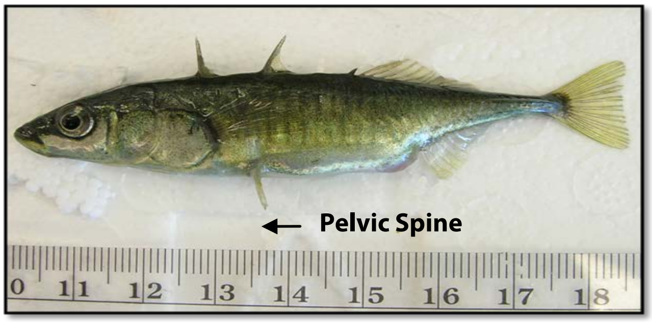
USING GENETIC CROSSES TO ANALYZE A STICKLEBACK TRAIT

INTRODUCTION

Geneticists breed organisms with different characteristics, or phenotypes, to answer questions about how the phenotypes are inherited. Is one phenotype dominant and one recessive? Is each phenotype controlled by a different version (allele) of a single gene, or are many interacting genes involved? In this activity, you will answer these questions by analyzing the outcome of breeding a stickleback with pelvic spines and one without pelvic spines—the same procedure, called a genetic cross, that Dr. David Kingsley described in the film *Evolving Switches, Evolving Bodies*.



The fish shown in the photo on the left is a marine threespine stickleback. Like all marine and sea-run stickleback, this fish has a pair of pelvic spines (only one is visible in the photo), which serve as a defense from large predatory fish. In some freshwater populations, such as in Bear Paw Lake, Alaska, stickleback lack pelvic spines. (The scale is in centimeters.)

How are these two phenotypes inherited?

MATERIALS

You will need:

- F_1 stickleback fish cards
- F_2 stickleback fish cards
- Calculator

PROCEDURE

1. Watch the short film entitled *The Making of the Fittest: Evolving Switches, Evolving Bodies*.
2. Proceed through each part of this activity as instructed by your teacher.

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

In this activity you will examine the offspring of a genetic cross between a stickleback from the ocean (marine stickleback) and a freshwater stickleback from Bear Paw Lake.

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 __ pelvic spines absent

   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?

   Marine

   Bear Paw Lake

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

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 below.

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

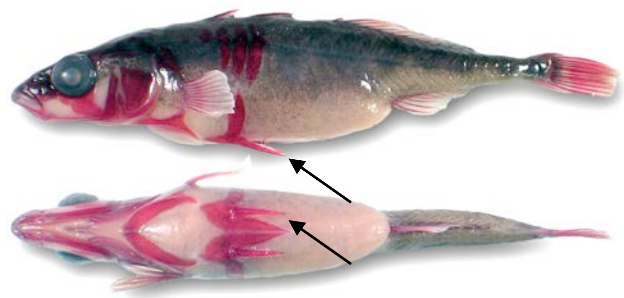
6. Using the Punnett square below, what do you expect would be the result of crossing two F_1 fish to produce the second filial (F_2) generation?

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

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?

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

In this part of the activity, you will use the fish cards provided by your teacher to see whether the result of the crosses described in Part 1 support your hypothesis. These cards show photographs of stickleback fish that were stained with a solution that turns bones red, making them easier to see. (If your cards are in black and white, the bones should still be easier to see as they appear darker.) There should be two sets of cards: the first set (16 cards) represents the first filial (F_1) generation and the second set (40 cards) the second filial (F_2) generation. You will be sorting these cards according to whether the fish have pelvic spines.



A stickleback with pelvic spines. The fish is shown in side (lateral) and belly (ventral) views, and the pelvic spines are indicated by the arrows. The pelvic spines are attached to the pelvic girdle.

A stickleback without pelvic spines. The fish has a reduced pelvic girdle (indicated by the circles) with no pelvic spines attached to it.

1. Sort the F_1 set of cards into two separate piles:
   Fish with pelvic spines
   Fish without pelvic spines
2. Repeat the same procedure with the F_2 set of cards.
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		
F_2		

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

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

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

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

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

In your crosses, you only looked at one family of 16 fish in the F_1 generation and one family of 40 fish in the F_2 generation. Researchers would typically conduct many more crosses.

William Cresko, a researcher at the University of Oregon, conducted several crosses between marine stickleback with pelvic spines and freshwater stickleback populations without pelvic spines, similar to the crosses described in Parts 1 and 2. The freshwater stickleback fish were collected from several Alaskan lakes, including Bear Paw Lake, Boot Lake, and Whale Lake. The F_1 generation results of those crosses are shown in Table 2 below. (Note that each cross is indicated with the letter "x".)

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

Parental Cross	**F1 Generation Total Offspring**	**With Pelvic Spines**	**Without Pelvic Spines**
Your Cross			
Marine x Bear Paw Lake	50	50	0
Marine x Boot Lake	33	33	0
Marine x Whale Lake	42	42	0
Total			

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

1. Add your data from the F_1 fish cards from Part 2 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?

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

Dr. Cresko then took the F_1 offspring and conducted several crosses between them to produce the F_2 generations. The results of the crosses with the F_1 progeny from the Marine x Bear Paw Lake cross are shown in Table 3. (Dr. Cresko also conducted crosses with the F_1 progeny from the Marine x Boot Lake and Marine x Whale Lake crosses, but those results are not shown here.)

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

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				
Family 1	98	71	27	
Family 2	79	62	17	
Family 3	62	49	13	
Family 4	34	28	6	
Family 5	29	24	5	
Family 6	23	17	6	
Family 7	21	17	4	
Family 8	19	18	1	
Family 9	15	11	4	
Family 10	12	10	2	
Family 11	12	10	2	
Family 12	4	3	1	
Total				

4. In Table 3, add your F_2 fish card data from Part 2 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 for the total population. (Round your numbers to the first decimal place.) Record these calculations in column D.

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

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7. 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?

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EXTENSION ACTIVITY: CHI-SQUARE STATISTICS

In Part 3 of this activity, you evaluated whether the ratio of fish with spines to fish with no spines suggests that the inheritance of the trait follows the inheritance of a phenotype controlled by a single gene with a dominant and recessive allele. That question is easy to answer if the F_2 ratio is a perfect 3:1, but what if the ratio is 2.6:1 or 3.4:1? Are such differences from a 3:1 ratio a true variation from the expected 3:1 ratio, which would mean a different pattern of inheritance, or could these differences simply have occurred by chance? The chi-square test can help us answer this question.

Remember that with our stickleback experiment the *experimental* hypothesis we were testing was that the pelvic spine trait follows the inheritance of a phenotype encoded by a single gene with two alleles. A statistical test does not test the experimental hypothesis but rather what is called the *null* hypothesis. In the case of the chi-square test, the null hypothesis is that the observed and expected outcomes are the same and that any deviations between them occurred by chance.

In this section you will perform three chi-square tests on some of the F_2 data from Part 3 to verify whether the differences between the observed and expected results of the crosses are statistically significant (in other words, not due to chance alone) or are NOT statistically significant (due to chance alone).

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

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2. Perform a chi-square statistics test on the data for the **Total Population** 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**
	Total =			Sum (χ^2) =

a. What is the chi-square value (χ^2)? ______________

b. Calculate the degrees of freedom (df). ______________

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

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

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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)**	**$(o - e)^2 / e$**
	Total =			Sum (χ^2) =

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

b. Calculate the degrees of freedom (df). ______________

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

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

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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)**	**$(o - e)^2 / e$**
	Total =			Sum (χ^2) =

a. What is the chi-square value (χ^2)? _____________

b. Calculate the degrees of freedom (df). _____________

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

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

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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.

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.

Critical Values Table

df \ p	**0.995**	**0.975**	**0.9**	**0.5**	**0.1**	**0.05**	**0.025**	**0.01**
1	0.000	0.000	0.016	0.455	2.706	3.841	5.024	6.635
2	0.010	0.051	0.211	1.386	4.605	5.991	7.378	9.210
3	0.072	0.216	0.584	2.366	6.251	7.815	9.348	11.345
4	0.207	0.484	1.064	3.357	7.779	9.488	11.143	13.277
5	0.412	0.831	1.610	4.351	9.236	11.070	12.832	15.086
6	0.676	1.237	2.204	5.348	10.645	12.592	14.449	16.812
7	0.989	1.690	2.833	6.346	12.017	14.067	16.013	18.475

ENRICHMENT ACTIVITIES

You now know something about the inheritance of the pelvic spine trait and which phenotype is dominant and which is recessive. You will now use this knowledge to further explore the genotype-phenotype connection.

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?

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

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

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:

Cross B:

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

   Cross A:

   Cross B:

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.

Activity 2—Crossing Different Parents

In the initial activity, the outcomes of the F_1 and F_2 generations would have been different if the parental generation had been a heterozygous fish with pelvic spines and a homozygous fish without pelvic spines.

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

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

   Genotype ratio:

   Phenotype ratio:

3. You 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		
Male without spines x female with spines		
Male without spines x female without spines		
Male with spines x female without spines		

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