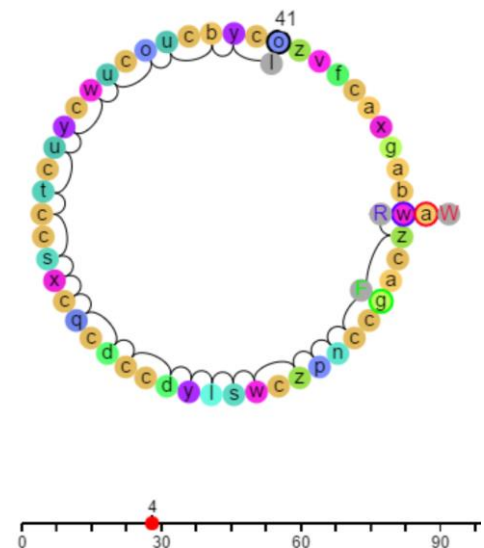
Assignment Tasks

• **Preparation.** Familiarize yourself with some of an Avidian's observable traits. While you have a population running in the Population Viewer you can select any single organism in the virtual Petri dish and see some information about what traits its genotype produces by looking in its Stats box. In the screenshot above, for instance, the selected ultramarine-colored organism in the dish (the organism's cell is outlined in white) with the genome named *aakvt* is displayed. The energy acquisition rate and offspring cost for that organism are two phenotypic traits that are listed, but the main traits that are of interest in this exercise are the functions in the table. In this case, the organism is negative for all of these functions except for one (*oro+*). The box to the right of this shows the prevalence of organisms with these functions in the population as a whole. Pressing the respective label highlights (with a green cell outline) all the organisms that are positive for that trait. (In the screenshot, the NOT button is pressed.)

You should already be familiar with how to examine the genome of an Avidian by dragging it into the Organism Viewer. In the Organism Viewer you will see another phenotype table that displays the functions that the organism can perform. Additionally, the x axis below the genome display shows when in the execution of the genetic sequence each function is completed (e.g. function 4 – ORO – at instruction cycle 28 and instruction position 41 in the figure below). Finally, you can observe some of the computational mechanism itself by looking at what is happening in the registers, stacks and input/output buffers while the genetic program is executing.

• **Demo:** Use the Population Viewer to see new phenotypes arising and spreading in the population.

- (i) Flip to Setup. Set the per site mutation rate to 5%. Drag in the “@ancestor” from the freezer to the *Ancestral Organism(s)* box. Set repeatability mode to Experimental. Flip back to Map.
- (ii) Click *Run* and watch the population until a variety of phenotypes have evolved. (It could take 1000 or more updates before two or three functions arise.) Pause the run.
- (iii) In the Population Viewer, explore the phenotypic traits of the organisms in your population using the methods described above.
- (iv) Save organisms (one each) with the following two phenotypic functions (*nan+*, *oro+*) to the Freezer. Be sure to give them identifiable names.





From Genotype to Phenotype

• **Observe:** In the Organism Viewer, record the requested data for each. Note: While the organism viewer is stopped, clicking on an instruction circle shows its position number. To record the final five instructions, start by finding the instruction at the cycle number when the function completes and then list it and the four instructions prior to it in the (clockwise) order they occur. In the previous figure, the function ORO completes at cycle 28 and position 41 (the o instruction) in the following sequence:

wzcagccnpszwslydcccqcxscctcuycwucouchyc...

In that case the “final five” instructions are cwuco.

- *Control organism (@ancestor):*

Genotype: Confirm the ancestral genome below.

Ancestor wzcagccczvfcaxgab

Phenotype: Nan-; Oro-
Other Traits:

- *Org #1 (Nan+ @1000):*

Genotype: Mark differences from ancestral genome below.

Position	1	10	20	30	40	50
Ancestor	wzcagccczvfcaxgab					

Phenotype: Nan+
Cycle# when Nan completes:
Final five instructions:
Other Traits:

- *Org #2 (Oro+ @1000)*

Genotype: Mark differences from ancestral genome below.

Position	1	10	20	30	40	50
Ancestor	wzcagccczvfcaxgab					

Phenotype: Oro+
Cycle# when Oro completes:
Final five instructions:
Other Traits:

• **Predict:** What do you expect to see if you extend the run and perform the same tests 1000 updates later?

- Will the *timing* of the trait occurrences remain the same? Yes / No
- Why?

- Will the *final four instructions* be the same? Yes / No
- Why?

- Will the *other traits* all remain the same? Yes / No
- Why?





From Genotype to Phenotype

- **Test:** Repeat steps (ii) and (iv) from the Demo two times, extending the duration of your original run by 1000 updates each time.

- Org #3 (Nan+ @2000):

Genotype: Mark differences from ancestral genome below.

Position	1	10	20	30	40	50
Ancestor	wzcagccccccccccccccccccccccccccccccccccccczvfcaxgab					

Phenotype: Nan+

Cycle# when Nan completes:

Final five instructions:

Other Traits:

- Org #4 (Oro+ @2000)

Genotype: Mark differences from ancestral genome below.

Position	1	10	20	30	40	50
Ancestor	wzcagccccccccccccccccccccccccccccccccccccczvfcaxgab					

Phenotype: Oro+

Cycle# when Oro completes:

Final five instructions:

Other Traits:

- Org #5 (Nan+ @3000):

Genotype: Mark differences from ancestral genome below.

Position	1	10	20	30	40	50
Ancestor	wzcagccccccccccccccccccccccccccccccccccccczvfcaxgab					

Phenotype: Nan+

Cycle# when Nan completes:

Final five instructions:

Other Traits:

- Org #6 (Oro+ @3000)

Genotype: Mark differences from ancestral genome below.

Position	1	10	20	30	40	50
Ancestor	wzcagccccccccccccccccccccccccccccccccccccczvfcaxgab					

Phenotype: Oro+

Cycle# when Oro completes:

Final five instructions:

Other Traits:





From Genotype to Phenotype

- **Results:**

- Were your predictions confirmed or disconfirmed? If disconfirmed, how would you now revise your expectation?

- When comparing the organisms drawn from different times, was the same phenotype always produced by the same genotype? What does that tell you about the relationship between genotype and phenotype?

- **Discussion:** What do your tests above reveal about how phenotypic variations arise in a population?

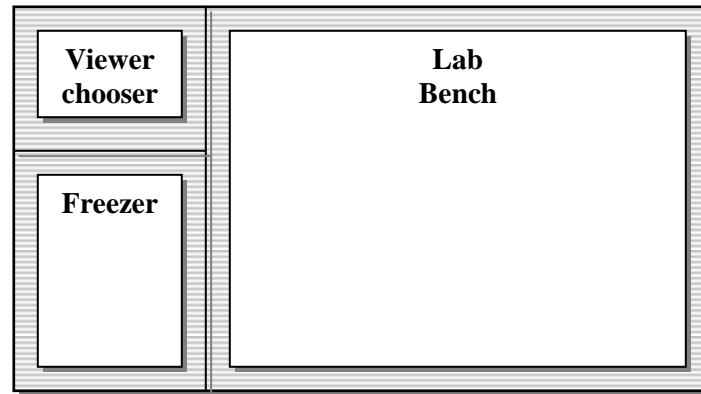
- **Supplemental Inquiry:** If you repeat the experiment again from the beginning, what patterns in the data do you expect to be the same and what do you expect to be different between the first and second replicates? Why?





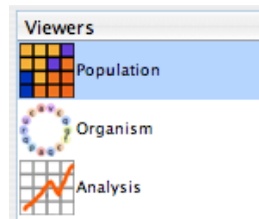
Avida-ED Quick Start User Manual

I. General Avida-ED Workspace



(A) Viewer chooser buttons

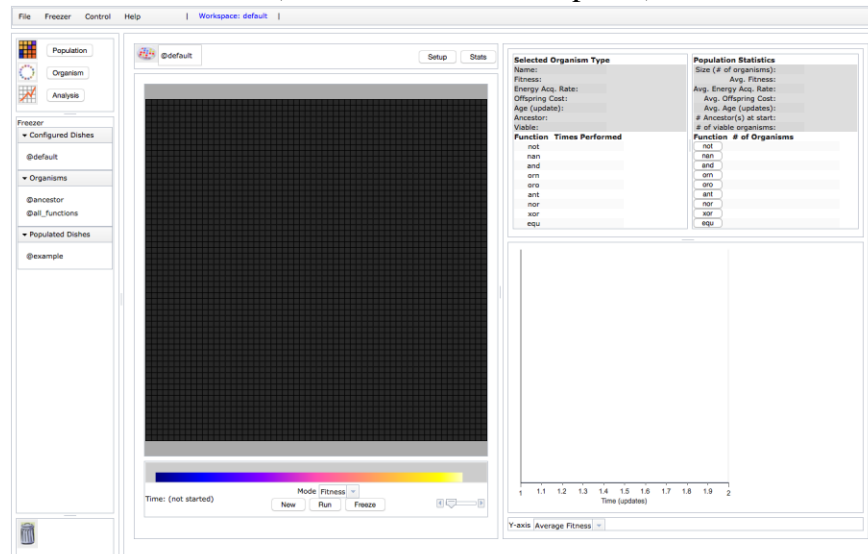
Switch between lab bench views



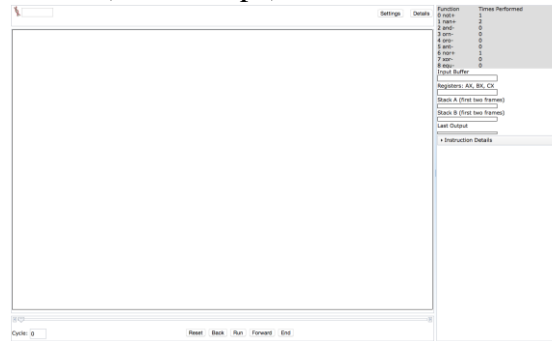
(B) Lab bench

Three lab bench options:

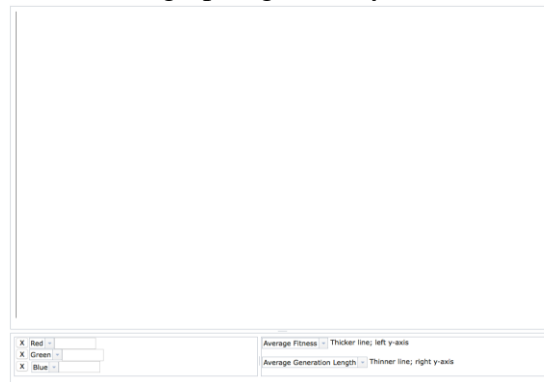
1. Population View Lab Bench (Petri dish & stats viewpanes)



2. Organism View Lab Bench (Genetiscope)

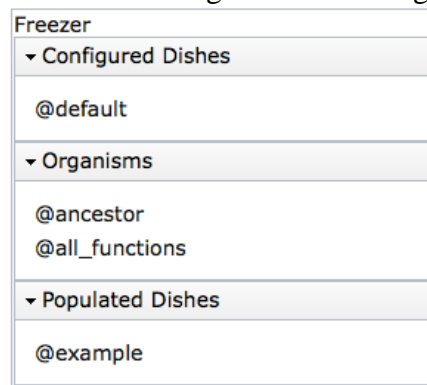


3. Analysis View Lab Bench (Data graphing & analysis)



(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

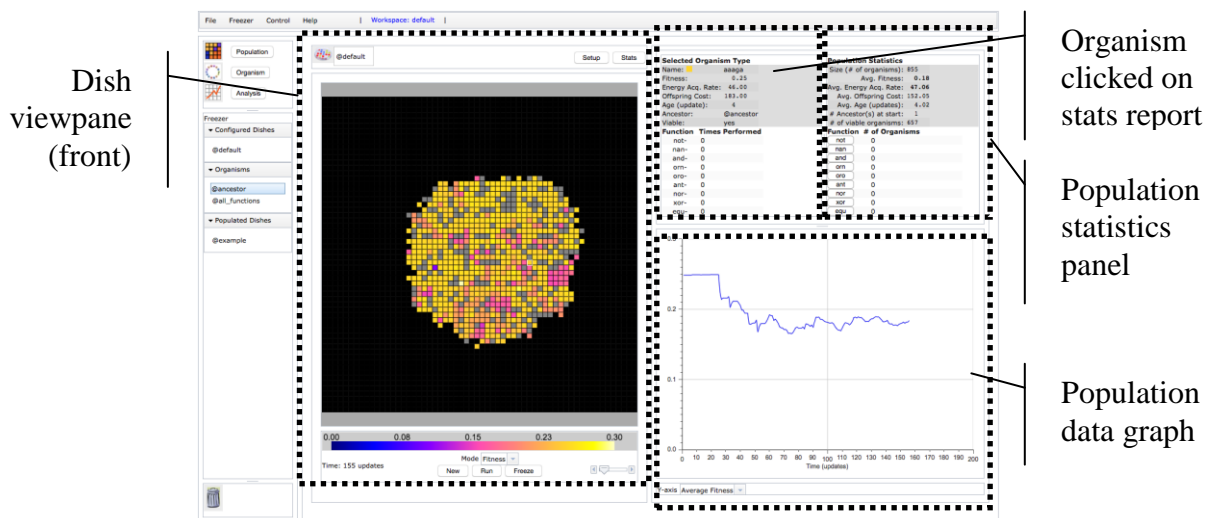
(1) Population Viewer (“Petri Dish” & environment settings)

Functionality

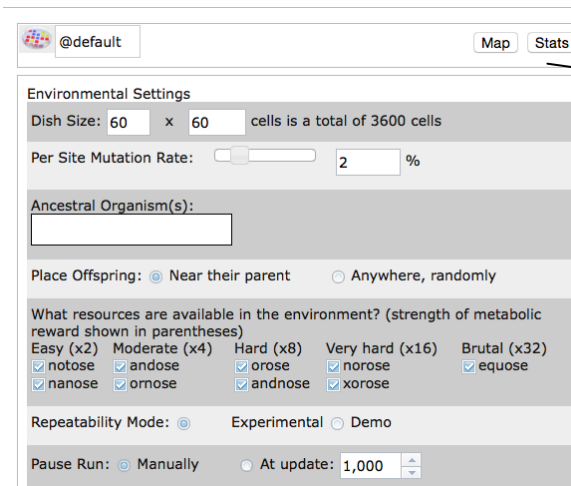
- Evolve a population in a Petri dish
- Click on an individual organism and observe its stats
- Observe whole population stats in real time
- Graph population stats in real time

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...]



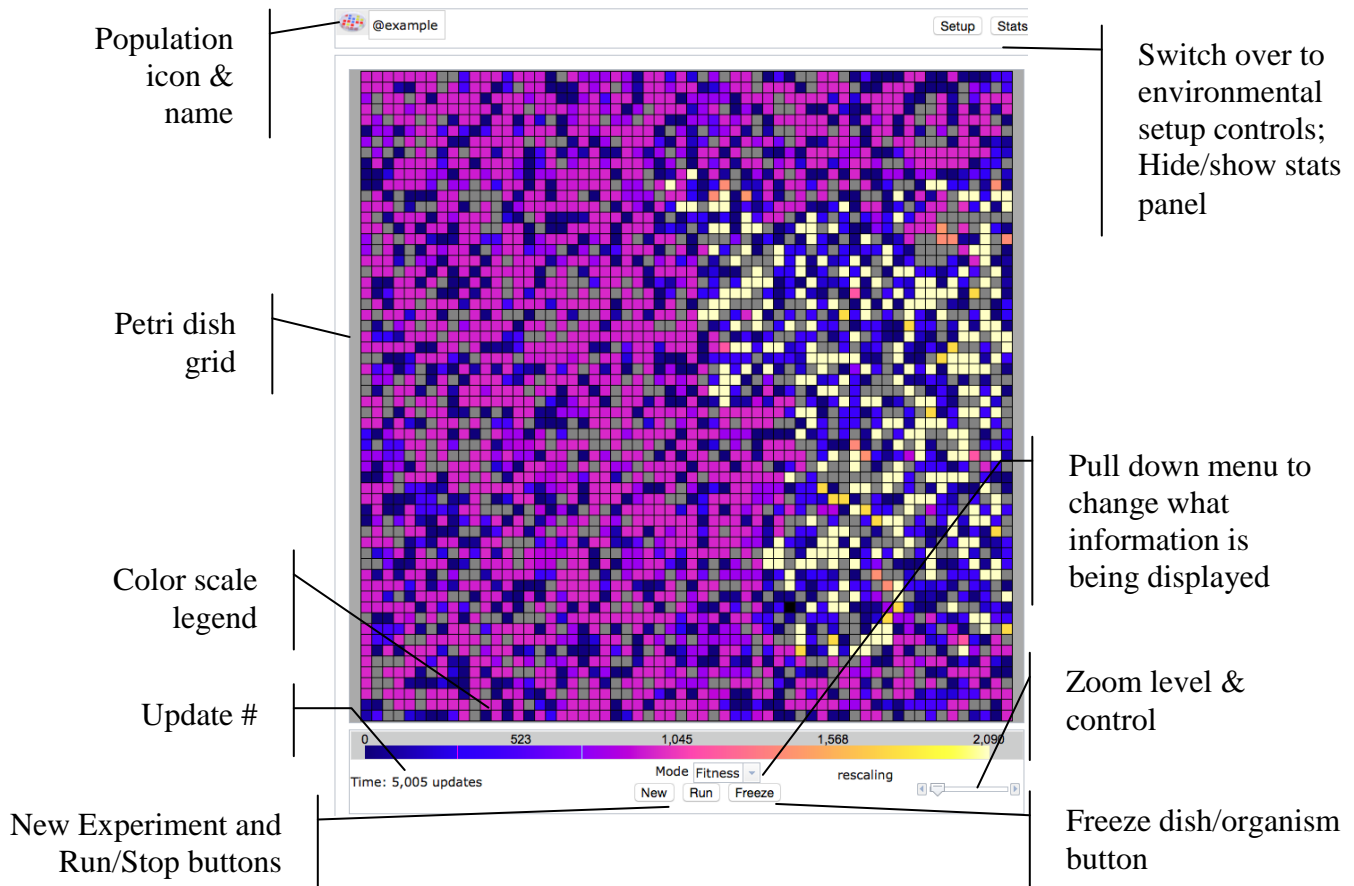
Dish
viewpane
(rear)
Environment
settings



The screenshot shows the 'Environment Settings' dialog box. It includes fields for 'Dish Size' (60 x 60 cells), 'Per Site Mutation Rate' (2%), and 'Ancestral Organism(s)'. There are radio buttons for 'Place Offspring' (Near their parent, Anywhere, randomly) and 'Repeatability Mode' (Experimental, Demo). A section titled 'What resources are available in the environment?' lists various resources with checkboxes for their availability. At the bottom, there are options for '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:** 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.

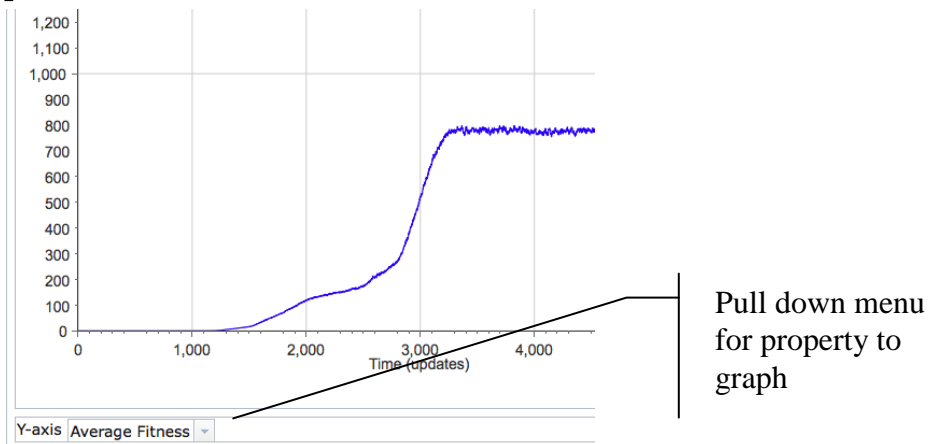
Population statistics

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	

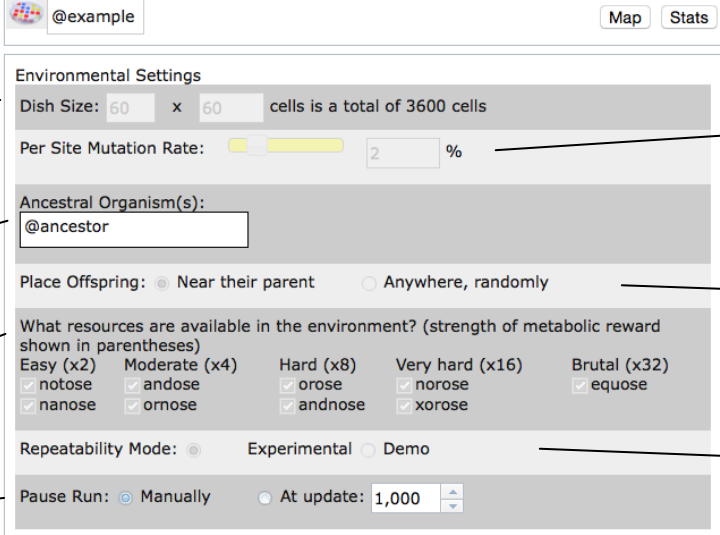
- These are the same functions as in the Org. Clicked on Report, but data is given for the population as a whole.
- Click a single function button to highlight organisms in the dish that have the given function. Clicking two or more function buttons highlights organisms that have ALL the selected functions.

Population data graph



- **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



The screenshot shows the 'Environmental Settings' dialog box in Avida-ED. Annotations on the left and right point to specific controls:

- Petri Dish size setting:** Points to the 'Dish Size' field, which is set to 60 x 60, with a note that 'cells is a total of 3600 cells'.
- Ancestral seed organism(s):** Points to the 'Ancestral Organism(s)' text box, which contains '@ancestor'.
- Environmental resources settings:** Points to the 'What resources are available in the environment?' section, which includes a table of metabolic rewards and checkboxes for various nutrients.
- Pause Run setting:** Points to the 'Pause Run' section, which has radio buttons for 'Manually' and 'At update'.
- Buttons to show Petri dish map; show/hide stats side panel:** Points to the 'Map' and 'Stats' buttons at the top right of the dialog.
- Per Site Mutation Rate & slide control:** Points to the 'Per Site Mutation Rate' slider, which is set to 2%.
- Offspring placement setting:** Points to the 'Place Offspring' section, which has radio buttons for 'Near their parent' and 'Anywhere, randomly'.
- Exact repeatability control:** Points to the 'Repeatability Mode' section, which has radio buttons for 'Experimental' and 'Demo'.

- **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.

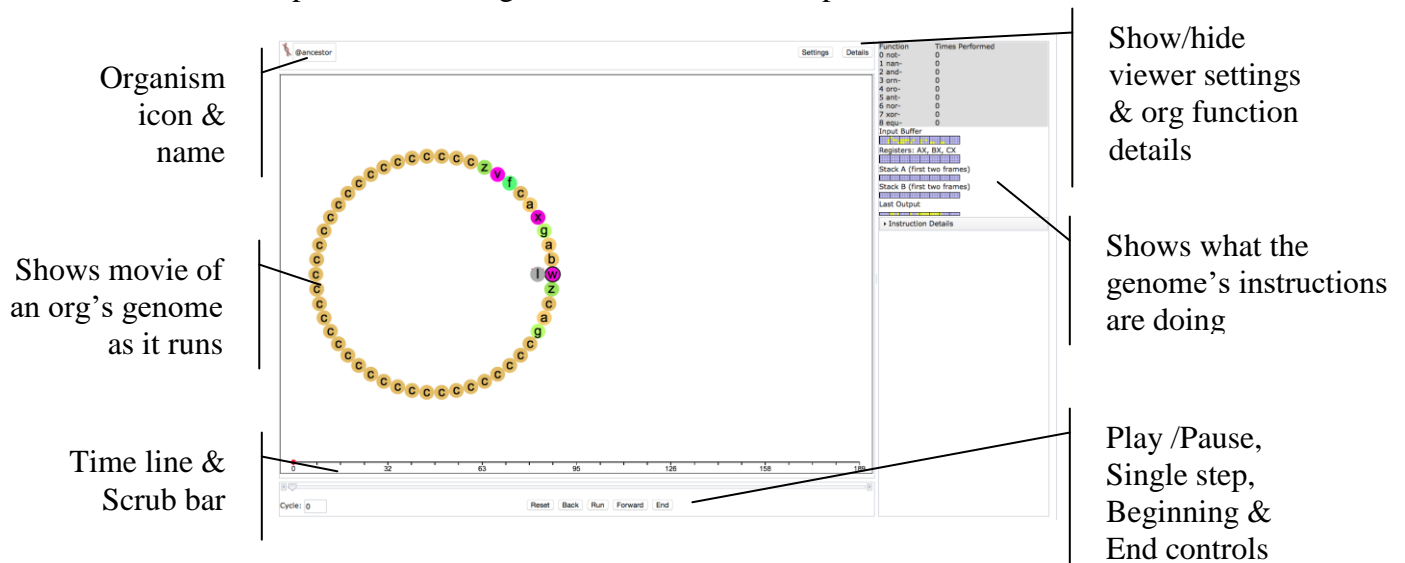
(2) Genetiscope (Organism genome viewer)

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.



- **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...]

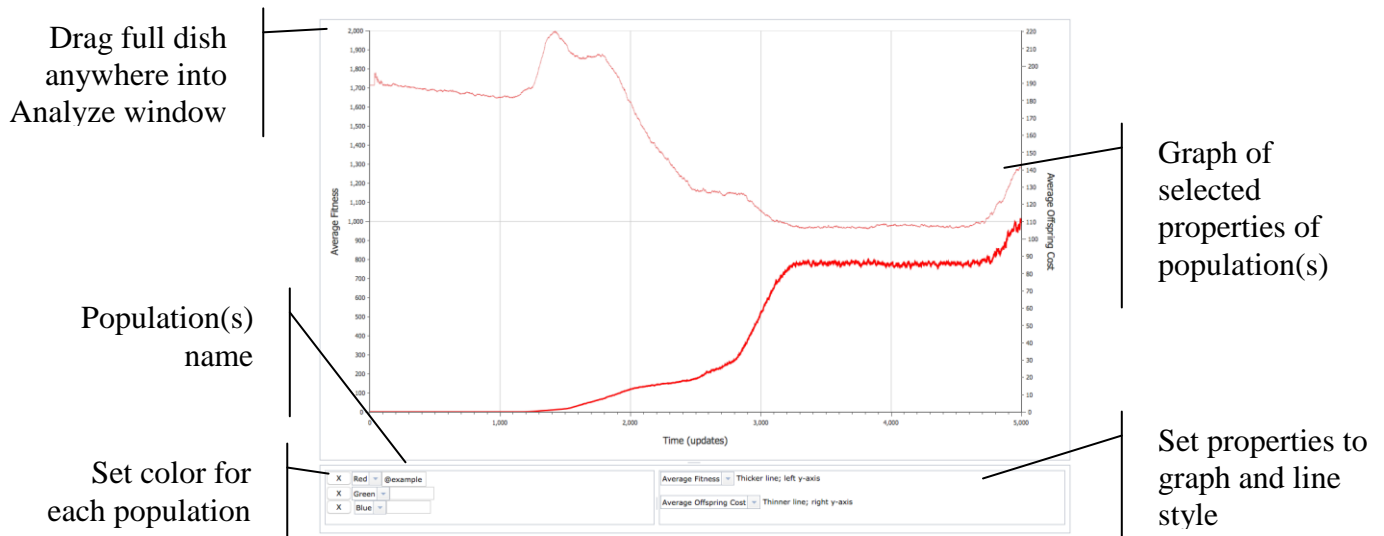
(3) Analyzer Lab Bench (Data graphing & analysis)

Functionality

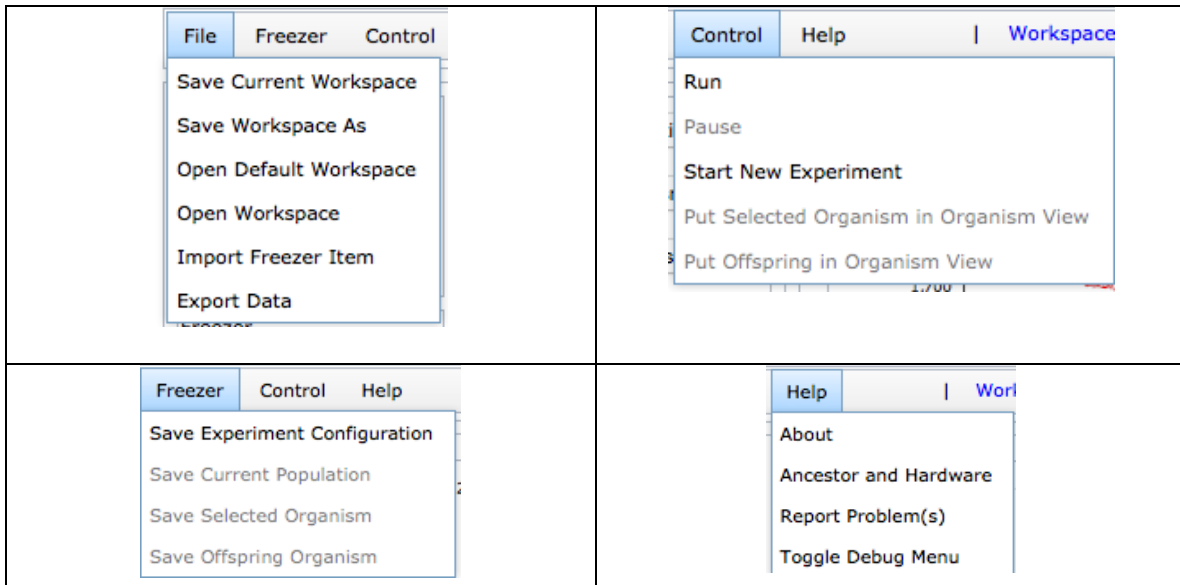
- Graph one or two data sets from a population.
- Graph and compare data from one to three populations.
- Export selected data for external analysis in Excel or other programs.
- Save viewer images.

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...]



(4) Menus



File Menu

- **Workspace controls:** All the settings and data generated in your workspace may be saved and reopened later.
- **Export / Import controls:** Data, images, configured and populated dishes, and individual organisms can be separately exported and then later imported into another workspace or used by some other program.

Freezer Menu

- Petri dish environmental configurations, individual selected organisms, or entire populated Petri dishes in your workspace may be saved to the freezer for later reuse.

Control Menu

- **Run/Pause** a population that is set up in the Petri Dish.
- **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

- **Report Problem(s):** Use to send a bug report to the Avida-ED software developer.
- **Toggle Debug Menu:** Turns on or off the Debug menu for developer use in Beta version.



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 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. Inviability organisms are those that are unable to replicate. In the fitness mode of the population viewer, inviable Avidians are shown colored grey.