Agents of Evolution

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In this introductory biology lab, each student in a lab group is assigned an agent of evolution (natural selection, gene flow, mutation, or genetic drift) to investigate. Each student completes a combination of specific tasks related to their agent—these include some combination of readings in a handout, online readings, videos, and at least one simulation they manipulate (all online resources are free). This can be done before lab, individually during lab, or in a group during lab. Students then meet with their lab group and share what they've learned; each student serves as an "expert" on their agent. As a group, students investigate a free online modeling simulation that allows them to manipulate multiple variables simultaneously. Students are asked to develop an experimental question, generate a hypothesis, and then test their hypothesis using the modeling simulation. The group work portion of the lab can be completed in approximately two hours. Student response to the lab was generally positive; students appreciated the chance to take an active role in the selection and investigation of an experimental question. We felt the lab was effective at teaching concepts and incorporating meaningful group work in an online situation, but could be used for in-person labs as well.

Keywords: evolution, simulation, online

Introduction

It is important for introductory college biology students to learn about evolution; while the teaching of evolution in high school is improving, it isn't yet part of every student's background (Plutzer et al. 2020). The goal of this lab is to help students understand the agents of evolutionary change: natural selection, gene flow, mutation, and genetic drift. (Some textbooks also include non-random mating as a separate agent, while others do not; non-random mating only changes genotype frequencies in populations, not overall allele frequencies.) These concepts are important for students to understand in conjunction with Hardy-Weinberg Equilibrium, which is based on the assumption that none of these agents are active. Students struggle with the concepts of natural selection (Nehm and Reilly 2007), gene flow, mutation (Zhao and Schuchardt 2019), and genetic drift (Andrews et al. 2012).

Evolutionary mechanisms are challenging to explore in a teaching lab because of the time required for multiple generations. Bacteria can be used (Krist and Showsh 2007; Serafini and Matthews 2009) but it can be challenging to identify phenotypes that can be easily tracked, and using molecular techniques to identify allele frequencies can be time-consuming. Several lab exercises have been developed using physical models (playing cards, dice, beads, coins, candy, etc.) to simulate evolutionary processes (Hongsermeier et al. 2017; Jensen 2015; Ketcham 2010; Lee et al. 2017; Malone et al. 2019; Marion 2020; Orrell et al. 2016; Pecor 2019; Staub 2002; Zeeh and Quell 2015), though these typically do not simulate all the mechanisms of evolution. Model simulations of gene flow and mutation are much less common than those involving natural selection and genetic drift. Physical simulations are also typically limited in population size and by the number of generations that can be followed during a lab period. Previous labs have also been published that utilize computer modelling to study evolution (Kosinski 2015; Sheehy 2015). A previous study compared physical simulations to virtual simulations and found that both were effective in helping students understand natural selection (Pope et al. 2017). Some labs incorporate both physical models and computer simulations (Andersen 1993; Barton 2000; Glase 1993). Some of the computer simulations described have fairly complex interfaces, and some focus on only a single mechanism of evolution. Some

computer simulations require downloading particular software, and others are outdated or unavailable.

The goals of this lab are to introduce students to the agents of evolutionary change and provide them with a solid understanding before they move on to consideration of Hardy-Weinberg Equilibrium in class. I don't assume students have prior knowledge of these concepts, but they do need to understand what alleles are. In designing this lab, I was looking for an all-online option to accommodate remote students due to the COVID-19 pandemic in 2020-2021. I wanted students to be able to access the simulations from any computer or tablet platform, without using specialized software. I wanted a way for students to engage fully with the agents of evolution, but I wanted to make sure they didn't confuse the different agents with each other (particularly genetic drift and gene flow, which sound so similar to students). I also wanted them to work in groups, and to use their lab time efficiently. Finally, I wanted students to start asking their own experimental questions and use a simulation to start getting answers to those questions.

The main simulation students use in this lab is PopGen Fishpond, originally developed as PopGen Fishbowl (Jones and Laughlin 2010). It uses HTML5, so it is available in a web browser, works on Mac, PC, and tablets, and is not reliant on Flash. Students can use it for free and do not need to register. All simulations for this lab are similarly free and available through web browsers on multiple platforms. Other simulations students use include AlleleA1 (developed by Jon C. Herron at the University of Washington for use with his textbook *Evolutionary Analysis* (Herron and Freeman 2014)), Peppered Moths (a predation game developed by Craig Tavis at the Ask a Biologist website), Population Genetics (designed by Jolene Pappas at Biology Simulations), Genetic Drift – Bottleneck (designed by Jolene Pappas at Biology Simulations), Genetic Drift – Founder Effect (designed by Jolene Pappas at Biology Simulations), Genie (a gene flow and genetic drift simulation (Castillo et al. 2021)), and Genetic Drift Simulation (from the *Principles of Life, Second Edition* (Hillis 2013) textbook website).

This lab was designed for introductory students, and takes place in multiple stages; the first stage is an exploration of a single agent of evolutionary change. This is followed by a sharing stage, in which students with different agents explain what they learned to each other. Then there is an experimental stage, in which the group works together to develop and test an experimental question. The exploration stage can be assigned to individuals to complete before the lab, or can be completed during the lab period, either individually or with a group. The timing is flexible; the exploration stage can be completed in less than an hour, and the remainder of the lab can be completed in two hours. The lab can also be expanded by asking each group to develop and conduct multiple experiments.

Student Outline

Part I: Learn About an Agent of Evolutionary Change

You will be assigned a particular agent of evolutionary change to investigate. You will serve as an "expert" on this agent, and you'll work with a group in lab to see how the different agents interact. Your lab group will be depending on you to complete the tasks in the Appendix for your agent of evolutionary change:

        Natural Selection: See Appendix A
        Gene Flow: See Appendix B
        Mutation: See Appendix C
        Genetic Drift: See Appendix D

Part II: Share What You've Learned

Once you've completed Part I, you'll move into an assigned group that consists of one person from each of the different agent groups.

1. Introduce yourselves to each other.

2. Take turns briefly summarizing what you learned about your agent of evolutionary change. You should:
        a. Describe what your agent of evolutionary change is.
        b. Describe the effects of your agent on the genetics of a population.
        c. Explain the reason your agent has these effects.

3. Check to make sure everyone in your group understands the different agents—ask questions if you are uncertain about the differences between the agents or how they affect a population.

Part III: Investigate Interactions Between Agents

Continue working in the same lab group you were in for Part II.

4. First, brainstorm a list of experimental questions that you might ask about how different agents of evolutionary change interact. For example, you might want to know how mutations affect populations that are undergoing natural selection. Record your list in a shared Google Doc.

5. As a group, decide on one of these questions to investigate further during lab today. Note which one you agree on in your Google Doc.

6. For your question, develop a hypothesis about how you think the agents will interact. Record this in your Google Doc.

7. One person should share their screen and operate the **PopGen Fishpond** (**http://virtualbiologylab.org/ModelsHTML5/PopGenFishbowl/PopGenFishbowl.html**) model for the group.

8. As a group, decide what experiments would be useful to answer your experimental question. Keep in mind that if you are looking at multiple variables, you'll want to develop appropriate controls. *Note: you should run multiple trials for each test you perform; the simulation will not give identical results each time.*

9. Run your experiments and record your results in your Google Doc in whatever format makes the most sense to you. What did you find?

    *Feel free to go back and run additional experiments to help answer your question. If you have concerns about the relatively small sample size available to you in this simulator, you can conduct your tests in **AlleleA1** (**https://evound.shinyapps.io/AlleleA1Shiny/**) from Jon Herron at the University of Washington (some of you used this model in your exploration of your agents earlier). This simulator runs with an infinite population by*

default, or you can choose a very large population size (up to 10,000) under the "Population size" setting. Do these results match the PopGen Fishpond model?

10. Do your results support your hypothesis? Include your answer in your Google Doc.

11. What is your explanation for your results? Include your answer in your Google Doc.

12. Check in with your lab instructor or TA; be prepared to share:
  a. your experimental question
  b. your hypothesis
  c. your results and whether these were consistent with your hypothesis
  d. your explanation for your results

13. Hand in your Google Doc after making any changes needed based on your conversation with your lab instructor or TA.

Materials

Each student should have a computer or tablet with Internet access and a web browser. If students are learning remotely, they will need the ability to interact using a platform (like Zoom) that allows for small groups and screen sharing. For in-person labs, the instructions can easily be modified for students in a group to share a computer instead.

Notes for the Instructor

Student Evaluations

Overall, student evaluations of this lab were positive. The lab was taught in Fall of 2020 and again in Winter of 2021. Both terms, students were asked how much they enjoyed this lab. In Fall, with 36 out of 67 students responding, 75% of students responded enjoying the lab "medium," "quite a bit," or "tons," with 25% responding "only a little" or "not at all." In Winter, with 53 out of 65 students responding, 72% responded favorably. We also asked how much students learned from the lab. In the Fall term, 84% of students responded medium/quite a bit/tons, and in the Winter, it was 79%.

In individual comments about the lab, most students agreed that they gained a good understanding of the agent they'd been assigned in Part I of the lab, but several noted that they didn't understand the other agents as well. It might be helpful to remind students that they have access to the resources for all the agents, so they can refer to these if they are confused later. It might also be useful to encourage students to be thorough and ask for questions when they are teaching others about their agent in Part II of the lab.

Optional Organizations for Part I

This lab was designed to engage students by helping them develop as an "expert" of a particular agent of evolutionary change and then asking them to teach others. The first term I taught the lab (Fall term 2020), I asked students to complete Part I before lab time, and they did a great job coming to lab prepared. I was really impressed by their ability to quickly engage with their group members to complete the lab.

By Winter term 2021, in the midst of the COVID-19 pandemic, I was concerned about students' ability to get work done outside of lab time, and so I gave them an hour at the beginning of lab to complete Part I individually. (We have a 10-week term, with a weekly 4-hour lab session; during the pandemic, all labs were taught remotely, and we tried to keep the synchronous portion of the lab closer to 2 hours.) One hour was plenty of time for almost all students to complete Part I with time left over; since students work at different paces, and not all the agents of evolution have the same number of simulations, some students used most of the hour and other students finished quickly. Setting an hour worked well; I was able to let students know they might have time to complete other work during that hour, and students didn't feel rushed to finish. One student commented in the lab evaluation "Something that was important to me in this lab was having the 1-hour time slot allocated for us to do individual work, rather than having a race where we all tried to finish as quickly as possible. I felt more relaxed because of the time allocation without the pressure of going very quickly." Students in lab this term were accustomed to having a chunk of scheduled lab time that was more asynchronous in nature, which I think also helped—while usually this time occurred at the end of the lab, I was able to warn them ahead of time that we'd be inverting that structure.

A third option, which was used at the ViABLE conference in 2021, is to ask students to complete Part I during lab in groups. These groups could be whatever size works best for your labs. This gives students a little more support than individually completing the work, but may not require as much active engagement. Students in these initial agent groups then are put into jigsaw groups (Aronson and Patnoe 2011) consisting of one student from each agent group.

Student Agency

This lab allows students to develop their own experimental questions and hypotheses, find a way to test them, repeat their experiment, and determine what they think their results mean. This allows for active participation in the process, and agency in deciding what to investigate. The project is open-ended but still bounded, so it is not overwhelming or high-stakes for students. If something doesn't work like they expect, there is plenty of time to revise a strategy and run the simulation again.

In our introductory class, we don't talk much about models or modeling biological systems; in this lab students get an introduction to the ways in which simulations and models can be really useful and allow them to look at complicated systems.

Assessment

I did not formally assess student work for this assignment; students were required to discuss their project with myself or an undergraduate teaching assistant during lab and turn in their final document by the end of lab. These were graded only for completion (all groups received full credit). I felt that the process benefitted the students more than

creating an accurate product; they had to navigate the identification of an experimental question and figure out what variables to test. They had to then see if their results agreed with their hypothesis, and interpret their results. This could easily be adapted for more sophisticated purposes, but my goal was just to give them more exposure to the agents of evolutionary change so they became more meaningful to the students.

Other Possible Modifications
Group Size
If the number of students in lab is not divisible by four, some of the groups for parts II and III can have five students. This can be accommodated by assigning two students to the Genetic Drift agent, and instructing one student to complete step #3 (looking at the genetic bottleneck) and the other student to complete step #4 (looking at the founder effect). Both students also complete steps #1-2 and #5-6 (see Appendix D). We used this modification successfully for multiple lab sections.

Experimental Questions
In Fall term 2020, we asked student groups to develop and carry out two separate experiments using the PopGen Fishpond. While most groups were able to complete this in a timely fashion, a few of the more methodical groups struggled with running out of time. We told those groups to stop after completing a single experiment; they had done a great job at staying on task, and had sometimes put more thought into planning or analyzing their results than the faster groups. In Winter term 2021, due to some timing concerns, I only required one experiment from all groups. Interestingly, some Winter term lab students indicated on evaluations that they weren't sure if they understood all the agents well because they had only completed one experiment. So, if there is time for groups to complete two experiments, or if you are comfortable letting some groups stop after a single experiment, this might be a good option for you.

Non-Random Mating
In the Fall of 2020, I was able to identify an online resource that allowed students to look at infinite and very large populations with non-random mating. However, the site I was using for this (the Population Genetics Simulator) was taken down between November of 2020 and January of 2021. I did not find a good alternative that allowed for intuitive assortative mating similar to that of PopGen Fishpond. We also saw some unpredicted results in PopGen Fishpond with assortative mating, possibly due to small population numbers. I decided to discontinue the inclusion of non-random mating in the lab, but it may be worth looking into again in the future.

Online Resources
Inevitably, some of the links in this document will become outdated, or the online resources will no longer be freely available. The links will need to be checked before the lab is taught each term or semester. This same lab structure could be used with your preferred online resources.

Suggestions from ABLE Workshop Participants
Participants in the workshop in June of 2021 had great suggestions about a number of aspects of the lab.

Improving Learning from Other Students
One suggestion was to make Part II (teaching and learning in the jigsaw group) more thorough by asking students to develop a set of slides to help them teach others about their agent. These might include annotated screen shots of their simulations to more concretely describe the agent and its effects. Another option would be to check in with individuals before they share with each other, to make sure good information is being passed along.

Providing Additional Structure
The lab as written is open-ended, with students given a lot of room to explore what they are most interested in. My students were working in established groups near the end of the term, and I was most focused on the process the students were going through. Several participants suggested that an excellent way to strengthen the lab would be to provide additional structure, including more directed questions to ensure students understand content, additional check-ins with instructors to make sure students are on track (e.g., experimental design approval), suggestions of experimental questions students might address, and specific guidance about how to use the PopGen Fishpond to answer their question. Providing additional structure might change the amount of time needed for the lab, but Part III could easily be shifted to a different lab period.

Participation and Engagement
Some workshop participants had concerns about making sure all students were contributing and engaged in learning. One useful suggestion was using Notability with a pdf of the lab questions, asking each student to answer questions individually and to include annotated screen shots to support and explain their answers.

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Acknowledgments

About the Author

Sarah Deel is a Senior Lecturer in Biology at Carleton College in Northfield, Minnesota, where she has been teaching and coordinating introductory biology labs since 1996. She received her B.A. in Biology from Grinnell College in 1991 and her M.S. in Zoology from Oregon State University in 1996.

Appendix A

Agents of Evolution Lab Part I: Natural Selection

Evolution refers to genetic changes in populations of organisms over time. Biologists have categorized four main types of factors that affect evolution: these are the four "agents of evolutionary change." You have been assigned Natural Selection, and will serve as your group's "expert" for that agent.

Below are the tasks you should complete so you are prepared to help your group during lab. *You'll want to make notes somewhere as you work through these. You are not required to take highly detailed notes on every simulation you run; unless we ask you to record specific values, you can describe your thoughts more generally. You won't be handing these in, but they may be useful for "teaching" your other group members.*

Note: If you are working in a group, please start your work by introducing yourselves. One of your group members should share their screen and you should work through these tasks together.

1. For a summary of the basics about natural selection, watch the Amoeba Sisters video (7:22) on Natural Selection (**https://www.youtube.com/watch?v=7VM9YxmULuo**).

2. Read through the Peppered Moths site (**https://askabiologist.asu.edu/peppered-moths-game/index.html**) and play the game there.

3. Determine the effects of natural selection on genotype frequencies and allele frequencies in a population.
   - a. Go to the Population Genetics simulation (**https://www.biologysimulations.com/population-genetics**) from the Biology Simulations website. You may need to make your browser window wider to correctly view the full page. This simulation models a gene in a diploid population that has two alleles: "red" and "blue." Since there are only two alleles in the population, their proportions always add up to 1. Click on the "Introduction" button to read about the simulation.
   - b. The default "Population size" and Number of Generations" are both very low. Use the sliders to set these as high as they will go. This will allow you to model 1000 individuals over each of 100 generations. The simulation will track the proportion of each allele in the population. You'll notice the default "Red Allele Starting Frequency" is 0.5: this means that the frequency or proportion of the red allele is 0.5 (50% of the alleles are red). Go ahead and run the model by clicking the "Run Simulation" button to see what it does. You'll notice that lines appear on the graph, the x-axis changes to indicate a run of 100 generations, and a legend appears to the right of the graph.
   - c. Because the population size is not infinite, there is some variability in the data. The variation you see is due to genetic drift, which is another agent of evolutionary change you'll learn more about in lab. In general, what happens to the allele frequencies over time? What is the range of values for each allele frequency? Make some notes about what you're seeing.
     You can hover your mouse over the lines in the graph to see the actual frequency values for any generation. If you just want to focus on particular lines, in the legend, click the lines you don't want to see. Note that the y-axis range changes automatically, so it may look like there is more variability in the data than there is.
   - d. Click the "Run Simulation" button again to see a different run with the same parameters. Note that these will not be identical: each time, the program models the results independently. Run the simulation several times to see how it changes. Do you see patterns that make sense when you compare the red allele frequency to the blue allele frequency? The blue phenotype frequency to the blue allele frequency? Make sure you understand these relationships.
   - e. To bring natural selection into play, change the "Red Survival Chance" slider to around 0.5. This means that each organism with a red phenotype will have only a 50% chance of surviving to reproduce and pass its alleles on to the next generation. Record notes about your results. What are the starting and ending frequencies of each allele?
   - f. Run this simulation with the same settings several times, to see if the pattern stays the same.
   - g. How do you explain your results? Record an explanation.
   - h. Explore the natural selection portion of the model a little more; what happens if the red survival chance is just below 1? What if it is less than 0.5? What if the red survival chance is back to 1 but the purple survival chance is less than 1? Remember to run each simulation several times—the pattern you see

based on a single run may not be the same for other runs. Take notes about your results. Are they all consistent with your understanding and explanation?

  i. (Optional if time) Feel free to explore other aspects of the model if you'd like (how do the results change with a different red allele starting frequency?). Other students in your lab group will be looking at the effects of population size and mutation, but you're welcome to play with these if you're curious.

4. Familiarize yourself with the simulation you'll be working with during lab today.
   a. Go to the PopGen Fishpond Virtual Biology Lab:
      http://virtualbiologylab.org/ModelsHTML5/PopGenFishbowl/PopGenFishbowl.html
   b. Read through the Background Information and Tutorial sections of this site.
   c. Go into the Run Experiments section and play around with the settings for your agent of evolutionary change. Note that the population size in this simulation is fairly limited; we suggest increasing the Carrying Capacity and Initial Size settings as high as possible.
      If you aren't sure which settings you should be changing for your agent, check in with your lab instructor or TA.
   d. Run a few simulations to see the effects; be sure to run multiple simulations at the same setting to check for variability. Does the model respond in the way you'd expect? If not, do you have a guess why that might be?
      Note: If the simulation stops working (I had a little bit of trouble after pausing and restarting simulations multiple times), just reload the page and it should work fine (you'll need to re-set any settings you've changed, but it should be quick).

5. Prepare to share what you've learned with your lab group, and to serve as your group's "expert" when they have questions about your agent of evolutionary change. You will want to be able to share the main points about what you've learned in 5-10 minutes. You'll be asked to, in your own words:
   a. Describe what your agent of evolutionary change is.
      You might consider if anything was particularly confusing for you, and figure out how to explain it clearly to others who are just hearing about it for the first time.
   b. Describe the effects of your agent of evolutionary change on the genetics of a population.
      You can demonstrate a simulation for your group to help with this.
   c. Explain the reason your agent of evolutionary change has these effects.

Appendix B

Agents of Evolution Lab Part I: Gene Flow

Evolution refers to genetic changes in populations of organisms over time. Biologists have categorized four main types of factors that affect evolution: these are the four "agents of evolutionary change." You have been assigned Gene Flow, and will serve as your group's "expert" for that agent.

Below are the tasks you should complete so you are prepared to help your group during lab. *You'll want to make notes somewhere as you work through these. You are not required to take highly detailed notes on every simulation you run; unless we ask you to record specific values, you can describe your thoughts more generally. You won't be handing these in, but they may be useful for "teaching" your other group members.*

Note: If you are working in a group, please start your work by introducing yourselves. One of your group members should share their screen and you should work through these tasks together.

1. Read the following background information about gene flow:
   > Gene flow occurs when individuals migrate into or out of a population. When this happens, it can change the genetics of the population by adding new alleles, removing alleles, or changing the proportion of the alleles in the population.

2. Determine the effects of gene flow on two populations with different alleles.
   a. Go to Genie (**https://cartwrig.ht/apps/genie/**), a simulation that models a gene in a population of haploid organisms. This gene has several possible alleles, but because each individual in the population is haploid, each individual only has one allele. Scroll down on the page and read the "About the simulation," "Controls," and "Barriers" sections *(we won't be using Mutations—those are a different agent of evolutionary change).* In each generation of the simulation, as each individual "dies," the allele for the "new" individual that replaces it is chosen at random from the alleles in the 8 cells surrounding that individual.
   b. Start the simulation and let it run for several generations. Pause the simulation after around 50 generations. What happened to the total number of alleles? Record what's happening (briefly is fine!) in your notes for today's lab. *(The main reason for the decrease in the number of alleles is genetic drift, which is a different agent of evolutionary change—one of your group members will explain more about this during lab.)*
   c. Add a barrier to separate your population into two separate sections of approximately equal size. You can do this by selecting individual boxes on the grid or by clicking and dragging your cursor across the boxes. It's okay if it isn't a perfectly straight barrier; just make sure there aren't any diagonal spots where the populations on either side of the barrier can mix. You can fill in those diagonals with additional barrier. Now you have two separate populations that can't interact with each other. You've set up a situation where there is no gene flow between these two populations.
   d. Continue your paused simulation by clicking "Start." Watch what happens over another 20 generations or so, and pause. Does the population look the same on either side of the barrier? Describe what's going on in your notes. Continue to let the simulation go until it is clear the populations look different (but you still have multiple alleles).
   e. Now break a hole in your barrier by clicking on some (5-6) of the black cells (they will turn white). This will allow gene flow (or migration) to occur between the two populations. Continue the simulation. What happens to the number and type of alleles in each population? Make notes about the effect of gene flow.
   f. Write an explanation for your results. *Don't make this more complicated than you need to!*
   g. (Optional if time) Feel free to manipulate the system and explore what happens. If you re-run a similar simulation do you see the same patterns? What happens if you build a barrier before you begin the simulation? What if the sizes of the populations are very different? Is your explanation from above consistent with your results?

3. Determine the effects of gene flow on the genotype frequencies (the proportions of particular genotypes) in a population.

    a. Go to AlleleA1 (**https://evound.shinyapps.io/AlleleA1Shiny/**), developed by Jon Herron at the University of Washington. This simulation models a gene in a diploid population that has two alleles: A1 and A2. Since these are the only two alleles in the population, their proportions always add up to 1.

    b. In the "Generations to run" section in the bottom right corner of the page, you'll notice that the default is set to run the simulation for 500 generations. This simulation will track the proportion of each allele or genotype in the population (A1 or A2, if you choose one of the allele options, or A1A1, A1A2, or A2A2 if you choose one of the genotype options) over 500 generations. You'll also notice in the "Set parameters" section of the page that the default "Starting frequency of allele A1" is 0.5: this means that the frequency or proportion of the A1 allele is 0.5 (50% of the alleles are A1). By default, the "Population size" is set to "Infinite," meaning there is an infinite population of organisms. You'll notice that on the graph, there is a line that runs for 500 generations at the "0.5" level when the "A1" radio button is selected. If you click through the other radio buttons and watch how the graph changes, you'll note that the A2 allele is also present at a frequency of 0.5. The heterozygous genotype, A1A2, is present at a frequency of 0.5 in this population, and each homozygous genotype (A1A1 and A2A2) is present at a frequency of 0.25. Think about why the frequency of A1A1 *genotypes* is only 0.25, even though you set the A1 *allele* frequency much higher, at 0.5.

    c. See what happens to these frequencies if you change the "Starting frequency of allele A1" to 0.6 in the "Set parameters" box. Check the A2 frequency. Does the new allele frequency of A2 make sense?

    d. If you want to see the actual frequencies, you can click the "Table" tab near the top of the page. With the A1 frequency still set to 0.6, record the genotype frequency for each genotype. Note that none of the frequencies change over the course of 500 generations.

    e. Next, see what happens to the population if there is gene flow into the population. Under the "Set parameters" section, look for the "Migration" column. The default condition is no migration, represented by a "Freq of Migrants" of 0. Set the "Freq of Migrants (each generation)" to 0.5, which means that new migrants will be 50% of the population in each generation. The default allele "Freq of A1 in source population" is 0, which means that all the alleles of the incoming migrants are A2. *Note that in this model the migrants do not have new alleles—they are coming from a population that has the same two alleles (A1 and A2), but not necessarily in the same proportion as the population we're studying.* Look at the results of the simulation.

    f. What has changed? Record the final allele frequency for each allele.

    g. There are some interesting things happening in the first few generations; under "Generations to run," decrease the number of generations to 25, and run the simulation again. Note what's going on over time. What happens to the frequency of each allele over time? What about each genotype over time?

    h. How do you explain these results? Record an explanation in your notes.

    i. Explore the simulation further. What happens if you reduce the Migration Rate? What happens if you drastically reduce the Migration Rate, to just above 0? Make notes about these results. Are they consistent with your understanding and explanation? *Adjust the number of generations as necessary to see the patterns.*

    j. (Optional if time) Feel free to explore the model a little more—what happens if you change the migrant allele frequency? Is your explanation also consistent with these results?

4. Familiarize yourself with the simulation you'll be working with during lab today.

    a. Go to the PopGen Fishpond Virtual Biology Lab:
       http://virtualbiologylab.org/ModelsHTML5/PopGenFishbowl/PopGenFishbowl.html

    b. Read through the Background Information and Tutorial sections of this site.

    c. Go into the Run Experiments section and play around with the settings for your agent of evolutionary change. Note that the population size in this simulation is fairly limited; we suggest increasing the Carrying Capacity and Initial Size settings as high as possible.
       If you aren't sure which settings you should be changing for your agent, check in with your lab instructor or TA.

    d. Run a few simulations to see the effects; be sure to run multiple simulations at the same setting to check for variability. Does the model respond in the way you'd expect? If not, do you have a guess why that might be?
       Note: If the simulation stops working (I had a little bit of trouble after pausing and restarting simulations multiple times), just reload the page and it should work fine (you'll need to re-set any settings you've changed, but it should be quick).

5. Prepare to share what you've learned with your lab group, and to serve as your group's "expert" when they have questions about your agent of evolutionary change. You will want to be able to share the main points about what you've learned in 5-10 minutes. You'll be asked to, in your own words:
      a. Describe what your agent of evolutionary change is.
        You might consider if anything was particularly confusing for you, and figure out how to explain it clearly to others who are just hearing about it for the first time.
      b. Describe the effects of your agent of evolutionary change on the genetics of a population.
        You can demonstrate a simulation for your group to help with this.
      c. Explain the reason your agent of evolutionary change has these effects.

Appendix C

Agents of Evolution Lab Part I: Mutation

Evolution refers to genetic changes in populations of organisms over time. Biologists have categorized four main types of factors that affect evolution: these are the four "agents of evolutionary change." You have been assigned Mutation, and will serve as your group's "expert" for that agent.

Below are the tasks you should complete so you are prepared to help your group during lab. *You'll want to make notes somewhere as you work through these. You are not required to take highly detailed notes on every simulation you run; unless we ask you to record specific values, you can describe your thoughts more generally. You won't be handing these in, but they may be useful for "teaching" your other group members.*

Note: If you are working in a group, please start your work by introducing yourselves. One of your group members should share their screen and you should work through these tasks together.

1. Read "Mutations are the Raw Materials of Evolution" from Nature Education Knowledge (**https://www.nature.com/scitable/knowledge/library/mutations-are-the-raw-materials-of-evolution-17395346/**). You've already learned about mutations in class, but this will help re-frame how you think about them and give you some sense of the rate at which mutations might occur.

2. Determine the effects of mutations on alleles in a population.
   a. Go to Genie (**https://cartwrig.ht/apps/genie/**), a simulation that models a gene in a population of haploid organisms. This gene has several possible alleles, but because each individual in the population is haploid, each individual only has one allele. Scroll down on the page and read the "About the simulation," "Controls," and "Mutations" sections *(we won't be using Barriers)*. In each generation of the simulation, as each individual "dies," the allele for the "new" individual that replaces it is chosen at random from the alleles in the 8 cells surrounding that individual.
   b. "Start" the simulation with the default settings and let it run for several generations. Pause the simulation after around 50 generations. What happened to the total number of alleles? Describe what's happening (briefly is fine!) in your notes. Click the "Start" button to continue the simulation. How many generations does it take before there are only two different alleles? Allow the simulation to go to around 500 generations (unless the population goes down to a single allele before that). *(The main reason for the decrease in the number of alleles is genetic drift, which is a different agent of evolutionary change—one of your group members will explain more about this during lab.)*
   c. Observe the effect of mutations on the population: reset the simulation, and set the mutation rate to 0.0002. This is a fairly high mutation rate, despite it being a pretty small number. Start the simulation. Record notes about differences between the population with no mutations and this population.
   d. Write an explanation for your results. *Don't make this more complicated than you need to!*
   e. Explore the model a little; what happens if you increase the mutation rate? If you decrease it? Record notes about your results. Is your understanding and your explanation consistent with these new results?

3. Determine the effects of mutation on the genotype frequencies (the proportions of particular genotypes) in a population.
   a. Go to AlleleA1 (**https://evound.shinyapps.io/AlleleA1Shiny/**), developed by Jon Herron at the University of Washington. This simulation models a gene in a diploid population that has two alleles: A1 and A2. Since these are the only two alleles in the population, their proportions always add up to 1.
   b. In the "Generations to run" section in the bottom right corner of the page, you'll notice that the default is set to run the simulation for 500 generations. This simulation will track the proportion of each allele or genotype in the population (A1 or A2, if you choose one of the allele options, or A1A1, A1A2, or A2A2 if you choose one of the genotype options) over 500 generations. You'll also notice in the "Set parameters" section of the page that the default "Starting frequency of allele A1" is 0.5: this means that the frequency or proportion of the A1 allele is 0.5 (50% of the alleles are A1). By default, the "Population size" is set to "Infinite," meaning there is an infinite population of organisms. You'll notice that on the graph, there is a line that runs for 500 generations at the "0.5" level when the "A1" radio button is selected. If you click through the other radio buttons and watch how the graph changes, you'll note that the A2 allele is also present at a frequency of 0.5. The heterozygous genotype, A1A2, is present at a frequency of 0.5 in this

population, and each homozygous genotype (A1A1 and A2A2) is present at a frequency of 0.25. Think about why the frequency of A1A1 *genotypes* is only 0.25, even though you set the A1 *allele* frequency much higher, at 0.5.
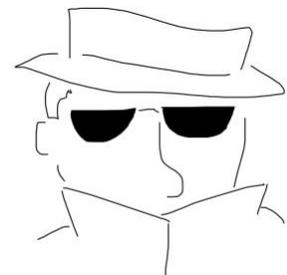
    c. See what happens to these frequencies if you change the "Starting frequency of allele A1" to 0.6 in the "Set parameters" box. Check the A2 frequency. Does the new allele frequency of A2 make sense?

    d. If you want to see the actual frequencies, you can click the "Table" tab near the top of the page. With the A1 frequency still set to 0.6, record the genotype frequency for each genotype. Note that none of the frequencies change over the course of 500 generations.

    e. Next, see what happens to the population if there is mutation within the population. In the "Set parameters" section, find the column labeled "Mutation rates." The default condition is no mutation, represented by a "From A1 to A2" rate of 0 and a "From A2 to A1" rate of 0. In this case, rather than introducing new alleles, the mutations will change an A1 allele to an A2 allele or an A2 allele to an A1 allele. Find out what happens when you set the A1 to A2 mutation rate to 0.0002 per generation.

    f. Can you tell what is going on? Record the final frequency for each allele and genotype in your notes. How do these compare to the simulation with no mutations?

    g. To get a better sense of what is happening over a longer time scale, change the "Generations to run" from 500 to 10000. What happens to the frequency of each allele over time? What happens to the frequency of each genotype over time?

    h. How do you explain these results? Write an explanation in your notes.

    i. Explore the simulation further. What happens if you increase or decrease the "From A1 to A2" mutation rate? What happens if you set the "From A1 to A2" mutation rate to 0 and increase the "From A2 to A1" mutation rate? What happens if both mutation rates are the same? Make notes about these results. Are they consistent with your understanding and explanation? *Adjust the number of generations as necessary to see the patterns.*

    j. (Optional if time) Feel free to explore the model a little more—what happens if you change the starting allele frequency? Is your explanation also consistent with these results?

4. Familiarize yourself with the simulation you'll be working with during lab today.

    a. Go to the PopGen Fishpond Virtual Biology Lab:
      http://virtualbiologylab.org/ModelsHTML5/PopGenFishbowl/PopGenFishbowl.html

    b. Read through the Background Information and Tutorial sections of this site.

    c. Go into the Run Experiments section and play around with the settings for your agent of evolutionary change. Note that the population size in this simulation is fairly limited; we suggest increasing the Carrying Capacity and Initial Size settings as high as possible.
      If you aren't sure which settings you should be changing for your agent, check in with your lab instructor or TA.

    d. Run a few simulations to see the effects; be sure to run multiple simulations at the same setting to check for variability. Does the model respond in the way you'd expect? If not, do you have a guess why that might be?
      Note: If the simulation stops working (I had a little bit of trouble after pausing and restarting simulations multiple times), just reload the page and it should work fine (you'll need to re-set any settings you've changed, but it should be quick).

5. Prepare to share what you've learned with your lab group, and to serve as your group's "expert" when they have questions about your agent of evolutionary change. You will want to be able to share the main points about what you've learned in 5-10 minutes. You'll be asked to, in your own words:

    a. Describe what your agent of evolutionary change is.
      You might consider if anything was particularly confusing for you, and figure out how to explain it clearly to others who are just hearing about it for the first time.

    b. Describe the effects of your agent of evolutionary change on the genetics of a population.
      You can demonstrate a simulation for your group to help with this.

    c. Explain the reason your agent of evolutionary change has these effects.

Appendix D

## Agents of Evolution Lab Part I: Genetic Drift

Evolution refers to genetic changes in populations of organisms over time. Biologists have categorized four main types of factors that affect evolution: these are the four "agents of evolutionary change." You have been assigned Genetic Drift, and will serve as your group's "expert" for that agent.

Below are the tasks you should complete so you are prepared to help your group during lab. *You'll want to make notes somewhere as you work through these. You are not required to take highly detailed notes on every simulation you run; unless we ask you to record specific values, you can describe your thoughts more generally. You won't be handing these in, but they may be useful for "teaching" your other group members.*

Note: If you are working in a group, please start your work by introducing yourselves. One of your group members should share their screen and you should work through these tasks together.

1. For a summary of the basics about genetic drift, watch the Amoeba Sisters video (4:37) on Genetic Drift (**https://www.youtube.com/watch?v=W0TM4LQmoZY**).

2. Determine the effects of genetic drift on genotype frequencies and allele frequencies in a population.
    a. Work through this general exercise on genetic drift (**https://www.whfreeman.com/BrainHoney/Resource/6716/SitebuilderUploads/Hillis2e/Student%20Resources/Animated%20Tutorials/pol2e_at_1502_genetic_drift_simulation/pol2e_at_1502_genetic_drift_simulation.html**). Read through the Introduction slide and go on to the simulation. This simulation models a gene in a diploid population that has two alleles: A and a. Since there are only two alleles in the population, their proportions always add up to 1.
    b. Follow the instructions in the simulation (the interface is pretty straightforward). Be sure to run the simulation several times with the same settings so you can see common patterns.
    c. Think through the questions that are part of the simulation. Record in your notes any explanations for yourself that you think might be helpful when you explain genetic drift to your lab partners.
    d. Genetic drift is occurring in populations of **all** sizes; include in your notes an explanation of what types of populations seem to be most strongly impacted by genetic drift.

3. Investigate the Genetic Bottleneck, a special case of genetic drift.
    a. Go to the Bottleneck simulation (**https://www.biologysimulations.com/genetic-drift-bottleneck-event**) from the Biology Simulations website. You may need to make your browser window wider to correctly view the full page. This simulation models a gene in a diploid population that has two alleles: "red" and "blue." Since there are only two alleles in the population, their proportions always add up to 1. Click on the "Introduction" button to read about the simulation *(you can ignore the information in the paragraphs above the simulation).*
    b. Click through the simulation once to get a sense of how it works. When you get to the comparison graph of the pre- and post- drought allele frequencies, hover over the data in the graph with your mouse to see the exact frequencies. In your notes, record the results. Were the pre-drought and post-drought allele frequencies the same or different?
    c. Click the "Restart Simulation" button and go through the simulation again; record the post-drought allele frequencies.
    d. Repeat the simulation a total of at least 5 times, recording the post-drought allele frequencies each time.
    e. What general pattern are you seeing in your results? In other words, what is the effect of a genetic bottleneck? Write a description in your notes.
    f. How do you explain your results? Write an explanation in your notes. Make sure your understanding includes the relationship between genetic drift and a genetic bottleneck.

4. Investigate the Founder Effect, a special case of genetic drift.
    a. Go to the Founder Effect simulation (**https://www.biologysimulations.com/genetic-drift-founder-effect**) from the Biology Simulations website. You may need to make your browser window wider to correctly view the full page. This simulation models a gene in a diploid population that has two alleles:

"red" and "blue." Since there are only two alleles in the population, their proportions always add up to 1. Click on the "Introduction" button to read about the simulation *(you can ignore the information in the paragraphs above the simulation)*.

b. Click through the simulation once to get a sense of how it works. When you get to the comparison graph of the mainland and island allele frequencies, hover over the data in the graph with your mouse to see the exact frequencies. Record the results. Are the allele frequencies the same or different?

c. Click the "Restart Simulation" button and go through the simulation again; record the island allele frequencies in your notes.

d. Repeat the simulation a total of at least 5 times, recording the island allele frequencies each time.

e. What general pattern are you seeing in your results? Is the result always the same? Describe this in your notes.

f. How do you explain your results? Write an explanation in your notes. Make sure your understanding includes the relationship between genetic drift and the founder effect.

5. Familiarize yourself with the simulation you'll be working with during lab today.

a. Go to the PopGen Fishpond Virtual Biology Lab:
   http://virtualbiologylab.org/ModelsHTML5/PopGenFishbowl/PopGenFishbowl.html

b. Read through the Background Information and Tutorial sections of this site.

c. Go into the Run Experiments section and play around with the settings for your agent of evolutionary change. Note that the population size in this simulation is fairly limited; we suggest increasing the Carrying Capacity and Initial Size settings as high as possible to begin with; because you're looking at genetic drift, you'll need to experiment with these settings further.
   If you aren't sure which settings you should be changing for your agent, check in with your lab instructor or TA.

d. Run a few simulations to see the effects; be sure to run multiple simulations at the same setting to check for variability. Does the model respond in the way you'd expect? If not, do you have a guess why that might be?
   Note: If the simulation stops working (I had a little bit of trouble after pausing and restarting simulations multiple times), just reload the page and it should work fine (you'll need to re-set any settings you've changed, but it should be quick).

6. Prepare to share what you've learned with your lab group, and to serve as one of your group's "experts" when they have questions about your agent of evolutionary change. You will want to be able to share the main points about what you've learned in 5-10 minutes. You'll be asked to, in your own words:

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b. Describe the effects of your agent of evolutionary change on the genetics of a population.
   You can demonstrate a simulation for your group to help with this.

c. Explain the reason your agent of evolutionary change has these effects.
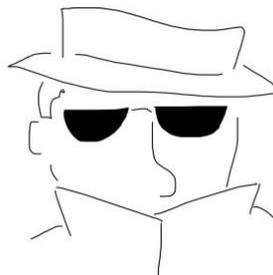
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