THE VIRTUAL EVOLUTION STICKLEBACK LAB

OVERVIEW

This worksheet was developed to support use of The Virtual Stickleback Evolution Lab in the classroom. The lab emphasizes quantitative measurement of phenotypic diversity in related stickleback populations and encourages inquiry into the role of natural selection and underlying genetic mechanisms. Before doing the virtual lab, students would benefit from watching the HHMI short film *The Making of the Fittest: Evolving Switches, Evolving Bodies*, available at http://www.hhmi.org/biointeractive/making-fittest-evolving-switches-evolving-bodies.

The worksheet is meant to provide students with structure and guidance as they perform the tutorials, experiments, and quizzes in the lab. There are two versions of the worksheet: an advanced version and a basic version. The only difference between them is that the basic worksheet does not contain the chi-square analyses portions of the lab. Each worksheet can be further modified by crossing out certain sections that students will not be performing. (For example, some students may not carry out one of the experiments in the lab.)

KEY CONCEPTS AND LEARNING OBJECTIVES

- Natural selection can drive the evolution not just of simple traits like coat color or body size, but also of complex traits like the size and shape of skeletons.
- Different environments—with different predators, food sources, or resource limitations—apply different selective pressures on the shape of animal bodies.
- Important evolutionary insights can be gained by comparing fossils of different ages and locations; living populations under different selective pressures; and fossils to living populations.
- Careful quantitative analysis of the traits of living populations can tell us about mechanisms of selection, including genetic mechanisms, whereas quantitative analysis of populations of fossilized specimens can provide a record of change over time and even the pace of specific adaptive changes.
- Statistical analysis is essential for gauging confidence in conclusions drawn from population data, which can include natural variation, populations in transition, and measurement error by the researcher.

After completing the lab, students will be able to:

- Assess phenotypic diversity among stickleback populations, focusing on the structure of the pelvis.
- Formulate hypotheses about how different habitats, each with potentially different predators, food sources, or resource limitations, apply distinct selective pressures on the anatomy of animal bodies.
- Examine fossilized fish specimens to identify skeletal structures.
- Use statistical analysis to gauge confidence in conclusions drawn from population data.
- Determine the rate of change of a trait over time based on data from the fossil record.

KEY TERMS

adaptation, evolution, fossil, natural selection, stickleback, variation

SUGGESTED AUDIENCE

The Virtual Stickleback Evolution Lab is appropriate for high school biology as an excellent companion to an evolution unit. Because the trait under study is fish pelvic morphology, the lab can also be used for lessons on vertebrate form and function. In an ecology unit, the lab could be used to illustrate predator-prey relationships and environmental selection pressures. The sections on graphing, data analysis, and statistical significance make the lab a good fit for addressing the "science as a process" or "nature of science" aspects of the curriculum.

HHMI
HOWARD HUGHES MEDICAL INSTITUTE

The Virtual Stickleback Evolution Lab

www.BioInteractive.org

Published October 2012
Updated September 2013
Page 1 of 11

PRIOR KNOWLEDGE

Students should know that some traits provide organisms with a greater chance to survive and reproduce. Inherited traits that are advantageous in a particular environment are more likely to be passed on from generation to generation.

TEACHING TIPS

- As they proceed through the virtual lab, students should complete specific sections of the worksheet, as assigned by their teachers. Teachers may choose to grade the worksheets or just use them to gauge students' understanding of the key concepts presented in the lab.
- The lab was designed to be modular; students may complete one, two, or all three experiments, each in its entirety or just portions of it. Each experiment includes a data-gathering section, a graphing section, a statistical analysis section, and a quiz. The lab also contains an overall quiz that tests knowledge acquired by working through all sections of the lab.
- The entire lab and worksheet take approximately 2.5 to 3.5 hours to complete, depending on the student. Teachers have a great deal of flexibility as to which portions of the lab and worksheet they will use and whether students should do the assigned sections at home or in class, working individually or in groups. Based on student evaluation data we have seen, they generally enjoy working in pairs or small groups, and they complete the lab more quickly when doing so.
- A link on the Introduction page of the virtual lab directs teachers to detailed information about how to implement the lab in the classroom, including tips from teachers who have already used the lab, and has links to various curricula.
- The lab can be used by students in different class periods. On the same computer, the lab can track the progress of 8 different students. . For directions on how to implement the multi-user mode, refer to the Teacher Resources section on the introduction page or the information under the Help tab.

ANSWER KEY

The answers below include more detail than would be provided by most students. They are meant to give teachers additional information that they may want to discuss with their students.

INTRODUCTION

1. *Define "model organism."* **Model organisms are nonhuman species that are extensively studied to understand certain biological phenomena.**

2. *How do spines protect ocean stickleback fish?* **Most stickleback fish living in the ocean sport heavy armor in the form of bony plates and spines projecting from the back and pelvis. When threatened, a stickleback can simultaneously flare out its pair of pelvic spines and three dorsal spines, making it difficult for predators to swallow them.**

3. *How did ancestral populations of ocean-dwelling fish come to live in freshwater lakes?* **At the end of the last ice age, new freshwater lakes formed as glaciers melted. These lakes provided new breeding grounds for stickleback swimming to these lakes from the ocean. As more and more ice melted, the land that had been depressed by the weight of the massive ice sheets became elevated, and the connections between some of the lakes and the ocean were cut off. Researchers think that stickleback populations became trapped in these lakes and adapted to living exclusively in fresh water.**

4. ***Watch the video about pelvic reduction in freshwater stickleback.*** *The loss of stickleback pelvic spines is similar to the loss of which body parts in some other four-legged vertebrates?* **Hind limbs.**

5. *From a researcher's perspective, what is the benefit of having access to hundreds of postglacial lakes?* **Because there are hundreds of postglacial lakes in the Northern Hemisphere, researchers can compare a trait like pelvic reduction in many different stickleback populations living in these lakes. The great number of lakes allows researchers to make predictions about which environmental conditions influence the trait.**

6. *Watch the video with evolutionary biologist Dr. Michael Bell. Why is the threespine stickleback a model organism for studies in evolution? (List at least two reasons.)* **Stickleback fish are small and have short generation times. These two characteristics make them easy to keep in a lab and useful for conducting genetic studies, since researchers can follow several generations of fish in a relatively short time. Also, stickleback fish populations occur throughout the Northern Hemisphere in a wide range of environments, so researchers can compare different populations and study how they have changed over time in response to different environmental pressures. Another reason that stickleback are useful model organisms for studies in evolution is that there are many stickleback fossils, so researchers can compare living populations to their ancestors.**

7. *Watch the video with Dr. David Kingsley explaining hind-limb reduction. Name two other vertebrate animals whose evolutionary histories included the loss of hind limbs.* **Hind-limb reduction has occurred in several vertebrate lineages, including manatees, snakes, and whales.**

OVERVIEW

1. *Click on the interactive stickleback fish. Describe where its spines are located.* **The threespine stickleback has three spines on its dorsal side and two spines projecting from the pelvis on its ventral side. The spines are modified fins.**

2. *What is the difference between marine, sea-run, and freshwater stickleback fish populations? Be specific.* **Marine stickleback populations live and breed strictly in the ocean. Sea-run stickleback fish are anadromous, meaning that they are born in fresh water, spend most of their lives in the ocean, and return to fresh water to breed. Freshwater stickleback fish live entirely in fresh water. (Students may also mention that marine and sea-run stickleback have heavy armor, whereas freshwater stickleback populations have reduced armor.)**

3. *Watch the video about the stickleback armor.*
    a. *In addition to the spines, what is another component of the "armor" of a stickleback fish?* **The row of plates on each side of the body.**

    b. *Explain how the stickleback armor protects the fish from some predators.* **The back spines stick up and the pelvic spine out at almost a perpendicular angle, making it hard for a predatory fish that catches a stickleback to swallow it. The plates also make stickleback fish difficult to eat. (Students may also mention that in lakes with different types of predators, such as dragonfly larvae, the pelvic spines actually make stickleback easier to catch.)**

TUTORIAL 1

1. *Describe the following structures:*
    a. *Complete pelvis:* **a pelvis with a full pelvic girdle with two pelvic spines**.
    b. *Reduced pelvis:* **a range of structures from a simplified girdle with no pelvic spines to an oval nub for a girdle**.
    c. *Absent pelvis:* **no pelvic girdle <u>and</u> no pelvic spines.**

2. ***Start Tutorial 1.*** *Continue to practice scoring fish until you have mastered the technique; then proceed to Experiment 1.*

EXPERIMENT 1

1. *Explain in your own words the overall objective of Experiment 1.*
   The objective of Experiment 1 is to analyze the pelvic structures of stickleback fish collected from two lakes around Cook Inlet, Alaska. The data collected will be used along with information about the lakes to suggest possible environmental factors that influence pelvic morphology.

2. ***Click on the link to the map of Alaska, then click on the blue pin "A" on the larger map.***
   Which lake is located between Bear Paw Lake and Frog Lake just to the north? **Prator Lake**

3. *In a population, what happens to organisms that are better adapted to the environment in which they live?*
   At the population level, individuals that are better adapted for a particular environment are more likely to live longer and reproduce, and pass their genetic variants to the next generation. Over many generations, these genetic variants and the associated traits increase in frequency in the population.

4. ***In the virtual lab window, complete Part 1: Staining the Fish.***
   a. *Why do you think it is important to empty the used stain, destaining solution, and water under a fume hood?* **The hood has a built-in vacuum, which helps limit a scientist's exposure to potentially toxic or hazardous fumes from the different chemicals used in the staining procedure.**

   b. *How were the fish you will be using in this virtual lab caught?* **To catch the fish, scientists throw special traps in the water, which they leave overnight. The traps are tube-shaped with funnel openings at each end. As they are swimming, some fish enter the traps, and don't always manage to swim back out. Researchers simply pull the traps out of the water to collect the live fish.**

5. ***In the virtual lab window, proceed to Part 2: Scoring the Fish.***
6. ***Before scoring the fish, watch the short video on Bear Paw and Frog Lakes.***
   According to Dr. Bell, what is an important difference between Bear Paw Lake and Frog Lake? **The main difference is that Bear Paw Lake is completely enclosed and does not drain at the surface. This means that predatory fish cannot readily enter the lake. On the other hand, Frog Lake is connected to the water system by a small stream, which allows predatory fish to gain access to the lake.**

7. ***In the window on the right, click on the link to read more about random sampling.***
   Why are random samples, rather than entire populations, used in most research studies? **In most cases, it would take too much time and money to study all individuals in a population. As a result, scientists study randomly collected subsets of individuals from a population, or random samples. Scientists study random samples to estimate properties of the population and infer conclusions about the population as a whole.**

8. *What is one advantage of studying larger-sized samples?* **Larger samples are usually more representative of the population as a whole than are smaller samples.**

9. *Give an example of sampling bias.* **Answers will vary. One example of sampling bias would be if researchers collected fish only in shallow water because it was easier to place traps there; as a result, they might miss individuals living in deeper water, which could have different traits because, for example, deep-water predators are different from those in shallow water. Another example would be that by using traps to catch fish, researchers might catch only the most daring and inquisitive individuals in a population; this might result in a sampling bias if researchers are studying traits associated with behavior.**

10. ***Complete Part 2 of the lab in the window on the left.***

11. *Why is it important that the labels included in specimen jars be made of special paper that does not disintegrate in alcohol over time?* **The labels record critical information about the specimens, such as the location (e.g., Bear Paw Lake) and date of collection. The labels are placed in the jars along with the specimens to make sure that they are not lost. Because fish are stored in 50% isopropyl alcohol, which disintegrates most types of paper, the labels have to be made of a special paper that does not disintegrate. If the label is lost or destroyed, scientists would not know what population they were studying, and the data they collected on this sample would be useless.**

12. *Examine the pelvic score data you just collected. Does the pelvic phenotype differ between Bear Paw Lake and Frog Lake fish? Explain.* **Yes, it appears that Bear Paw Lake contains mostly stickleback fish that lack pelvic spines, and Frog Lake contains stickleback fish that have pelvic spines.**

13. ***Complete the graphing exercise as instructed by your teacher. After graphing, verify your data.*** *How do your data compare to those obtained by Dr. Bell and colleagues?* **Answers will vary, but if students scored the fish accurately, their data should be similar to Dr. Bell's data.**

14. ***Complete the Experiment 1 Quiz. Take time to read the explanation for each correct choice provided after each question.***

15. *Explain why the stickleback fish in Frog Lake are more similar to ocean and sea-run stickleback than they are to the stickleback fish in Bear Paw Lake.* **Fish in marine and sea-run populations have complete pelvises; the pelvic spines protect stickleback fish from larger fish that prey on them in the ocean. Most stickleback fish in Bear Paw Lake have either a reduced or an absent pelvis, whereas those in Frog Lake have a complete pelvis. Thus, the stickleback population in Frog Lake is more similar to marine and sea-run stickleback populations.**

16. *In addition to predators, what other environmental factors might be responsible for the differences between Bear Paw Lake and Frog Lake stickleback populations? How would you test your prediction?*
    Answers will vary. For example, students could mention fresh water is not as rich in calcium and other minerals needed to form pelvic spines. Students could test this prediction by measuring the mineral content of fresh water and comparing it to that of ocean water. Another example would be that unlike the open ocean, freshwater lakes have more vegetation and more places where stickleback can hide, in which case having less armor may make stickleback more agile and better able to quickly dart into hiding places. Students may come up with other ideas.

17. ***After completing the quiz, click on Experiment 1 Analysis.***

18. *For this analysis, what is your null hypothesis?* **The null hypothesis is that a random sample of stickleback from a population would contain roughly the same number of individuals with and without pelvic spines.**

19. Complete the following tables as you perform the chi-square calculations in the lab.

Bear Paw Lake

Phenotype	Observed (o)	Expected (e)	(o-e)	(o-e)²	(o-e)²/e
Pelvic Spines Present	0	10	10	100	10
Pelvic Spines Absent	20	10	10	100	10
	Total = **20**	Total = **20**			χ^2 = **20**

Frog Lake

Phenotype	Observed (o)	Expected (e)	(o-e)	(o-e)²	(o-e)²/e
Pelvic Spines Present	0	10	10	100	10
Pelvic Spines Absent	20	10	10	100	10
	Total = **20**	Total = **20**			χ^2 = **20**

Morvoro Lake

Phenotype	Observed (o)	Expected (e)	(o-e)	(o-e)²	(o-e)²/e
Pelvic Spines Present	8	10	2	4	0.4
Pelvic Spines Absent	12	10	2	4	0.4
	Total = **20**	Total = **20**			χ^2 = **0.8**

20. For each chi-square calculation, how many degrees of freedom are there? **df=1**
    How did you arrive at this number? **df = Number of phenotypes – 1 = 2 – 1 = 1**

21. What are the p values for the data from each lake?
    a. Bear Paw Lake **0.01**
    b. Frog Lake   **0.01**
    c. Morvoro Lake **0.5**

22. Do you "reject" or "fail to reject" the null hypothesis for the data from each lake?
    a. Bear Paw Lake   **Reject**
    b. Frog Lake   **Reject**
    c. Morvoro Lake   **Fail to reject**

23. *Complete the Chi-Square Analysis Quiz. Take time to read the explanation for each correct choice provided after each question.*

24. *Explain what it means to reject the null hypothesis.* **If the null hypothesis can be rejected that means that any difference between the observed data (the data you collected) and the expected data distribution of 50:50 is unlikely to be due to chance.**

25. *Explain what it means if the null hypothesis cannot be rejected.* **If the null hypothesis cannot be rejected, the chi-square test was unable to detect a statistically significant difference between observed and expected data.**

26. *Explain the difference between the results of the chi-square calculations for Bear Paw Lake and Morvoro Lake.* **The null hypothesis was rejected for the Bear Paw Lake population. This test result, together with our data, tells us that, in this population, there are significantly more fish with pelvic reduction than fish with pelvic spines. Further studies can be conducted to determine if there are ecological or other explanations for this observation. In Morvoro Lake, we cannot reject the null hypothesis. This may or may not mean that the null hypothesis is true.**

27. *What do you think might be an explanation for why Morvoro Lake contains fish with and without pelvic spines?* **Answers will vary. It is possible that the population in Lake Morvoro is in the process of changing from a population of fish with mainly pelvic spines to one without pelvic spines, or vice versa. Perhaps there was a predatory fish in the lake when the stickleback population first colonized the lake, but that predator is no longer there (e.g., all the trout were caught).**

TUTORIAL 2

1. *What score would you assign to a fossil specimen that has only one pelvic spine visible?* **Complete.**

2. *A stickleback fossil may show no signs of pelvic structures. What are possible sources of error associated with scoring the pelvis of such a fossil as "absent"?* **Animals that are preserved as fossils may have been damaged by decay or by physical processes. As a result, certain body structures may not be visible in the fossil.**

3. *Start Tutorial 2. Continue scoring the fossil specimens until you are confident in your scoring abilities; then proceed to Experiment 2. (Most people find that it is more difficult to accurately score fossils than the preserved fish specimens. You may have to practice on 20 specimens or more before you feel you have mastered the technique.)*

EXPERIMENT 2

1. *In your own words describe the overall objective of Experiment 2 and explain what the data you collect will allow you to estimate.* **In this experiment, students will characterize the pelvic structures of fossil stickleback fish from the Truckee Formation in Nevada—the site of a lake that existed about 10 million years ago. The fossils represent fish that lived in the lake during a 15,000-year period. The data collected in this experiment will allow students to estimate the rate of change in the frequency of the complete pelvic phenotype over time for this population.**

2. *Watch the short video on the Truckee Formation in Nevada. What does each sedimentary rock layer of the Truckee Formation represent?* **Each rock layer presents about a year of deposition. When the Truckee Formation was a lake, diatoms (microscopic algae) died each year, and their skeletal remains sank to**

the bottom. There, they mixed with clay and silt, forming annual layers of thick sediments. Over time, the lake dried up and these sediments became sedimentary rock layers that are like growth rings in a tree. (Note that in this lab, students examine fossils from rock samples spaced about 3,000 years apart.)

3.   *What is one type of information that researchers can gain from studying fossils that they cannot obtain from living populations?* **Studying fossils can tell researchers about long-term patterns (i.e., on a time scale of thousands to millions of years) of evolutionary change and could allow researchers to calculate the rate of change for certain traits**.

4.   ***Begin the experiment in the window on the left. Complete Part 1: Preparing Fossils.*** *(You may skip this step by clicking on "Skip Part1: Fossil Prep.")*

5.   ***Proceed to Part 2: Scoring Fossils.*** *In this virtual lab, you will graph fossil data from six rock layers. Approximately how many years apart are any two adjacent samples?* **Each layer analyzed in this lab is spaced 3,000 years from the next. (Note that students only scored pelvic structures of fossils from layers 2 and 5. They were provided with the scores of fossils from other layers.)**

6.   *You will collect data on pelvic structures using fossils from rock layers 2 and 5. Approximately how many years of deposition separate these two layers?* **9,000 years.**

7.   *Which layer is older, 2 or 5? Explain your answer.* **The layers are numbered from oldest (1) on the bottom to youngest on the top (6); therefore, 2 is older than 5.**

8.   ***Complete Part 2 of the lab in the window on the left.***
9.   *Based on the pelvic phenotypes you measured, do the fossils in layer 2 differ from those in layer 5? Explain how.* **Yes, it appears that pelvic spines were more prevalent in fossil stickleback from rock layer 2 than from those in rock layer 5. Layer 5 contained mostly fish without pelvic spines**.

10. ***Complete the graphing exercise as instructed by your teacher.***
11. ***After graphing, verify your data***. *How do your data compare to those collected by Dr. Bell and colleagues?* **Answers will vary. If students scored the fossils correctly, their results should be similar to those obtained by Dr. Bell's lab**.

12. ***Complete the Experiment 2 Quiz. Take time to read the explanations for each correct choice provided after each question.***

13. *What can be inferred about the presence or absence of predatory fish when the Truckee Formation was a lake? Describe the evidence.* **Because the frequency of fossil stickleback with pelvic spines decreased over the 15,000-year time span, we can infer that pelvic spines did not provide an advantage to stickleback fish that had them. Because pelvic spines in living three-spined stickleback populations provide a defense against large predatory fish, we can infer that there probably were no predatory fish in the ancient lake. Dr. Bell and colleagues have found fossils of other fish in the quarry, but most of them were small species that could not eat stickleback fish.**

14. ***After completing the quiz, click on Experiment 2 Analysis.***

15. Complete the tables below as you perform the rate calculations. (*The link to the instructions is very helpful.*)

Sample Layer	Number of Fish with a Complete Pelvis	Total Number of Fish Sampled	Relative Frequency of Complete Pelvis Trait in Population Sampled
1	20	20	1.00
2	18	20	0.90
3	3	20	0.15
4	1	20	0.05
5	0	20	0.00
6	0	20	0.00

Time	Decrease in Percentage of Complete Pelvis Trait per Thousand Years (Rate of Change)
First 3,000 years (Layer 1 to Layer 2)	-0.033 = -3.3%
Next 3,000 years (Layer 2 to Layer 3)	-0.25 = -25%
Next 3,000 years (Layer 3 to Layer 4)	-0.033 = -3.3%
Next 3,000 years (Layer 4 to Layer 5)	-0.017 = -1.7%
Next 3,000 years (Layer 5 to Layer 6)	0.00
Total 15,000 years (Layer 1 to Layer 6)	-0.067 = -6.7%

16. *What does it mean when the rate of change is a negative number?* **A negative number indicates a decrease in the quantity being studied. In this case, there was a decrease in the relative frequency of fossil fish with pelvic spines over time**.

17. ***Complete the Analysis Quiz. Take time to read the explanation for each correct choice provided after each question.***

18. *Describe the trend in the data over time.* **The relative frequency of the presence of pelvic spines decreased over time. This decrease was rapid in the first 9,000 years and then slowly leveled off toward the end of the 15,000-year period, after which point no fish with a complete pelvis were found in the fossil record**.

19. *Why is it important to calculate the rate of change over time?* **Calculating rates of change in fossil fish can be used to infer some aspects of the evolution of traits in living fish. It is not usually possible to study the rates of change of traits in living populations because of the long time scale of most evolutionary processes**.

20. *In what way is the change in the complete pelvis phenotype in the fossils from the Nevada lakebed similar to what might have occurred in Bear Paw Lake from Experiment 1?* **The reduced pelvic phenotype has never been observed in marine and sea-run populations. Most stickleback fish in Bear Paw Lake have either a reduced or an absent pelvis, suggesting that the frequency of the pelvic spine phenotype in Bear Paw Lake decreased over time, just as the fossil data suggest happened in the ancient Nevada lake. We**

cannot study how the pelvic phenotype changed over time in Bear Paw Lake because this event happened before researchers discovered this population, but we can study this change in the Nevada lake because it has been recorded in the rock layers.

EXPERIMENT 3

1. *In your own words describe the overall objective of Experiment 3.* **In this experiment, students analyze the reduced pelvis trait in more detail by scoring stickleback fish from two lake populations. Students will focus on pelvic asymmetry: whether the reduced pelvis is consistently larger on one side (left or right) in a given population.**

2. *What is one function of the* Pitx1 *gene?* **Pitx1 is a major gene involved in the development of the pelvic girdle and pelvic spines of stickleback fish and of the hind limbs of some four-legged terrestrial vertebrates.**

3. ***Watch the video segment from the HHMI short film, "Evolving Switches, Evolving Bodies."***
   a. *What is the relationship between changes in body form, the process of development, and genes?*
   Changes in the physical forms of all animals arise from changes in development. Because genes control development, changes in forms are ultimately due to changes in genes.
   b. *Why did Dr. Kingsley do genetic crosses with stickleback fish?* **Dr. Kingsley performed genetic crosses between marine and freshwater stickleback fish in order to pinpoint which gene(s) are responsible for pelvic reduction in freshwater fish.**

4. ***In the virtual lab window, click on the blue gloves and perform Part 1: Staining the Fish.*** *You can skip the staining procedure if you already completed it in Experiment 1.*
5. ***Proceed to Part 2: Scoring Pelvic Asymmetry.***
   Which one of the three pelvic girdle phenotypes is analyzed in more detail in this experiment? **The reduced pelvis phenotype.**

6. *What is the difference between left-biased and right-biased pelvic asymmetry?* **When a pelvis is reduced, the remaining pelvic vestiges are rarely the same size. After careful measurement, there are usually two outcomes: the pelvic vestige is larger on the left (left bias) or larger on the right (right bias).**

7. ***In the virtual lab window, complete Part 2: Scoring Pelvic Asymmetry.***
8. ***Complete the graphing exercise as instructed by your teacher. After graphing, verify your data.*** *How do your data compare to those collected by Dr. Bell and colleagues?* **Answers will vary. If students scored the pelvises correctly, their results should be similar to those Dr. Bell's lab obtained.**

9. ***Complete the Experiment 3 Quiz. Take time to read the explanation for each correct choice provided after each question.***
10. *The pelvic asymmetry observed in stickleback fish from Bear Paw Lake and Coyote Lake is biased toward which side?* **Left.**

11. *Based on previous research conducted in mice, what does this observed bias among Bear Paw Lake and Coyote Lake stickleback suggest about the genetic mechanisms of pelvic reduction in these two populations?*
   Experiments showed that mice lacking *Pitx1* gene function did not develop complete hind limbs. These animals just had the remnants of a pelvis and hind limbs, and what was left of these structures was larger on the left than on the right side. Therefore, the observed left bias in Bear Paw Lake and Coyote Lake stickleback suggests that pelvic reduction in these two populations is associated with mutations that affect the function of the same gene: *Pitx1*.

12. ***After completing the quiz, click on Experiment 3 Analysis.***
13. *For this analysis, what is the null hypothesis?* **The null hypothesis is that in a given population with pelvic reduction, you would expect a roughly equal number of fish with left bias as fish with right bias.**

14. Complete the following tables as you perform the chi-square calculations in the lab.

Bear Paw Lake

Phenotype	Observed (o)	Expected (e)	(o-e)	(o-e)^2	(o-e)^2/e
Left Bias	**18**	**10**	**8**	**64**	**6.4**
Right Bias	**2**	**10**	**8**	**64**	**6.4**
	Total = **20**	Total = **20**			χ^2 = **12.8**

Coyote Lake

Phenotype	Observed (o)	Expected (e)	(o-e)	(o-e)^2	(o-e)^2/e
Left Bias	**20**	**10**	**10**	**100**	**10**
Right Bias	**0**	**10**	**10**	**100**	**10**
	Total = **20**	Total = **20**			χ^2 = **20**

15. *For each chi-square calculation, how many degrees of freedom are there?* **df = 2-1 = 1**

16. *What are the p values for the data from each lake?*
    a. *Bear Paw Lake*   **0.01**
    b. *Coyote Lake* **0.01**

17. *Do you "reject" or "fail to reject" your null hypothesis for the data from each lake?*
    a. *Bear Paw Lake*   **Reject**
    b. *Coyote Lake* **Reject**

18. ***Complete the Chi-Square Analysis Quiz. Take time to read the explanation for each correct choice after each question.***
19. *What does it mean to reject the null hypothesis for Bear Paw and Coyote Lakes?* **If the null hypothesis can be rejected that means that any difference between the observed data and the expected 50:50 distribution of left to right bias is unlikely to be due to chance. There could be a biological explanation of why most fish in these populations have pelvic asymmetry.**

AUTHOR

Ann Brokaw

Rocky River High School

Rocky River, Ohio