USING GENETIC CROSSES TO ANALYZE A STICKLEBACK TRAIT

OVERVIEW

This hands-on activity serves as an extension of *The Making of the Fittest* short film entitled *Evolving Switches, Evolving Bodies.* Students apply the principles of Mendelian genetics to analyze the results of genetic crosses between stickleback fish with different traits, or phenotypes. Students will use photos of actual research specimens to obtain their data; they will then analyze additional research data from the scientific literature.

KEY CONCEPTS AND LEARNING OBJECTIVES

- Scientists breed individuals with different phenotypes—a procedure known as a genetic cross—to determine the pattern of inheritance of those phenotypes.
- By applying the principles of Mendelian genetics, the results of a genetic cross can reveal whether a particular phenotype is controlled by one or more genes.
- If a phenotype is controlled by one gene with two alleles, the results of a genetic cross can reveal which allele, and resulting phenotype, is dominant or recessive.
- Chi-square analysis is used to determine whether the data from a genetic cross are compatible with the expectations of the hypothesis.

After completing this activity students should be able to:

- characterize stickleback fish according to the presence or absence of pelvic spines;
- use Punnett squares to predict the frequencies of genotypes and phenotypes in the offspring of a genetic cross, based on the genotypes of the parents;
- develop hypotheses about whether a phenotype is dominant or recessive;
- evaluate the evidence in support of or against a hypothesis;
- perform a test cross to determine the genotype of an individual with a dominant phenotype; and
- use chi-square analysis to determine the significance of genetic data.

CURRICULUM CONNECTIONS

Text/Curriculum	Curriculum Topics
Next Generation Science Standards	MS-LS1.B, MS-LS3.A, MS-LS3.B, MS-LS4.C HS-LS1.A, HS-LS3.A, HS-LS3.B, HS-LS3-3, HS-LS4.B, HS-LS4.C
AP® (2012–2013 Standards)	1.A.1, 1.A.2, 1.A.4, 1.C.2, 2.D.1, 2.E.1, 3.A.4, 3.B.1, 3.C.1
IB (2009 Standards)	4.1, 4.3, 5.4, D.2
Common Core*	CCSS.ELA-Literacy.WHST.6-8.1, CCSS.ELA-Literacy.WHST9-12.1, CCSS.ELA-Literacy.RST.6-8.7, CCSS.ELA-Literacy.RST.9-12.5, CCSS.ELA-Literacy.RST.9-12.7, CCSS.Math.Practice.MP2, CCSS.Math.Content.HSS.IC.A.1

KEY TERMS

Mendelian genetics, Punnett square, trait, phenotype, genotype, F_1 generation, F_2 generation, test cross, chi-square, statistical analysis

HHMI
HOWARD HUGHES MEDICAL INSTITUTE

Using Genetic Crosses to Analyze a Stickleback Trait

www.BioInteractive.org

Published April 2013
Updated May 2015

Page 1 of 9

TIME REQUIREMENT

This activity requires a minimum of one 50-minute class period with the expectation that students will complete the activity as homework. If students watch the short film and complete the entire activity in class, then a maximum of two 50-minute class periods will be required.

SUGGESTED AUDIENCE

This activity was designed for a first-year high school biology course (honors or regular), but would also be useful to students in more advanced courses as a review of Mendelian genetics.

PRIOR KNOWLEDGE

Students should understand the basics of Mendelian genetics, including the terms genotype, phenotype, homozygous, heterozygous, dominant, recessive, F_1 and F_2 generations, and test crosses. In addition, they should be familiar with completing Punnett squares to make predictions about expected phenotype and genotype ratios. They should also know how to calculate ratios and proportions. If the extension activity is assigned, students should have a basic understanding of chi-square statistical analysis.

MATERIALS

Students will need:
- F_1 stickleback fish cards (16)
- F_2 stickleback fish cards (40)
- Calculator

TEACHING TIPS

- Students should watch the short film *Evolving Switches, Evolving Bodies* before doing this activity. You may consider showing the film up to the point where Dr. David Kingsley describes how genetic crosses using stickleback fish were done (4 min. and 45 sec. after the start of the film). You could stop the film at that point, have the students complete this activity, and then finish showing the film.

This activity uses "fish cards," which were developed using photographs of stained and preserved fish specimens provided by Dr. Michael Bell at SUNY, Stony Brook, and Dr. Peter Park at Nyack College. The fish cards should be printed in landscape view, using a "no margin" option on the printer. This ensures that eight cards of equal size are printed on each page and will make the cards easier to cut.

- Students could work in pairs or small groups for Part 2 of the activity, when they are sorting the F_1 and F_2 fish cards and collecting data. This approach will cut down the number of cards to print and students will help each other in assigning the correct phenotype to each fish.

- The chi-square statistics portion of this activity is optional. If you teach a course in which chi-square analysis is not required knowledge, you may remove the chi-square section from this activity; it has been placed on separate pages for that reason.
  - If some students have been exposed to chi-square analysis while others have not, create groups that have chi-square "experts" to explain chi-square analysis.
  - You may also refer to the math and statistics guide on the BioInteractive website (http://www.hhmi.org/biointeractive/teacher-guide-math-and-statistics).

- The activity also uses research data published by Dr. William Cresko at the University of Oregon. The original paper and supplementary data may be obtained here: http://www.pnas.org/content/101/16/6050.long.
- Students may have some questions about Dr. Cresko's data. For example, in the F_2 generation, some families have only four fish whereas others have close to 100. According to Dr. Cresko, there is great variability in clutch size among stickleback fish. In general, smaller females have smaller clutches.
- One family in the F_2 generation, Family 8, has a phenotype ratio that is significantly different from the expected 3:1 ratio. This may be due to sampling variation. Family 8 has 19 individuals, which is a relatively small sample, and results may not be representative of the entire population.
- If your students have completed The Virtual Stickleback Evolution Lab, they might be confused about the fact that in the lab they scored three different phenotypes whereas in this activity they are only scoring two. The "absent" and "reduced" phenotypes in the virtual lab are equivalent to the "without pelvic spines" phenotype in this activity. The "complete" phenotype in the virtual lab is equivalent to the "with pelvic spines" phenotype in this activity. The reduced phenotype does not represent heterozygous individuals. The genotype for stickleback fish without pelvic spines (both absent and reduced fish in the virtual lab) is homozygous recessive**.**

ANSWER KEY

Part 1—Stating the Hypothesis: Which Phenotype Is Dominant?

1. Based on what you learned in the film, what are the phenotypes of these two parental stickleback fish? Indicate your choice with a check mark.

Marine   ✔ pelvic spines present   __without pelvic spines

Bear Paw Lake   __ pelvic spines present   ✔ pelvic spines absent

*2. If we start with the simplest assumption that the presence or absence of pelvic spines is controlled by a single gene with two alleles, how would you denote the genotype of the two **homozygous** parents?*

The phenotypes may be indicated as PP and pp. Students could choose to use any letter they wish; it's important that they include one homozygous dominant parent and one homozygous recessive parent.

3. What is your hypothesis for which phenotype is dominant and which one is recessive?

Students may say that the presence of pelvic spines is dominant (PP) and the absence of pelvic spines is recessive (pp), or vice versa. Either response is appropriate.

4. Based on your hypothesis, what would you expect to be the results of the cross between the marine and Bear Paw Lake stickleback parents? Make your prediction using the Punnett square to the right.

	P	P
p	Pp	Pp
p	Pp	Pp

5. What would be the ratio of stickleback with pelvic spines to stickleback without spines in the first filial (F_1) generation?

Depending on which phenotype the students chose to be dominant, the ratio should be 1:0 or 0:1.

6. *Using the Punnett square to the right, what do you expect would be the result of crossing two F_1 fish to produce the second filial (F_2) generation?*

	P	p
P	PP	Pp
p	Pp	pp

1PP: 2Pp: 1pp

7. *What would be the ratio of stickleback with pelvic spines to stickleback without spines in the F_2 generation?*

Depending on which phenotype the students chose to be dominant, the ratio should be either 3:1 or 1:3.

8. *If you had 40 offspring in the F_2 generation, approximately how many would you expect to have pelvic spines and how many to lack pelvic spines?*

Depending on which phenotype the students chose to be dominant, the ratio should be either 30 with and 10 without or 30 without and 10 with.

Part 2—Obtaining the Data: Do the Results from the Experiment Support Your Hypothesis?

3. *Count and record the total number of fish with each phenotype in the table below.*

Table 1. Results of a Cross Between Marine and Bear Paw Lake Stickleback

Generation	Fish With Pelvic Spines	Fish Without Pelvic Spines
P	1	1
F_1	16	0
F_2	31	9

4. *What is the ratio of fish with pelvic spines to fish without pelvic spines in the F_1 generation?*

If students scored the fish correctly, they should indicate that for the F_1 generation the ratio is 1: 0. Because students might not categorize all the fish correctly, answers may differ slightly.

5. *What is the ratio of fish with pelvic spines to fish without pelvic spines in the F_2 generation?*

If students scored the fish correctly, the ratio should be 3.4:1.

6. *Do these results support the hypothesis that the presence or absence of pelvic spines is controlled by a single gene? Explain using evidence.*

Answers will vary. Students should realize that the F_2 ratio is not exactly 3:1, but it's close. All the F_1 offspring have one phenotype and the F_2 phenotype ratio is close to 3:1; these observations are consistent with the hypothesis that a single gene with two alleles is mainly responsible for the presence or absence of pelvic spines.

7. *According to these results, which phenotype is dominant and which is recessive? Explain using evidence.*

All the F_1 offspring had pelvic spines and most of the F_2 offspring had pelvic spines; these results are consistent with the hypothesis that the presence of pelvic spines being the dominant phenotype.

Part 3—Analyzing Additional Experimental Data: Do the Results Agree?

Table 2. Crosses Between Several Marine and Freshwater Stickleback: The F_1 Generation

Parental Cross	F_1 Generation Total Offspring	With Pelvic Spines	Without Pelvic Spines
Your Cross	**16**	**16**	**0**
Marine x Bear Paw Lake	50	50	0
Marine x Boot Lake	33	33	0
Marine x Whale Lake	42	42	0
Total	**141**	**141**	**0**

(Source: 2004 *Proc. Natl. Acad. Sciences* **101**: 6050-6055.)

1. Add your data from the F_1 fish cards in the top row and total the F_1 fish with and without pelvic spines in the bottom row of Table 2.

2. If you look at the total number of F_1 fish, what is the ratio of fish with pelvic spines to fish without pelvic spines? **1:0**

3. Compare the outcome of your cross from Part 2 to the F_1 data from Dr. Cresko's experiments. Describe similarities and differences.

Students should realize that although the numbers are different, their results are consistent with the crosses done by Dr. Cresko. In both cases, all fish in the F_1 generation had pelvic spines.

Table 3. Crosses Between F_1 Generation Stickleback from the Marine x Bear Paw Lake Parental Cross: The F_2 Generation

F_2 Generation	(A) Total Offspring	(B) With Pelvic Spines	(C) Without Pelvic Spines	(D) Ratio of Pelvic Spines to No Pelvic Spines
Your cross	**40**	**31**	**9**	**3.4:1**
Family 1	98	71	27	**2.6:1**
Family 2	79	62	17	**3.6:1**
Family 3	62	49	13	**3.8:1**
Family 4	34	28	6	**4.7:1**
Family 5	29	24	5	**4.8:1**
Family 6	23	17	6	**2.8:1**
Family 7	21	17	4	**4.2:1**
Family 8	19	18	1	**18:1**
Family 9	15	11	4	**2.7:1**
Family 10	12	10	2	**5:1**
Family 11	12	10	2	**5:1**
Family 12	4	3	1	**3:1**
Total	**448**	**351**	**97**	**3.6:1**

4. In Table 3, add your F_2 fish card data to the first row. Then add the totals for columns A, B, and C and record these values in the last row.

5. Calculate the ratio of fish with pelvic spines to fish without pelvic spines (for example, 3:1 or 2.8:1) for each family and the total population. Record these calculations in column D.

Students should calculate ratios by dividing the numbers in columns B and C by the number in column C. That also applies for the last (Total) row. The ratio of the total F_2 generation should not be an average of all the ratios since some of the ratios were calculated from a very small number of offspring. You might want to discuss this point with your students.

6. Look at the phenotype ratio for all of the crosses combined (bottom of column D). What does that ratio suggest about the inheritance of the pelvic spine phenotype?

The ratio is 3.6:1. Students should indicate that number is close to a 3:1 ratio of fish with pelvic spines to fish with no pelvic spines. An acceptable answer would be that the trait follows the inheritance of a trait controlled by a single gene.

7. You will notice that the phenotype ratios vary from family to family. Explain why every family does not show the same ratio.

There are several possible answers. (1) The sample sizes may not be large enough. With each birth there's a 3:1 CHANCE that stickleback fish will have a pelvic spine. For example, just because there is a 1:1 chance of getting heads or tails with a flip of a coin, that does not guarantee that if it was heads on the first flip, it will be tails on the second flip. The larger the sample size, the more you approach the predicted ratio. Only in an infinite sample size would you expect actual data to be exactly the same as predicted (if the hypothesis is correct). (2) If the sample size is large, it's possible that the hypothesis is wrong, or (3) There is natural variability in every experiment that is outside our control.

EXTENSION ACTIVITY: CHI-SQUARE STATISTICS

1. State the null hypothesis. Use the numbers of the total F_2 generation results in Table 3 in your response.

The null statistical hypothesis is that the observed outcome of 351 fish with pelvic spines and 97 fish without pelvic spines is not significantly different from the expected 3:1 ratio; any deviation from the expected 3:1 ratio likely occurred by chance alone.

*2. Perform a chi-square statistics test on the data for the **Total Population** sampled in Table 3. Use the following tables to aid your calculations:*

Total Fish Population:

Phenotype	Observed (o)	Expected (e)	(o − e)	$(o - e)^2 / e$
Pelvic Spines	351	336	15	0.670
No Pelvic Spines	97	112	-15	2.009
	Total = **448**			Sum (χ^2) = **2.679**

a. What is the chi-square value (χ^2)? ***χ^2 = 2.679***

b. Calculate the degrees of freedom (df). ***df = 2 − 1 = 1***

c. Using the Critical Values Table below, determine whether the null hypothesis is rejected. Explain how you determined your answer and what it means.

Since p > 0.05, we cannot reject the null hypothesis. This result suggests that the difference between the observed and expected data is not statistically significant; this difference is thus likely to be solely due to chance.

d. *What do the results of this chi-square analysis tell you about the results obtained with these crosses?*

The observed outcome of 351 fish with spines and 97 without spines is not statistically different from a 3:1 ratio.

3. *Chi-square analysis can also be used to analyze the results from each individual cross. Using the F_2 generation data from Table 3, perform a chi-square statistics test for **Family 1**.*

Family 1:

Phenotype	Observed (o)	Expected (e)	(o − e)	(o − e)^2 / e
Pelvic Spines	71	73.5	-2.5	0.085
No Pelvic Spines	27	24.5	2.5	0.255
	Total = **98**			Sum (χ^2) = **0.34**

a. *What is the sum chi-square value (χ^2)?* χ^2 **= 0.34**

b. *Calculate the degrees of freedom (df).* **df = 2 − 1 = 1**

c. Based on these results, is the null hypothesis rejected? Explain how you determined your answer and what it means.

Since p > 0.05, we cannot reject the null hypothesis. This result suggests that the difference between the observed and expected data is not statistically significant; this difference is thus likely to be solely due to chance.

d. *What do the results of this chi-square analysis tell you about the results obtained with these crosses?*

In practical terms this means that the observed outcome of 71 and 27 is not statistically different from a 3:1 ratio.

4. *Using the F_2 generation data again, perform a chi-square test for **Family 8**.*

Family 8:

Phenotype	Observed (o)	Expected (e)	(o − e)	(o − e)^2 / e
Pelvic Spines	18	14.25	3.75	0.986
No Pelvic Spines	1	4.75	-3.75	2.961
	Total = **19**			Sum (χ^2) = **3.947**

a. *What is the chi-square value (χ^2)?* χ^2 **= 3.947**

b. *Calculate the degrees of freedom (df).* **df = 2 − 1 = 1**

c. *Based on these results, is the null hypothesis rejected? Explain how you determined your answer and what it means.*

Since p < 0.05, we reject the null hypothesis. This suggests that the difference between the observed and expected data is statistically significant and unlikely to be solely due to chance.

d. *What do the results of this chi-square analysis tell you about the results obtained with these crosses?*

This suggests that the observed outcome of 18 and 1 is statistically different from a 3:1 ratio. The difference between the observed and expected outcome is unlikely to be due to chance.

5.   If you had only carried out the Family 8 cross, what would you conclude about the inheritance of the pelvic spine phenotype? Explain your answer.

Students should make the point that the results are statistically different from a 3:1 ratio. Thus these data do not support the hypothesis that the pelvic spine trait is encoded by a single gene with two alleles. They would need to do more crosses to determine the inheritance of this phenotype.

6.   If you look at the total population sampled, which includes 448 F_2 fish, do the data support or refute the hypothesis that the pelvic spine trait follows the inheritance of a phenotype encoded by a single gene with two alleles, one dominant and one recessive? Explain in detail.

For most students, the failure to reject the null hypothesis means that the results are not statistically different from a 3:1 ratio. Thus, the results support the experimental hypothesis that the pelvic spine trait follows the inheritance of a phenotype encoded by a single gene with two alleles. In addition, the results indicate that the presence of pelvic spines is the dominant phenotype.

ENRICHMENT ACTIVITIES

Activity 1—Test Crosses Reveal Genotypes of F_2 Generation Fish

1.   Find the card for the $F_2$1 fish. Can you determine the pelvic spine genotype of this fish simply by looking at it? If so, what is the genotype?

Yes, it is pp. Students can choose any letter to use. It is important that they indicate that the genotype is homozygous recessive.

2.   Now find the card for the $F_2$8 fish. Can you determine the pelvic genotype of this fish? Explain.

No, this fish could be PP or Pp.

3.   What type of fish would you cross the $F_2$8 fish with to determine its genotype? Explain.

You would cross the $F_2$8 fish with a fish that is homozygous recessive (pp), which has no spines. This is called a test cross and can be used to determine the genotype of a phenotypically dominant individual.

4.   Based on your answer to question 3 above, show the two possible outcomes of that test cross using the Punnett squares below.

Cross A.

	P	P
p	**Pp**	**Pp**
p	**Pp**	**Pp**

Cross B.

	P	p
p	**Pp**	**pp**
p	**Pp**	**pp**

5.   What are the expected phenotype ratios for the two crosses above?

Cross A: 100:0 pelvic spines to no pelvic spines

Cross B: 50:50 pelvic spines to no pelvic spines

6. *After crossing fish $F_2 8$ with a fish lacking pelvic spines, imagine that 48 out of 100 offspring lack pelvic spines. What can you assume about the genotype of the $F_2 8$ fish based on this result? Explain.*

If some of the offspring lack pelvic spines, the $F_2 8$ fish must have been heterozygous (Pp). The offspring that lack pelvic spines are homozygous recessive (pp). In order for an individual to be pp, that individual must inherit a recessive allele from each parent. Therefore, fish $F_2 8$ must have a recessive allele to pass on to the offspring.

Activity 2—Crossing Different Parents

1. *In the space below, complete the Punnett square with the parents described above and show the F_1 generation.*

	P	**p**
p	**Pp**	**pp**
p	**Pp**	**pp**

2. *For the cross above, indicate the expected phenotype and genotype ratios for the F_1 generation.*

Genotype ratio: 1Pp : 1pp

Phenotype ratio: 1 pelvic spines : 1 no pelvic spines

3. *Now take two fish from your F_1 generation and cross them. For each cross described in the table below, fill in the possible outcomes. (Note: the letter "x" represents a cross.)*

F_1 Experimental Cross	What is the chance of having an F_2 offspring with spines?	What will be the genotype ratio of the F_2 generation for each cross?
Male with spines x female with spines	**75%**	**1PP:2Pp:1pp**
Male without spines x female with spines	**50%**	**2Pp:2pp**
Male without spines x female without spines	**0%**	**4pp**
Male with spines x female without spines	**50%**	**2Pp:2pp**