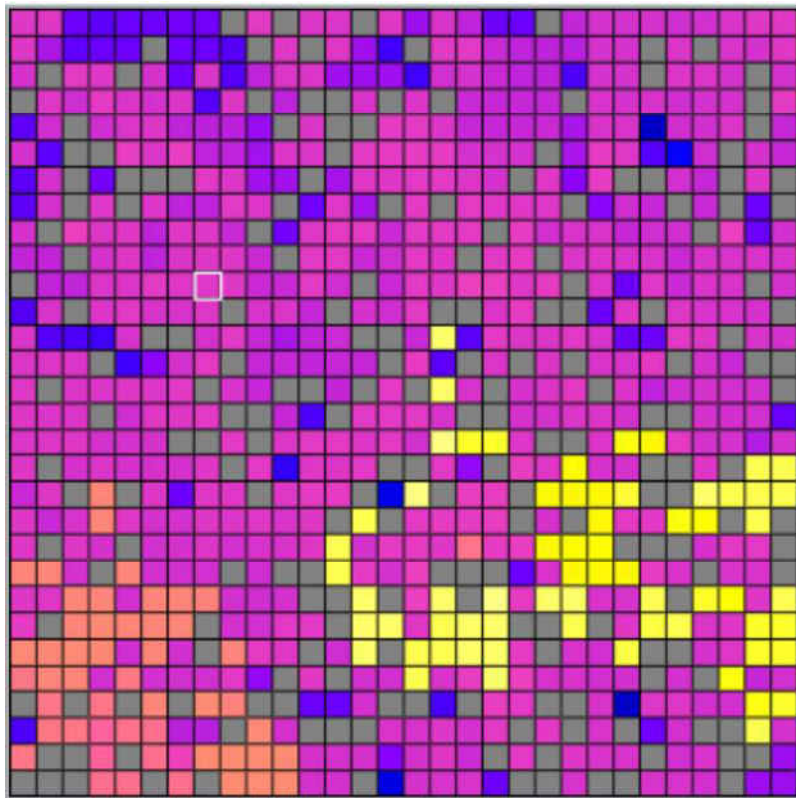# Avida-ED Lab Book
Fall 2016

Avidians in Action

Avida-ED Project Curriculum Development Team
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http://avida-ed.msu.edu
http://bit.do/avida-ed

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.

a. Go to http://avida-ed.msu.edu/.
b. Click on "App Download".
c. Select the radio button for the version you want to download.
d. Click on "Download Avida-ED now".
e. 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_avida/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.
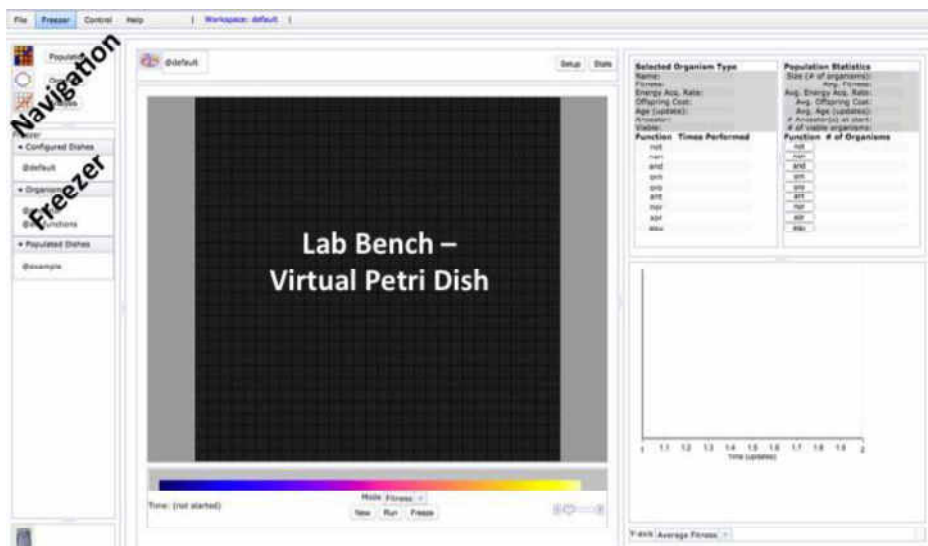

Figure 1. Screen shot of the Avida-ED Workspace in the "Population" map view.
The virtual Petri Dish is where Avidians will grow and divide. The "Navigation" and "Freezer" areas are on the left.
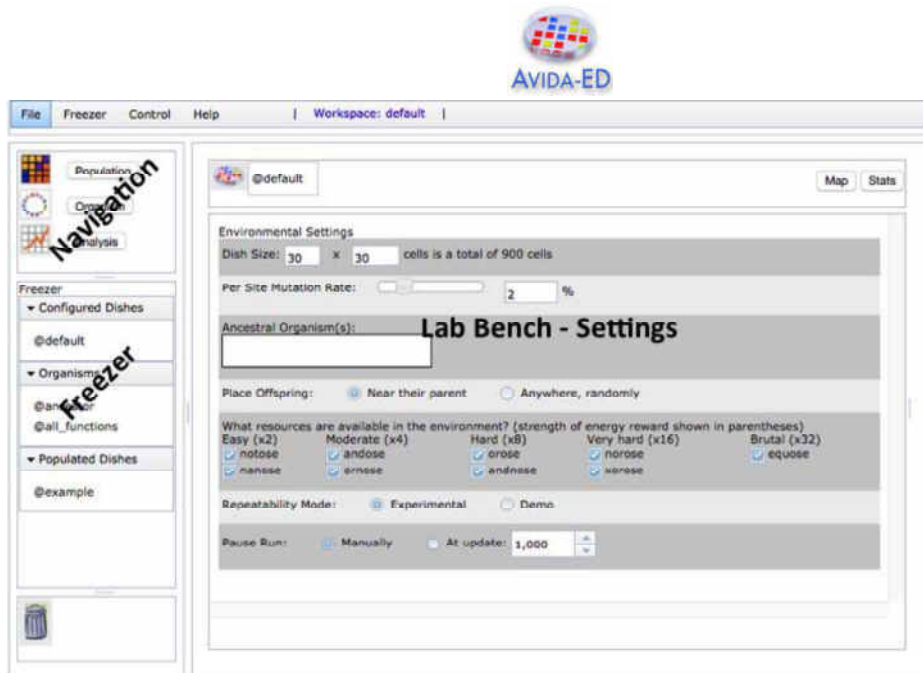
Figure 2. Screen shot of the Avida-ED Lab Bench Setting in the "Population" view.
Several parameters of the experimental set-up can be manipulated.

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).
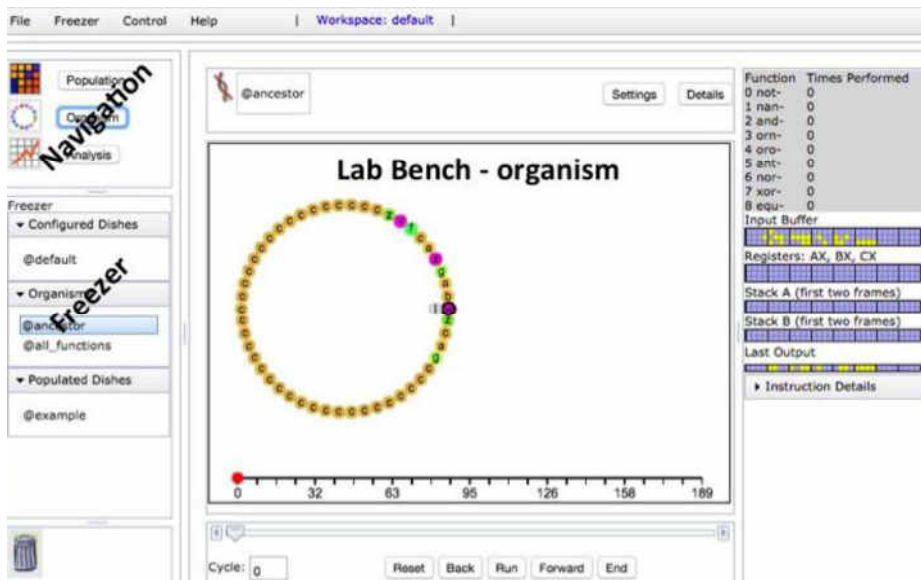

Figure 3. Screen shot of the Avidian "@ancestor" in the "Organism" view. The genome is circular and represented by colored letters. Each letter is a specific command. Notice that most of the instructions in "@ancestor" are tan-colored C's (these are "no operation" commands and here essentially serve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

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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     wzcagcccccccccccccccccccccccccccccccccccczvfcaxgab
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.
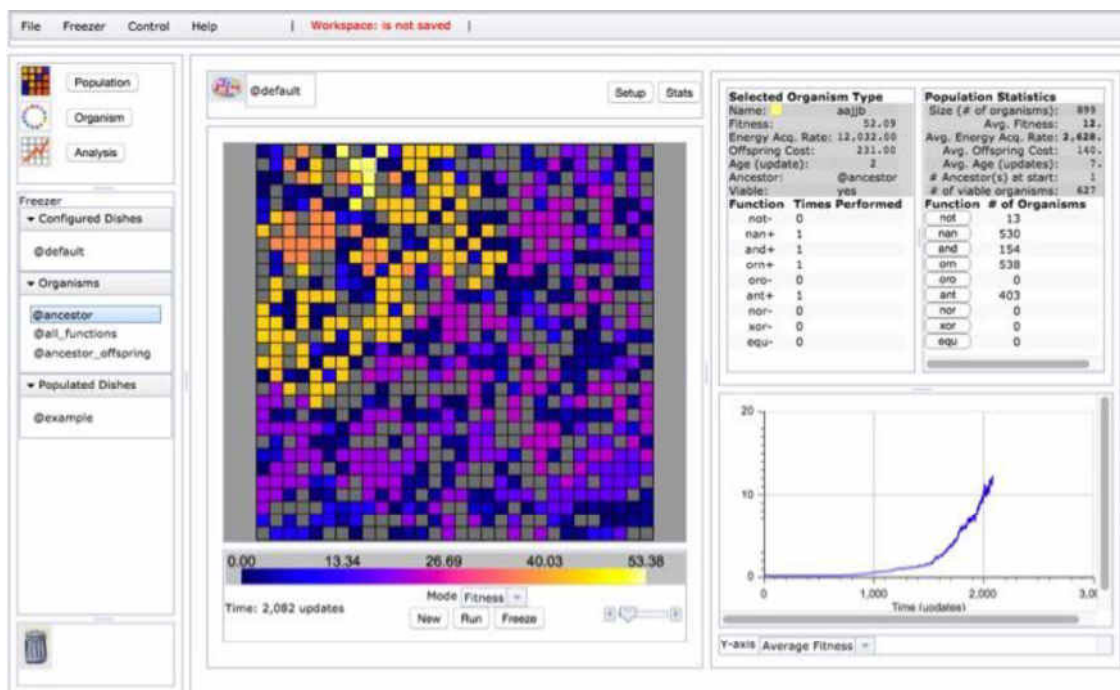

Figure 4. Screen shot of the panels displaying population and individual statistics. The upper right panel shows basic population statistics, plus how many individuals in the population perform each function. The panel just to its left does the same for a selected individual. The lower panel graphs a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

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?
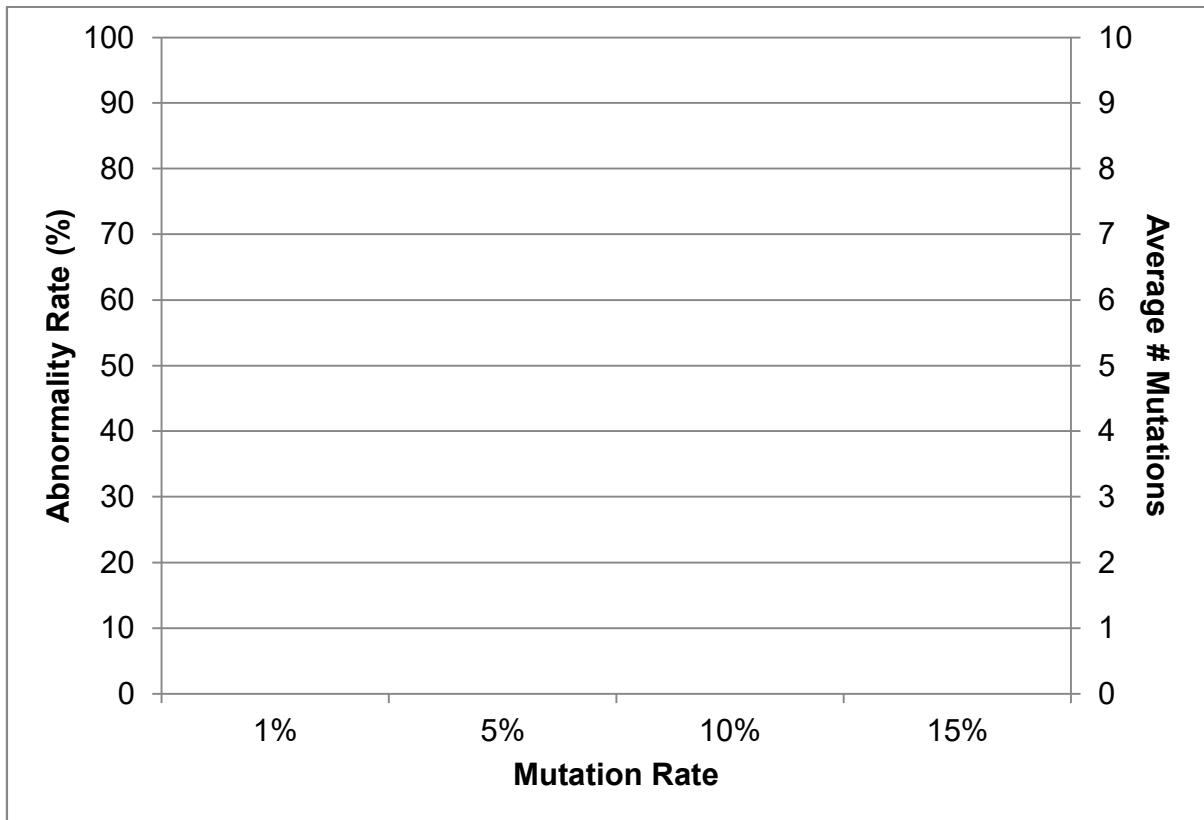
Exploring the Effects of Mutation Rate on Individuals

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

Offspring	Mutation Rate							
	1%		**5%**		**10%**		**15%**	
	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

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Data Chart. Plot your data to reveal patterns.

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).

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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

I F 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

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

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

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

QUESTION #1: WHAT GOOD IS HALF AN EYE?

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

'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 eburneum* (1b); *Masdevallia welischii* (1c); *Coryanthes speciosa* (1d); *Paphiopedilum hookerae* (2a); *Paphiopedilum armeniacum* (2b); *Psychopsis papilio* (2c); *Paphiopedilum tigrinum* (2d); *Paphiopedilum malipoense* (3a); *Paphiopedilum sukhakulii* (3b); *Paphiopedilum callosum* (3c); *Paphiopedilum delenatii* (3d); *Phalaenopsis amabilis* (4a); *Cypripedium acaule* (4b); *Paphiopedilum fairrieanum* (4c); and *Paphiopedilum venustum* (4d).

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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

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

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'

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resources

9 R & D

"Flash." For more about eggs dividing without sperm, see "Phospholipase Cζ Causes Ca²⁺ Oscillations and Parthenogenetic Activation of Human Oocytes." N. T. Rogers et al. in *Reproduction* 128 (2004), pages 697–702.

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

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

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

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

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

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

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

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

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

"When the Brain Gets Out of Tune." "Neural Synchrony Indexes Disordered Perception and Cognition in Schizophrenia." Kevin M. Spencer et al. in

Proceedings of the National Academy of Sciences, Vol. 101: 17288–17293; published online November 16, 2004 at www.pnas.org/cgi/doi/10.1073/pnas.0406074101.

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

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

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

18 EMERGING TECHNOLOGY

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

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

20 THE BIOLOGY OF . . . CRYOGENICS

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

28 TESTING DARWIN

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

"Developmental Cheating in the Social Bacterium *Myxococcus xanthus.*" G. J. Velicer, L. Kroos, and R. E. Lenski in *Nature* 404, pages 598–601; April 6, 2000.
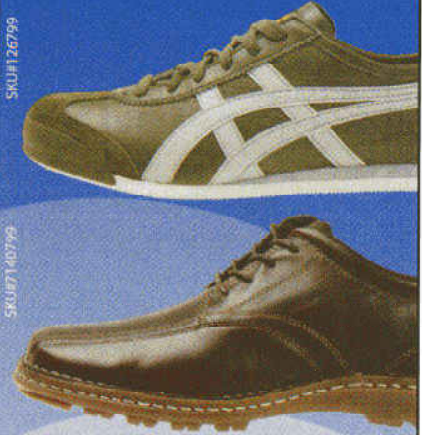
Orchid pictures were provided by Eric Hansen, author of *Orchid Fever: A Horticultural Tale of Love, Lust, and Lunacy* (Vintage Books, 2001). His latest book is *The Bird Man and the Lap Dancer: Close Encounters With Strangers* (Pantheon, 2004).

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

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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.