Measuring Evolution: Peppered Moths

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This lab was designed to be done by students asynchronously using two websites that each differently illustrate the classic natural selection example of the peppered moth. The learning outcomes for this lab are to experience natural selection through the first simulation, to use Hardy-Weinberg equilibrium as a null hypothesis for populations at specific times and evaluate if populations have experienced evolution (significant changes in allele frequencies) over time using the second model. While a peppered moth simulation was available on the NetLogoWeb site, it did not accurately represent the population or genetics of the moths as it used asexual reproduction. We modified the program to accurately simulate the population and provide students with a more realistic representation of these difficult evolutionary processes. Qualitative feedback from students indicated that they felt they learned a lot from this activity.

Keywords: Evolution, peppered moths, Hardy-Weinberg, null hypothesis

Introduction

This lab is designed to be done by students asynchronously using two websites that each differently illustrate the classic natural selection example of the peppered moth (*Biston betularia*), one of which was reprogrammed to meet our requirements. The exercise was developed as on online alternative to Marion's (2020) lab. While Marion (2020) was the inspiration, we modified the direction of the lab to best fit the online environment in combination with our learning goals. This activity is the fifth activity in a sequence looking at evolution, occurring after students have been introduced to the topic via NOVA, phylogenetics, the HHMI lizard evolution lab, and a predator escape lab (Pentz *et al.* 2015).

While teaching these activities at the introductory level, we noticed that students are very quick to pick up on the mechanisms of natural selection and a lesser degree mutation but are not as aware of genetic drift. By contrasting the outcomes of population growth simulations that include natural selection and mutation with those that do not, students are exposed to the random nature of genetic drift. This helps to address our learning goals which are:
1. to evaluate possible mechanisms of evolution,
2. to use Hardy-Weinberg equilibrium as a null hypothesis for populations at specific times and
3. evaluate if populations have experienced evolution (significant changes in allele frequencies) over time.

A peppered moth simulation available on the NetLogo Web site was modified through editing of the original programing to meet these criteria. Our resulting new model represents the population growth more accurately through sexual reproduction and follows Punnett square ratios in the allele transmission of the peppered moth population.

We faced several challenges with deciding how to best quantitatively analyze the data collected. Discussion during the conference suggested that the best approach for the statistics appropriate to an introductory course would be to look if the populations were in Hardy-Weinberg equilibrium at certain time points using a chi-square analysis via sums or specific runs. To determine if the populations were experiencing evolution, the best approach suggested was to use the runs as replicates in a paired t-test of the p allele frequency between an initial and final time.

For the future, we are planning to run this lab synchronously and add a more hands on simulation as in Marion (2020) that should complement the analysis presented here. We also look forward to being able to work through examples with students in person, which we think would improve the learning

experience. We did need to use video explanations to support the asynchronous presentation of the lab, as the calculations did overwhelm some students in our mixed major and non-major population.

Student Outline

Objectives
Evaluate possible mechanisms of evolution
Use Hardy-Weinberg equilibrium as a null hypothesis for populations at specific times
Evaluate if populations have experienced evolution (significant changes in allele frequencies) over time

Introduction
This activity looks at the theme of evolution in the context of a population at a certain time, and the change in allele frequencies over time. This activity simulates the effect of natural selection through predation and introduces you to the evaluation of statistical evidence as part of the scientific method. You will be collecting and interpreting data from a modelling program to assess the evolution of populations. The Background below will give you some more detailed information on measuring evolution including an introduction to the null hypothesis for evolution, the Hardy-Weinberg principle.

Background on Evolution:
Evolution in populations occurs through natural selection, genetic drift, gene flow, and mutation; all of these processes will change the allele frequencies over time. How would we demonstrate evolution in a population? In general, good scientific method requires comparison of results to a control to demonstrate an effect by a variable or a difference in conditions. The control conditions for evolution were published independently at the same time by two researchers, G.H. Hardy and W. Weinberg, so the principle has a hyphenated name to honor both. The Hardy-Weinberg mathematical model defines the genetic makeup of a population that is not undergoing evolution, or what the null hypothesis would be for evolution (Russell et al. 2019). The principle also defines the conditions that are necessary to prevent allele frequencies from changing between generations.

Key concept:
The null hypothesis is defined as the expression of what researchers expect to observe if a particular treatment or factor has no effect (Russell et al. 2019). It is also a basic requirement for most statistical tests to compare to the null hypothesis to the alternative hypothesis, usually a permutation of the research hypothesis that is under study.

The conditions necessary for a stable, non-evolving population are:
1. there is no migration into the population,
2. there is an infinite number of individuals in the population,
3. no mutation occurs,
4. all genotypes survive and reproduce equally well, and finally,
5. individuals in the population mate randomly with respect to genotype.

To summarize, the processes of natural selection, genetic drift, gene flow, and mutation all violate the conditions above.

The simulations we will look at in this activity simplify the model that defines allele frequencies between the generations by looking at allele frequencies of one gene with two different alleles (forms of the gene). This is a complex topic that can take some time to understand and internalize, so you may find that you need to research resources outside of this write up that may help you to get a handle on this topic.

For this activity we will use a simplified example where there are two alleles *A* and *a* in the populations in the population at a single locus, and p and q represent the relative frequency of these alleles. We have three genotypes: homozygous for *A*, homozygous for *a*, and heterozygous *Aa*. Where there are dominant and recessive alleles, p is the frequency of the dominant allele (*A*) and q is the frequency of the recessive allele (*a*). Allele frequencies in the population must add up to 100%, or will add up to one, so

$p + q = 1.$

You will also need to use the Hardy-Weinberg formula that summarize the genotypes frequencies in the population:

Genotype frequencies in the population must also add up to 100% (one), so if we assume a large population and random mating, the Hardy-Weinberg principle dictates that [(p + q) sperm genotype frequencies] $\times$ [(p + q) egg genotype frequencies] = 1; or

$$p^2 + 2pq + q^2 = 1$$

To determine if the population is in Hardy-Weinberg equilibrium, we must

1. determine the allele frequencies from the observed genotype frequencies (or be provided the allele frequencies). For example, the observed allele frequency for *A* is the proportion of 2 x *A* homozygotes (*AA*) plus the number of heterozygotes (*Aa*) relative to the total number of alleles in the population.

2. calculate what the expected <u>genotype</u> frequencies would be if the population was in Hardy-Weinberg equilibrium using p^2 : $2pq$: q^2 from the observed allele frequencies.

3. compare the observed and expected genotypes in the population statistically. A chi square (χ^2) analysis must be done on the actual number of genotypes in the population, as we can't do this analysis on frequencies.

You should also consider and assess the conditions (also termed assumptions) that are necessary for Hardy-Weinberg equilibrium: that natural selection is not acting on the gene, that the population is so large that genetic drift (i.e., random allele frequency changes) cannot happen, that there are no changes in the structure of the alleles of the gene (i.e. mutation or immigration), and that random mating is occurring (Freeman et al. 2019). If you see a change in allele frequencies over time, then it is likely that one or more of these assumptions is not valid. When you consider real populations in nature, it is likely that any combination of these assumptions could be false.

Natural History:

Peppered moths (*Biston betularia*) are annual insects that overwinter in cocoons and hatch into moths in the spring (Tevis 2003). They feed at night, and in the day rest on tree trunks in England (Tevis 2003). The first collectors of these moths found that the phenotypes were generally light colored with speckles (= peppered); the phenotype *typica* (Cook 2003). This phenotype is effectively camouflaged against trees with light colored bark such as birch trees common in the area (Tevis 2003). Early in the 19th century, a dominant mutation was observed in the population that resulted in a dark form of the moth, the phenotype *carbonaria* (Kettlewell 1955). This coincided with trees becoming dark from coal dust deposits, the result of pollution from the use of coal to power industry during the industrial revolution (Kettlewell 1955). From 1848 to 1970 the proportion of *carbonaria* increased to be the dominant phenotype in the environment, but the proportion of *carbonaria* decreased after 1970 following the institution of environmental controls that reduced particulate air pollution (Cook et al. 1999).

Studies on the moths in most of the areas studied by Dr. Kettlewell revealed that there are two alleles for moth color (Cook 2003), as in our formulas presented in the background information for Hardy-Weinberg models. Therefore, it is possible to determine if the population of peppered moths is undergoing evolution by using a simulation that models populations over time where we can add a scenario that has both selection and mutation (Wilensky 1997). This should allow you to assess if selection and mutation together are significant drivers of evolution.

Methods and Data Collection

Part A: "Peppered Moths Natural selection in action"

(https://askabiologist.asu.edu/peppered-moths-game/)

This site will take you through the background of the peppered moth, including its lifecycle. This kind of summary is an important part of any biological study as it may impact the experimental method and interpretation of the results. You should also read about the work of Dr. Kettlewell on the site - if you would rather watch a video, check out The Peppered Moth: An Example of Evolution in Action at https://www.youtube.com/watch?v=q64iUBPC8Mo). Then try the game where you are a bird eating moths - you must try it on the dark and the light background. You can print your results to a .pdf after each trial and record the results in Table 1 below.

Table 1. Moth population results

Tree Background:	# of moths eaten	Light Moth Population %	Dark Moth Population %
Light			
Dark			

- Did the dark or light phenotype ever completely disappear from the population? What implications does this have for the persistence of the less favorable genotype?
- Could you determine if either of the simulated populations was experiencing evolution? Why or why not? Justify your answer by discussing the definition of evolution.

Part B: Peppered Moth Sexual Model

(http://ccl.northwestern.edu/netlogo/models/community/Peppered%20Moths%20sexual))
The original version of this model had several assumptions that did not match real life - moths bred asexually and their color was determined by their single parent (Wilensky 1997). We had a computer programmer update the model so that it would more accurately reflect real life.
Updated version of the model description adapted from Wilensky (1997):

WHAT IS IT?
This project models a classic example of natural selection - the peppered moths of Manchester, England. The peppered moths that have the same coloration as their background are camouflaged from the birds that would eat them. (Note that in this model, the birds act invisibly.) Historically, light-colored moths predominated because they blended in well against the variegated white bark of the trees they rested on.

However, due to the intense pollution caused by the Industrial Revolution, Manchester's trees became discolored with soot, and the light-colored moths began to stick out, while the dark-colored moths blended in. Consequently, the darker moths began to predominate.

Now, in the past few decades, pollution controls have helped clean up the environment, and the trees are returning to their original color. Hence, the lighter moths are once again thriving at the expense of their darker counterparts.

HOW IT WORKS
This model simulates these environmental changes, and how a population of moths, initially with the two phenotypes (three genotypes) changes under the pressures of natural selection.

HOW TO USE IT
The NUM-MOTHS slider controls how many moths are initially present in the world. Originally their coloration was randomly distributed over the possible colors of the world (white to black), but we have modified the model so both the homozygous dominant moths and the heterozygous moths appear to be black, as in nature (i.e. you will not see grey moths in the center window). Simply select how many moths you'd like to begin with (around 200 is good, **but at least 150**), and press the SETUP button. Then press the GO button to begin the simulation.

The MUTATION slider controls the rate of mutation at birth. For the purposes of the simulation, the mutation rate is much higher than it might be in real life. When MUTATION is set to 0, moth phenotypes follow Mendelian probabilities of the alleles inherited from their parents. When it is set to 100, there is no correlation between a parent's alleles and the alleles of its children. (Starting out you should set MUTATION between 10 and 15, but experiment with the rate and watch what happens. **When asked, you should use a mutation level of 15**.)

The SELECTION slider determines how moths are harvested by the birds that feed on them. SELECTION incorporates many factors that determine the survivability of a species - how many birds there are, how hungry they are, and just how important camouflage is to escaping predation. SELECTION provides a probabilistic window - the lower the level of the slider, the wider this window. At 0, a moth's color ceases to matter = no selection. At 100, a moth needs to be perfectly camouflaged to avoid being seen (and thus devoured). **When asked, you should run the model with SELECTION set to at 25.**

The POLLUTE and CLEAN UP buttons, along with the CYCLE-POLLUTION? switch, control the pollution levels in the environment. To watch the cycle described above - from clean environment to industrial revolution to

pollution control - set CYCLE-POLLUTION? to on. To directly manipulate the pollution levels in the environment, set CYCLE-POLLUTION? to off, and use the POLLUTE and CLEAN UP buttons to add and remove pollution from the environment.

The SPEED slider controls just how rapidly pollution levels change. As you might guess, 1 is slow, and 100 is fast. **A good speed to start with is 10**.

Finally, there are six monitors, all of which are straightforward. TICKS reports how much time has elapsed. TOTAL MOTHS displays how many moths are present in the world. LIGHT MOTHS, MEDIUM MOTHS, and DARK MOTHS report the total numbers of moths with each color gradation = **phenotypes**. The moth population is initially divided into roughly ½ homozygotes (¼ light and ¼ dark) and ½ heterozygotes (dark moths). POLLUTION reports the pollution level in the environment on a scale from 0% (no pollution) to 100% (maximum pollution).

THINGS TO NOTICE

The most important thing to watch is how the entire set of moths seems to change color over time. Let the model run by itself the first time - watch the world change from white to black back to white. Then see how manipulating the sliders affects the populations of moths.

Notice that during the first few initial time-steps, the moth population booms. You might then see the moth population fluctuate between different levels, some of which are quite large. The moths give birth to many offspring, but the world in which they live is finite --- it has finite space and resources. If the population exceeds the available resources (carrying capacity), the moths tend to die a lot faster than they would otherwise. Under normal circumstances, the average population will tend to stay constant, at a level dependent on the speed and selection rates.

Watch what happens when a drastic change in the environment occurs. (You can force this with the POLLUTE-WORLD and CLEAN-UP-WORLD buttons.) Can you kill off all of the moths in a matter of a few time-steps?

You can watch the ratios between the types of moths change either in the monitors, or graphically in the plot. The yellow line represents the lighter-colored moths (recessive homozygotes), the green line represents the intermediate moths (heterozygotes), and the blue line represents the darker-colored moths (dominant homozygotes).

THINGS TO TRY

How do different levels of mutation and selection change the population? How does the speed of the model affect the rate at which the moths change? Is there a speed at which the moths can't keep up, i.e. the world changes faster than small pockets of discolored moths or mutants can help keep the population up to size? The upper-bound for the moth population is defined as a global variable, upper-bound. It is initially set to 4 * the moth population, but you can change it and watch what happens.

NETLOGO FEATURES

Note that all the commands given to the moths are in a block of code that begins ask moths. This is because each moth is given a breed, moths. This makes the code far easier to modify, especially if you want to add a different kind of animal, say, the birds that eat the moths. You would then add a new breed, birds, and put all code that birds are to execute in the body of ask birds.

CREDITS AND REFERENCES

The peppered moths of Manchester, England as a case study in natural selection were originally studied by British scientist H. B. D. Kettlewell.

In 1998, Michael Majerus of the University of Cambridge re-examined Kettlewell's work and found that though his experimental design was questionable in some respects, his conclusions were likely correct nonetheless. In any case, the mechanism of natural selection illustrated by this model is not in doubt.

Running the Model:

For this next component, you need to go directly to http://ccl.northwestern.edu/netlogo/models/community/Peppered%20Moths%20sexual and click "Try It in NetLogo Web". We also recommend reading the model information above. The changes we have made are summarized following:

In our modified model, there are male and female moths, and they have 2 gene alleles that will generate offspring according to Punnett square frequencies. Note that in the visual of the model, heterozygous individuals will appear dark as this would be their phenotype in nature (i.e. the *typica* gene (*c*) is recessive to the *carbonaria* gene (*C*)), however, you can see that they are still reported as medium moths (*Cc*) in the yellow boxes and on the graph. Moths will mate with opposite sex individuals that are near to them - so some runs of the model will result in moth extinctions very quickly as they did not find a mate soon enough. For our learning purposes you can ignore

these runs, but you may want to think about proximity of mates and how that could influence evolution of populations. To increase your chances of moths finding mates, you should set the number of moths (num-moths) to at least 150. You will need to set the other bars as recommended in the title of each table, and you can adjust the speed of the model as you like.

Table 2. Scenario 1 - Model outcome selection = 0, mutation = 0, on default light background.

Run number:	Time:	# light moths (*cc*):	# medium moths (*Cc*):	# dark moths (*CC*):	Total moths:	p = *C* allele frequency:	q = *c* allele frequency:
1	0						
	100						
2	0						
	100						
3	0						
	100						
4	0						
	100						
5	0						
	100						
	Sum of runs 1-5 for time 0						
	Hardy-Weinberg predicted initial (summed data):						
	Sum of runs 1-5 for time 100						

	Hardy-Weinberg predicted final:						

Data Analysis Scenario 1
For full marks, you should show all your work.
Time 0:
Allele Frequencies for summed data at time 0 are p = _____ and q = _____. Use four significant figures.

Chi-square (χ^2) results: _____ d.f. = 2; critical value (α=0.05) = 5.991

Significant statistical result? ☐ Yes   ☐ No

Is the population in Hardy-Weinberg at time 0? I.e.do the predicted genotypes from the allele frequencies at time 0 in the Hardy-Weinberg equilibrium differ from the initial observed genotypes recorded?

Time 100:

Allele Frequencies for summed data at time 100 are p =_____ and q = _____. (= p_f and q_f)

Chi-square (χ^2) results: _____ d.f. = 2; critical value (α=0.05) = 5.991

Significant statistical result? ☐ Yes   ☐ No

Is the population in Hardy-Weinberg after 100 generations? I.e.do the predicted genotypes from the allele frequencies at time 100 in the Hardy-Weinberg equilibrium differ from the final observed genotypes recorded?

Overall:
Evolution is the change in allele frequencies over time. To evaluate this, we will compare the frequency of *C* alleles (i.e., p) in the original population to the final population and see if that has changed. Therefore, p from each simulation at time 0 to time 100 will be used as a replicate. As we have an initial and a final frequency that are linked through time, we will be using a paired t-test. This can be done in Excel or using the calculator found at https://www.socscistatistics.com/tests/ttestdependent/default2.aspx.

Table 3. Initial and final p frequencies Scenario 1

Run #	Time 0 p (initial)	Time 100 p (final)
1		
2		
3		
4		
5		

Paired t-test results: _____ d.f. = 4; critical value (α=0.05) = 2.776

Significant statistical result? ☐ Yes   ☐ No

Conclusion: Do you see evidence (i.e. statistical support) for evolution in the scenario here?

Table 4. Scenario 2 - Model outcome selection = 25, mutation = 15, on default light background.

Run number:	Time:	# light moths (*cc*):	# medium moths (*Cc*):	# dark moths (*CC*):	Total moths:	p = *C* allele frequency:	q = *c* allele frequency:
1	0						
	100						
2	0						
	100						
3	0						
	100						
4	0						
	100						
5	0						
	100						
	Sum of runs 1-5 for time 0						
	Hardy-Weinberg predicted initial (summed data):						
	Sum of runs 1-5 for time 100						
	Hardy-Weinberg predicted final:						

Data Analysis Scenario 2
For full marks, you should show all your work.
Time 0:
Allele Frequencies for summed data at time 0 are p = _____and q = _____. Use four significant figures.

Chi-square (χ^2) results: _____ d.f. = 2; critical value (α=0.05) = 5.991

Significant statistical result? ☐ Yes   ☐ No

Is the population in Hardy-Weinberg at time 0? I.e.do the predicted genotypes from the allele frequencies at time 0 in the Hardy-Weinberg equilibrium differ from the initial observed genotypes recorded?

Time 100:
Allele Frequencies for summed data at time 100 are p =_____ and q = _____.

Chi-square (χ^2) results: _____ d.f. = 2; critical value (α=0.05) = 5.991

Significant statistical result? ☐ Yes   ☐ No

Is the population in Hardy-Weinberg after 100 generations? I.e. do the predicted genotypes from the allele frequencies at time 100 in the Hardy-Weinberg equilibrium differ from the final observed genotypes recorded?

Overall:
Evolution is the change in allele frequencies over time. To evaluate this, we will compare the frequency of *C* alleles (i.e., p) in the original population to the final population and see if that has changed. Therefore, p from each simulation at time 0 to time 100 will be used as a replicate. As we have an initial and a final frequency that are linked through time, we will be using a paired t-test. This can be done in Excel or using the calculator found at https://www.socscistatistics.com/tests/ttestdependent/default2.aspx.

Table 5. Initial and final p frequencies Scenario 2

Run #	Time 0 p (initial)	Time 100 p (final)
1		
2		
3		
4		
5		

Paired t-test results: _____ d.f. = 4; critical value (α=0.05) = 2.776

Significant statistical result? ☐ Yes   ☐ No

Conclusion: Do you see evidence (i.e. statistical support) for evolution in the scenario here?

Data Analysis Scenario 3
Run the model with selection = 25, mutation = 15, and click cycle pollution. Run this scenario 5 times, making notes about the relative numbers of the light, dark, and heterozygote (medium) phenotypes.
        What phenotype was favored in this scenario? Were any phenotypes lost?

Discussion

        Did you observe evolution in any of the NetLogo scenarios using the modeled populations of peppered moths? You should structure your answer as a 150-250 word paragraph using claim evidence reasoning and the Hardy-Weinberg principle to support your claim. To receive full credit for your answer, you should discuss the processes from the background information that are at work in at least Table 2 and 3 scenarios as reasoning and justification.

Background on scientific argument and writing:

Scientific argument and writing require the use of claim evidence reasoning - or creating a logical argument from a statement you have made (like evolution occurred in the populations of anoles observed on different islands) (Sampson and Phelps Walker 2012). You then provide the evidence (e.g., changes in phenotypic characters observed), and the reasoning behind your claim (e.g., evolution is the change in allele frequencies; the frequency of alleles changed, changing the genotypes and phenotypes, therefore evolution was occurring). Where most people struggle is the reasoning which requires the use of the background theory and evidence. In most cases, creating a good scientific argument requires looking at the evidence from the original study and applying to your claim (ideally from data reported in peer reviewed journals).

The background theory and evidence for this lab may be summarized by your lab instructors (lab background), or come from posted materials online, the lab manual, or from your textbook. In these labs, you should cite the author that wrote what you read (not the authors they got their information from)

Some Background video links that may help:

Null hypothesis and statistical testing overview:
https://www.youtube.com/watch?v=YSwmpAmLV2s
Hardy-Weinberg equilibrium:
https://www.youtube.com/watch?v=7S4WMwesMts
Peppered moth background:
https://www.youtube.com/watch?v=q64iUBPC8Mo
Solving Hardy-Weinberg equations:
https://www.youtube.com/watch?v=xPkOAnK20kw
Claim Evidence Reasoning:
https://www.youtube.com/watch?v=5KKsLuRPsvU

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Materials

A computer with Internet access to two freely available websites:

Peppered moths: Natural selection in action (https://askabiologist.asu.edu/peppered-moths-game/) and NetLogo Web (Wilensky 1999) with the Peppered Moths sexual model (http://www.netlogoweb.org/launch#http://ccl.northwestern.edu/netlogo/community/Peppered%20Moths%20sexual.nlogo). There is another Peppered moth model that does not have sexual reproduction or Punnett square offspring.

Notes for the Instructor

We felt this was an understandable, easy to use model that was relatively engaging for students. Starting with the Ask a Biologist simulation allows students to experience a fun, game style simulation of natural selection by birds on the moths. As this model is relatively simple, students are not able to conclude whether evolution would be occurring in a quantifiable way. However, they are able to intuitively grasp that it should occur over time. The NetLogo model allows the simulation to occur over several generations and students can collect data for calculations. The modifications we made to the original moth program allow a more realistic simulation of the population dynamics and illustrate the effects of selection relative to no selection. Note that you could run more trials and tease apart the effects of mutation compared to selection, but we focused on the two together.

We did use a common rubric so that there was consistency in the evaluation of the paragraph assigned and guidance to students of our expectations (Appendix A). This rubric equally emphasizes biological concepts and scientific communication but could be adapted for other goals.

Setup notes:

We the link of the model to our LMS (Learning Management System) for students to run in NetLogoWeb (Wilensky 1999). We also do a video introduction to the program and walk through the calculations required.

Analysis notes:

We have set this up to pool the data from five runs of the program to look for Hardy-Weinberg equilibrium at time 0 and time 100. We then ask students see if the frequency of the dominant allele for color (p) has changed significantly from time 0 to 100 using each run of the model as a replicate. We do ask that they communicate their findings in a final paragraph that discusses evolution without perpetrating common misconceptions and uses statistical evidence as justification for their claims. Students are evaluated on their writing using a rubric that is used throughout the lab activities (see Appendix A). To assist the checking of student numbers, we set up an Excel file that is shared with Laboratory Instructors that will automatically calculate results for the assignment.

We did struggle with the analysis of the data, as taking multiple runs of the simulation leads to more complex statistics than is desirable for an introductory class. We are comfortable with having students assess the sums of the runs done in each scenario, however, it may work for others to do a more qualitative look at the data or only look at one or two runs. For the evaluation of evolution occurring over time, we do think that the paired t-test is the simplest and most applicable analysis, but others may feel that a non-parametric paired comparison would be better.

We are especially delighted when students see that the populations at time 0 and 100 are in Hardy-Weinberg equilibrium, but that evolution has occurred according to the paired t-test. This is a nice intellectual puzzle and they can be led to understand that the equilibrium is not necessarily 0.5 p and 0.5 q but can be at many different values that satisfy the equation and shifts under different mechanisms. It really does have them understand at a deeper level and has become a good quiz question (see Appendix B, Table 3 answer key).

This lab could also be increased in rigor if more scenarios were run by students. We felt that after two tables that students were capable of extrapolating to other scenarios and that this workload was appropriate for our situation. We did find that marking the data analysis was time intensive, and are working towards an online, self-marking quiz that would ask students to do the same calculations and enter an answer to see if they have mastered the concepts.

Student Evaluations:

305 students were surveyed about the activities that they experienced during the fall 2020 term on their final quiz (an assessment worth 5% of their overall course grade). Students received a 0.5 bonus mark for completing the questions. Out of 6 activities, this activity was ranked #2 when students were asked "Which of the activities done this term did you learn the most from? List the activities below in

order of most helpful to not so helpful" (n=302). Students were also asked what their favorite activity was, and this ranked #4 out of 6 (activities that ranked 1, 2, and 3 were in-person, on campus activities). When directly asked "The best online learning occurred during which activity?", this activity was identified as the best by 1/3 of the students (103 choose this activity, compared to 95 and 102 for the other activities). The other online activities were an introduction to phylogenetics by NOVA labs, PBS (Evolution Lab https://www.pbs.org/wgbh/nova/labs/lab/evolution/) and the HHMI Lizard Evolution Virtual Lab (https://www.biointeractive.org/classroom-resources/lizard-evolution-virtual-lab).

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Acknowledgments

About the Authors

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Appendix A

Rubric for written answer:

Criteria	Excellent/Exceptional 2.5 Points	Good/Very Good 2 Points	Adequate/Good 1.5 Points	Marginal 1 Points	Inadequate 0.5 Points
Biological Content	An accurate and relevant description of biological concepts and information based on correct interpretation of scientific evidence from the lab manual.	Relevant biological concepts and information are described. The information was generally correct, although may not always be accurate or go beyond application level of critical thinking.	The information provided may be incorrect in minor ways relative to the main points. Descriptions may be superficial or lack complexity. There may be reference to unrelated/incorrect information.	The biological concepts and information provided may be incorrect, inaccurate or extremely superficial.	Very little use was made of biological information.
Communication Effectiveness	A scientific argument/rationale is effectively demonstrated showing understanding of information relevant to the question. Writing was appropriate to scientific writing: i.e. clear, effective and added to the delivery of the argument. There were very few, or no, grammatical or spelling errors with correct punctuation, word usage, and verb tense. Scientific names of organisms followed conventions.	Writing is clear and concise. Overall, the question follows conventions of a good scientific argument and has relatively few errors. There were minor grammatical, spelling, sentence structure, or scientific convention errors but they did not significantly detract from the quality of the answer.	Minor elements of answer may be lacking but overall, the question is complete and main conclusions are communicated. The word count was exceeded by a significant amount (50-100 words). Writing conveyed the basic message but did detract somewhat from the delivery of the answer or main points. There were errors in grammar, word use, spelling, punctuation, or scientific convention.	The response is generally lacking. An answer is provided but is not adequately presented or discussed. Little or no scientific rationale could be identified. Writing interfered with the communication of the answer due to errors in grammar, word use, spelling, punctuation, or scientific convention. Informal expressions may be present.	The answer may not have addressed the question or is just wrong. It could be that the reader could not understand the answer as written in places due to errors in grammar, word use, spelling, punctuation, or scientific convention.

Appendix B

Sample data/ answer key to analysis:

Table 1. Moth population results

Tree Background:	# of moths eaten	Light Moth Population %	Dark Moth Population %
Light	32	83	17
Dark	38	8	92

- Did the dark or light phenotype ever completely disappear from the population? What implications does this have for the persistence of the less favorable genotype? (2 marks)

  Usually, they do not get all of one color; the less favorable genotype would persist in the population for a while as it takes time for natural selection to act. If the less favorable genotype can remain in the population, then if the environment changes, and the genotype persists, the population can adapt towards the now more favorable genotype.

- Could you determine if either of the simulated populations was experiencing evolution? Why or why not? Justify your answer by discussing the definition of evolution. (3 marks)

  No, because evolution takes place over a longer period of time, across generations, leading to a large and stable shift in genotypic frequencies. Great student answer: This shows natural selection, not evolution, as evolution requires time/generations while natural selection is the differential survival of some individuals. Overall, I would say no because we don't see the changeover generations but part marks for a good argument.

Table 2. Scenario 1 - Model outcome selection = 0, mutation = 0, on default light background.

Run number:	Time:	# light moths (cc):	# medium moths (Cc):	# dark moths (CC):	Total moths:	p = C allele frequency:	q = c allele frequency:
1	0	40	75	35	150	0.4833	0.5167
	100	111	266	253	630	0.6127	0.3873
2	0	42	70	38	150	0.4867	0.5133
	100	67	275	257	599	0.6586	0.3414
3	0	37	76	37	150	0.5	0.5
	100	369	183	89	641	0.2816	0.7184

4	0	44	74	32	150	0.46	0.54
	100	240	307	73	620	0.3653	0.6347
5	0	35	71	44	150	0.53	0.47
	100	22	265	333	620	0.7508	0.2492
	Sum of runs 1-5 for time 0	198	366	186	750		
	Hardy-Weinberg predicted initial (summed data):	193.5	374.9	181.6			
	Sum of runs 1-5 for time 100	809	1296	1005	3110		
	Hardy-Weinberg predicted final:	682.6	1548.8	878.6			

Data Analysis Scenario 1:
All analysis below is based on Table 2 - For full marks, you should show all your work.
Time 0:

Should do chi square for p^2, $2pq$ and q^2 between sum of the runs compared to predicted to HW and should yes, it is in HW (no statistical significance) –the simulation set up to generate numbers that conform to Hardy-Weinberg for the initial population.

Allele Frequencies for summed data at time 0 are p = _0.492_ and q = _0.508__. (= p_i and q_i) Use four significant figures as rounding affects things, and use the percent of average # of moths.

Chi-square (χ^2) results:__0.423__ d.f. = 2; critical value (α=0.05) = 5.991
Significant statistical result? ☐ Yes   ☐ **No   Should be no – computer generates the numbers that way initially**

Is the population in Hardy-Weinberg initially? I.e.do the predicted phenotypes from the allele frequencies at time 0 in the Hardy-Weinberg equilibrium differ from the initial observed phenotypes recorded?
Yes, there is no reason to reject H-W, they do not differ.

Time 100:

Allele Frequencies for summed data at time 100 are p =__0.5315_ and q = _0.4685__. (= p_f and q_f)

Chi-square (χ^2) results:_82.9__ d.f. = 2; critical value (α=0.05) = 5.991
Significant statistical result? ☐ **Yes**   ☐ No

Is the population in Hardy-Weinberg after 100 generations? I.e.do the predicted phenotypes from the allele frequencies at time 100 in the Hardy-Weinberg equilibrium differ from the final observed phenotypes recorded? **Could go either way here, depends on the numbers.**

Overall:

Evolution is the change in allele frequencies over time. To evaluate this, we will compare the frequency of *C* alleles (i.e. p) in the original population to the final population and see if that has changed. Therefore, p from each simulation at time 0 to time 100 will be used as a replicate. As we have an initial and a final frequency that are linked through time, we will be using a paired t-test. This can be done in Excel or using the calculator found at https://www.socscistatistics.com/tests/ttestdependent/default2.aspx.

Table 5. Initial and final p frequencies Scenario 2

Run #	Time 0 p (initial)	Time 100 p (final)
1	0.4833	0.6127
2	0.4867	0.6586
3	0.5	0.2816
4	0.46	0.3653
5	0.53	0.7508

Paired t-test results: __-0.4944__ d.f. = 4; critical value (α=0.05) = 2.776

Significant statistical result? ☐ Yes   ☐ **No**

Conclusion: Do you see evidence (i.e., statistical support) for evolution in the scenario here?

It may or may not be at the end, so students should think about the possible violations of Hardy-Weinberg that could have occurred. Generally, we are attributing it to genetic drift if it is significantly different, as natural selection and mutation are turned off and this is a relatively small population. If a student thinks to question the random mating that would be a fair answer as mating in this model is based on proximity.

Table 3. Scenario 2 - Model outcome selection = 25, mutation = 15, on default light background.

Run number:	Time:	# light moths (*cc*):	# medium moths (*Cc*):	# dark moths (*CC*):	Total moths:	p = *C* allele frequency:	q = *c* allele frequency:
1	0	37	70	43	150	0.52	0.48
	100	457	128	11	596	0.1256	0.8742
2	0	29	84	37	150	0.5267	0.4733
	100	454	140	0	594	0.1178	0.8822
3	0	37	82	31	150	0.48	0.52
	100	506	102	0	608	0.0839	0.9161
4	0	37	75	38	150	0.5033	0.4967
	100	553	108	5	666	0.0886	0.9114

	5	0	39	74	37	150	0.4933	0.5067
		100	484	111	11	606	0.1097	0.8903
		Sum of runs 1-5 for time 0	179	385	186	750		
		Hardy-Weinberg predicted initial (summed data):	184.0	374.0	191.0			
		Sum of runs 1-5 for time 100	2454	589	27	3070		
		Hardy-Weinberg predicted final:	2460.7	575.6	33.7			

Data Analysis Scenario 2:
All analysis below is based on Table 3 - For full marks, you should show all your work.
Time 0:

Allele Frequencies for summed data at time 0 are p = _0.5047__and q = __0.4953__. (= p_i and q_i) Use four significant figures as rounding affects things.

Should do chi square for p^2, $2pq$ and q^2 between sum of the runs compared to predicted to HW and should yes, it is in HW (no statistical significance) –the simulation set up to generate numbers that conform to Hardy-Weinberg for the initial population.

Chi-square (χ^2) results: __0.537__ d.f. = 2; critical value (α=0.05) = 5.991
Significant statistical result? ☐ Yes   ☐ **No**

Is the population in Hardy-Weinberg initially? I.e.do the predicted phenotypes from the allele frequencies at time 0 in the Hardy-Weinberg equilibrium differ from the initial observed phenotypes recorded?

Yes, there is no reason to reject H-W, they do not differ.

Time 100:
Allele Frequencies for summed data at time 100 are p =___0.1047__ and q = _0.8953_. (= p_f and q_f)

Chi-square (χ^2) results: _1.648__ d.f. = 2; critical value (α=0.05) = 5.991
Significant statistical result? ☐ Yes   ☐ **No**
Is the population in Hardy-Weinberg after 100 generations? I.e.do the predicted phenotypes from the allele frequencies at time 100 in the Hardy-Weinberg equilibrium differ from the final observed phenotypes recorded?

Yes, in this case there is no reason to reject H-W, they do not differ. It would depend on the student data.

Overall:

Evolution is the change in allele frequencies over time. To evaluate this, we will compare the frequency of *C* alleles (i.e. p) in the original population to the final population and see if that has changed. Therefore, p from each simulation at time 0 to time 100 will be used as a replicate. As we have an initial and a final frequency that are linked through time, we will be using a paired t-test. This can be done in Excel or using the calculator found at https://www.socscistatistics.com/tests/ttestdependent/default2.aspx.

Table 5. Initial and final p frequencies Scenario 2

Run #	Time 0 p (initial)	Time 100 p (final)
1	0.54	0.1354
2	0.5243	0.1072
3	0.4938	0.1107
4	0.4861	0.0798
5	0.4947	0.0816

Paired t-test results: __68.7__ d.f. = 4; critical value (α=0.05) = 2.776
Significant statistical result? ☐ **Yes**   ☐ No
Conclusion: Do you see evidence (i.e. statistical support) for evolution in the scenario here?
Yes, it should be significant unless they messed up the simulation parameters of the analysis.

Data Analysis Scenario 3
Run the model with selection = 25, mutation = 15, and click cycle pollution. Run this scenario 5 times, making notes about the relative numbers of the light, dark, and heterozygote (medium) phenotypes.
        What phenotype was favored in this scenario? Were any phenotypes lost?
Should see that the medium phenotype becomes favored and that the light can disappear more often as recessive. The graph, visual image and genotype frequencies all indicate that the heterozygous genotype remains the majority regardless of where the pollution cycle is at.

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