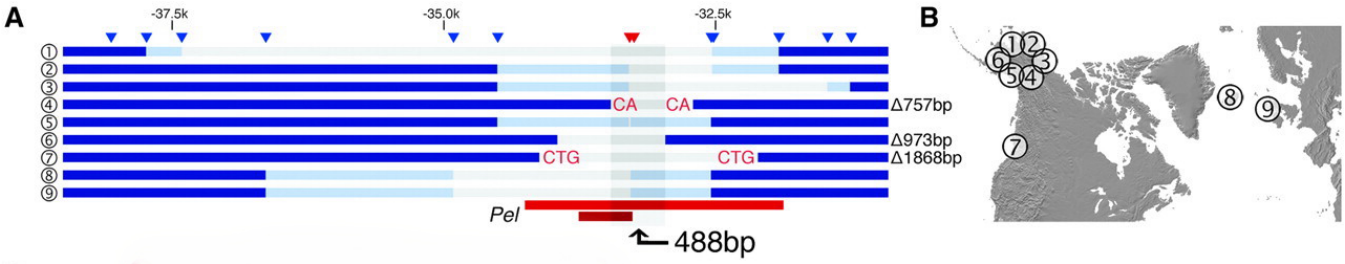
HOW TO USE THIS RESOURCE

Show the figure below to your students along with the caption and background information. The "Interpreting the Graph" and "Discussion Questions" sections provide additional information and suggested questions that you can use to guide a class discussion about the characteristics of the graph and what it shows.



Caption: The results of SNP genotyping in nine stickleback fish populations with reduced or absent pelvises (source locations shown in Figure B). Triangles indicate SNP markers that were used to identify the approximate location of deletion mutations. Dark blue bars indicate that the SNP markers on both sides of that section were present, thus that section of DNA was present. Light blue bars indicate that only one of the two SNP markers on either side of the section was present, meaning that the deletion started somewhere in that section. Light gray horizontal bars indicate that both SNP markers on each side of that section were missing, meaning that the section of DNA was wholly deleted. Three sequences, 4, 6, and 7, were sequenced completely and the lengths of the deletions are indicated to the right. Red bars indicate the location of two noncoding regions (Pel-2.5-kb and Pel-501-bp), based on DNA constructs from marine fish, which are able to drive gene expression specifically in the pelvis. The gray-shaded vertical rectangle indicates the 488-bp region where all the deletions overlap, which includes SNP markers indicated in red.

BACKGROUND INFORMATION

Species can undergo major changes in body morphology as they adapt to their environments, but the molecular mechanisms responsible for these changes are not always known. A classic example of a major evolutionary change in morphology is the modification, and sometimes even complete loss, of limbs and fins. Pelvic hind limbs have been lost in many species, including whales, manatees, and some amphibians, reptiles, and fish. Most members of a fish species called the threespine stickleback (*Gasterosteus aculeatus*) are marine fish that have a pelvis which supports prominent serrated spines that protrude from the underside, deterring predatory fish from eating them. But over two dozen geographically isolated freshwater stickleback populations either partially or completely lack the pelvis. One reason that pelvis and spine loss may occur is because some freshwater populations live in low calcium environments where building a pelvis may be metabolically costly, and are preyed upon by insects that can grasp onto spines, rather than by fish that can be deterred by the spines.

To determine the genetic mutations responsible for stickleback pelvic reduction and loss, and to explain how these changes arose separately in different populations, scientists looked for genetic changes in a chromosome region that controls most of the differences in pelvis size when marine and freshwater sticklebacks are crossed with each other in the laboratory. The key chromosome region contains a gene called *pituitary homeobox transcription factor 1* (*Pitx1*), which is required for both pituitary and hind limb development. Sequencing and expression studies have shown that the *Pitx1* gene of freshwater fish encodes a normal protein, but it fails to be expressed in the developing pelvic region, suggesting a possible regulatory change. The scientists conducted SNP (single-nucleotide polymorphism) genotyping of nine different stickleback populations with reduced pelvises to find genetic mutations that may be responsible for altered *Pitx1* expression in freshwater fish. SNP genotyping identified a variety of deletion mutations in a noncoding region of the genome called *Pel,* located upstream of the *Pitx1* gene. *Pel* normally enhances *Pitx1* expression during pelvic fin development but fails to do so when mutations render it nonfunctional. The above figure shows the locations of the deletion mutations.

INTERPRETING THE GRAPH

This figure shows a summary of DNA genotyping results from nine different freshwater populations of stickleback fish, together with a geographic map indicating the different lakes around the world where the fish with reduced pelvises were found. The approximate size and locations of genomic deletions were determined using SNP genotyping. This process involves searching for single-nucleotide polymorphisms (SNPs, marked by blue and red triangles) in the DNA samples. When a SNP marker is completely undetectable in a DNA sample, it indicates that the region of DNA containing it has been deleted. Mapping these missing SNPs allows researchers to map the approximate location and length of the deletion mutations. Gray horizontal bars indicate regions where SNPs on both sides are missing. Light blue bars indicate regions where a SNP is only missing on one side (which means that the deletion starts or ends somewhere in this region). Dark blue bars indicate regions where SNPs are present on both sides. Follow-up sequencing in three of the populations (4, 6, and 7) confirmed the accuracy of the SNP genotyping procedure. The scale numbers at the top of the figure indicate thousands of bases upstream of the place where the sequence of the *Pitx1* protein begins. For example, "-35k" refers to a position 35,000 DNA bases upstream of the position that encodes the first amino acid in *Pitx1*.

Although the locations of the deletions are different in each population, they nonetheless caused similarly reduced or absent pelvic and spine structures. This suggests that DNA in the area where the deletions overlap–a 488-bp segment (gray-shaded vertical box)–is likely involved. To further test the function of this region, the scientists built two transgenic stickleback fish by introducing different DNA constructs into a strain of fish in which the pelvis is fully developed. Each DNA construct contained an intact sequence from the *Pel* region of a marine fish (indicated by red bars at the bottom of the graph) fused to a green fluorescent reporter gene from jellyfish. Both transgenic fish showed green fluorescence in the pelvis, indicating that the sequences from the *Pel* region contain a noncoding regulatory enhancer region that can drive gene expression specifically in the developing pelvis (see Figure 2 below). When *Pel* is either fully or partially deleted, *Pitx1* expression thus drops in this region of the body, and the pelvis and spine appendages do not fully develop.

If *Pel* is indeed the regulatory region of the *Pitx1* gene and its loss leads to pelvic reduction, restoring this regulatory region should restore the pelvis. The researchers created a DNA construct consisting of a 2.5-kb *Pel* region of a population of fish with pelvises, fused to a *Pitx1* gene from coding exons of stickleback without pelvises. This DNA construct was injected into fertilized eggs of fish that normally fail to develop a pelvis. The transgenic fish showed enhanced development of pelvic spines (Figure 3A below) compared to siblings that did not receive the construct (Figure 3B below). This provides more evidence that *Pel-Pitx1* drives the formation of the pelvis in stickleback fish.

It is important to remember here that phenotypic variations can arise not only from mutations that disrupt the protein coding portions of genes, but also from mutations that alter where and when genes are expressed. Protein-coding and regulatory mutations can both be a basis of morphological evolution. However, regulatory mutations often produce more specific phenotypes, because they confine the alterations in gene function to a particular location in the body, while preserving many other functions of the gene.

Teacher Tip: Prompt your students to explain the following:

- Figure Type: Figure A—Gene map showing DNA sequence deletions in nine different stickleback fish populations; Figure B—Geographic map of fish populations
- Y-Axis: Each of the nine fish populations (one row per population)
- X-Axis: The base pair (bp) position on the chromosome where the sequences are located. The zero position is not shown, but it represents the start site of the *Pitx1* protein in the genome. Negative refers to position upstream of the start site. "k" refers to thousands of bases.
- Triangles: Blue triangles indicate the SNP marker locations. The red triangles highlight the SNP markers within a region that was deleted across all nine populations.
- Bar Colors: Gray bars indicate the regions of the gene that were deleted. Light blue bars indicate regions in which the deletion starts or ends. Dark blue bars indicate nonmutated regions. The red bars indicate the

positions of *Pel*-2.5-kb and *Pel*-501-bp regions that were cloned from intact marine DNA and tested for enhancer activity. The gray vertical rectangle denotes a 488-base pair shared region that is removed by overlapping deletions in all the freshwater populations with reduced pelvises.

- Map: Partial geographic map of the northern hemisphere showing the nine sampling locations of freshwater stickleback populations with reduced pelvises.

Figure 2

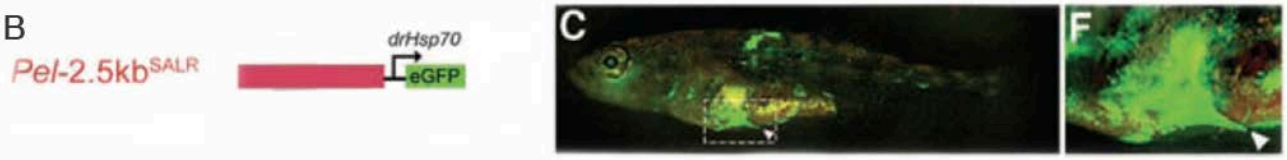
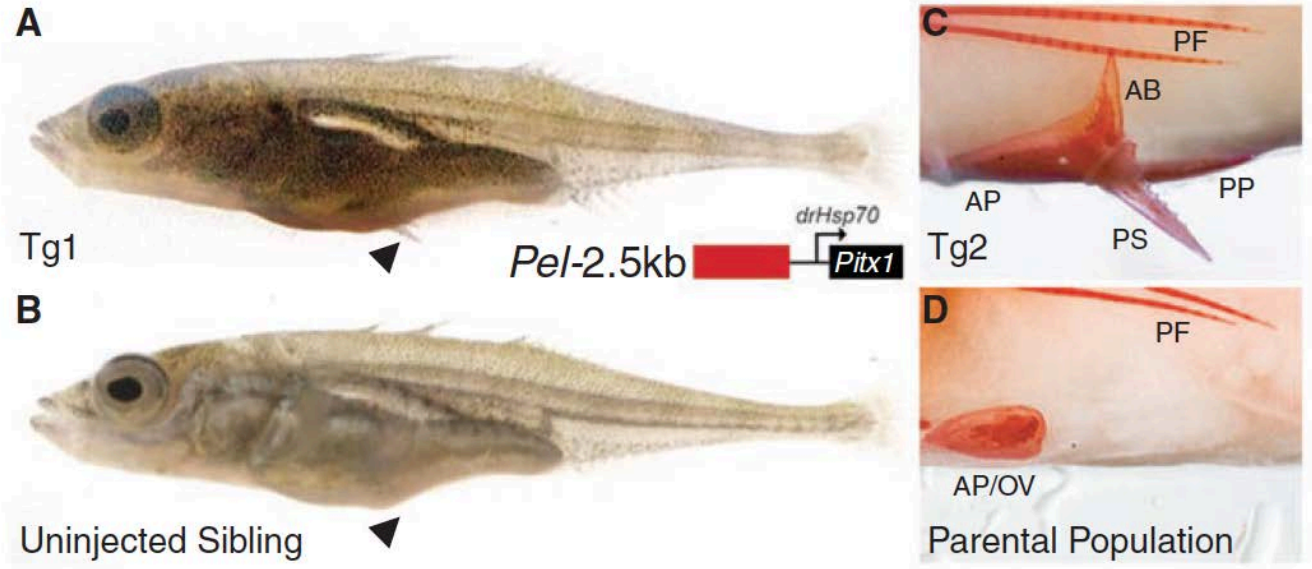


Figure 3



DISCUSSION QUESTIONS

- Describe the similarities and differences in the deletion mutations among the nine stickleback populations.
- What is the significance of the region in the gray-shaded vertical box?
- Would you predict that each mutation would lead to the same phenotypic change? If the phenotypes are different, which mutation would you predict might have the mildest phenotypic change? Explain your reasoning.
- How could it be possible that similar morphological changes evolved in nine geographically distinct locations? Use your knowledge of how DNA mutations occur and evidence from the figure to support your answer.
- How could a deletion in a noncoding region of the genome lead to a lack of pelvis and spines in stickleback fish?
- How does SNP mapping help scientists pinpoint mutations that are common among different populations?
- What do the delta values shown to the right of populations 3, 6, and 7 indicate? Why do you think the researchers performed gene sequencing on three of the stickleback populations?

- Two different *Pel* enhancer constructs are shown in red. Describe their size and location in relation to the 488-base pair region shaded in gray. Based on this comparison, what can you conclude about the importance of the position and length of a deletion in altering gene expression?
- If a deletion removes the protein-coding region of a gene, how many of the normal functions of that gene would you expect to be affected? If a deletion removes a tissue-specific enhancer of a gene, how many functions of the gene would you expect to be affected?
- The *Pitx1* gene is also expressed in other parts of the stickleback body, including the pituitary gland. Would you expect to find similar deletions in the enhancers in these regions? Why or why not?
- If the deletions in the *Pel* region were repaired by genetic engineering, how would this affect the morphology of the sticklebacks?
- Is this an observational or an experimental study? Is it possible to infer causality from patterns of association in observational studies? Why or why not?
- What experiments could be done in this system to test whether the deletion of *Pel* causes impaired pelvic development?
    - After your students make their predictions, show them figure 2, provided in the Supplemental Images document. In this experiment, the researchers built transgenic fish using a DNA construct with the *Pel* region from marine fish attached to a green fluorescent reporter gene. What does this experiment tell you about pelvic development? What further study could provide additional evidence of the role of *Pel* in pelvis formation?
    - Show your students figure 3, provided in the Supplemental Images document. In this experiment, researchers created a DNA construct using the *Pel* region from fish with pelvises and *Pitx1* from fish without pelvises and inserted it into fish that would normally not develop a pelvis. What new information does this experiment provide that the previous experiment did not? What can you conclude about the role of *Pel* in pelvis development?
- How do reduced or absent spines benefit these freshwater stickleback fish populations? What type of ecological pressures commonly lead to a population with reduced or absent spines?

KEY TERMS

enhancer, evolution, genotype, mutation, natural selection, noncoding region, phenotype, Pitx1, SNP, stickleback

SOURCE

Figure 4A & 4B:

Chan, Y. F., Marks, M. E., Jones, F. C., Villarreal, G., Shapiro, M. D., Brady, S. D., … Kingsley, D. M. (2010). Adaptive Evolution of Pelvic Reduction in Sticklebacks by Recurrent Deletion of a Pitx1 Enhancer. *Science*, 327(5963), 302–305. https://doi.org/10.1126/science.1182213

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