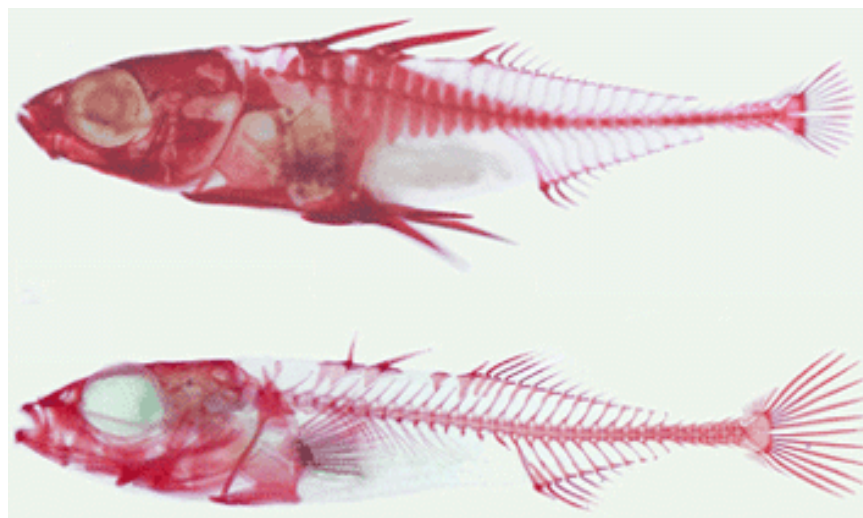


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## Evolution's Mirror in a Fish's Spines



**Image Title:** Stickleback fish (bottom) in some freshwater lakes have lost the large pelvic hindfin seen in their ocean ancestors (top). - David M. Kingsley, HHMI at Stanford Univ. School of Medicine

Howard Hughes Medical Institute researchers at Stanford University are closer to understanding one of evolution's biggest questions: How do genetic changes contribute to the generation of new traits in naturally occurring species?

By studying related populations of small fish, called sticklebacks, the scientists have learned how a variety of animals might have lost their hindlimbs during evolution. The researchers discovered that relatively small changes in the regulation of specific genes can lead to a phenomenon called hindlimb reduction. The work demonstrates that rapid skeletal changes can occur in one body structure without disrupting the essential role of the same genes elsewhere in the body.

The research team, led by Howard Hughes Medical Institute investigator David M. Kingsley, published its findings in the April 15, 2004, issue of the journal *Nature*. Kingsley and his colleagues at the Stanford University School of Medicine collaborated on the studies with researchers from the Fred Hutchinson Cancer Research Center, the Institute of Freshwater Fisheries in

Iceland and the University of British Columbia.

“One of the central mysteries of evolutionary biology has been the relationship between microevolution and macroevolution,” wrote Neil H. Shubin and Randall D. Dahn of the University of Chicago in an accompanying perspective article in *Nature*. “[The researchers] might have discovered a smoking gun—a real example of a type of macroevolutionary change that is produced by genetic differences between populations.”

According to Kingsley, hindlimb reduction is a trait that has evolved repeatedly in different animal groups, including mammals, such as dolphins and whales that have returned to the sea, snakes, reptiles, amphibians and many fish species. “It’s a major morphological change in the vertebrate skeleton,” Kingsley said. “And despite the fact that it has occurred repeatedly, the mechanisms responsible are not understood.”

Over the years, various laboratories studying gene mutations in animals have identified genes that govern limb development. But these mutations were almost invariably lethal, said Kingsley, leaving open the question of whether changes in those genes could possibly underlie skeletal evolution. Some scientists argued that since animals must always remain genetically fit, only subtle changes in genes that are relatively low in the hierarchy of developmental control could cause skeletal evolution.

“We felt that to understand what was happening in nature, we had to find a genetic model system where we could study naturally occurring populations instead of lab mutants,” said Kingsley. “We wanted examples that had evolved in nature and had been subject to whatever constraints of viability and fitness that any organism that has evolved in the wild would have.”

Kingsley and his colleagues found such an animal in the threespine stickleback fish. These small fish typically live in the ocean but breed in coastal streams. After the last ice age ended some 11,000 years ago, populations of sticklebacks rapidly colonized newly formed freshwater streams and lakes - through a process known as adaptive radiation.

The many stickleback populations underwent disparate and parallel evolutionary changes, among them partial or complete loss of their pelvic spines. These spines are thought to protect the fish from being devoured by predators. As Kingsley points out, however, pelvic spines may be a disadvantage if the fish live in environments that have very low calcium levels available for building the skeletal structures, or in locations with many large predatory insects that hunt sticklebacks by grabbing hold of the spines. “Although vastly different morphologies have evolved in different stickleback populations, they have evolved recently enough that you can still take those different populations, cross them and actually let the genetics of the trait guide you to the underlying events that have controlled the process,” said Kingsley.

The Stanford group collaborated with senior co-author Dolph Schluter at the University of British Columbia, and Bjarni Jo'nsson at Holar Agricultural

College in Iceland, to set up multiple crosses between marine and freshwater stickleback populations that exhibited significantly different body structures, such as the presence or absence of the pelvic spine. By analyzing the genetic differences among the progeny with regard to spine development, Kingsley and his colleagues sought to understand the genetic basis of reshaping the hindfin in different populations.

That analysis revealed that a single region on the fish's chromosomes was responsible for most of the changes in spine and pelvic morphology. The researchers then began a search of that chromosome region for candidate genes known to be involved in hindlimb development in other animals. They ended their search when they identified the stickleback homolog of a gene known as *Pitx1*.

The researchers were able to determine the DNA sequence of the *Pitx1* gene in marine fish with a normal hindfin and in freshwater fish with hindfin reduction. Although no changes were seen in the portions of the gene that code for the *Pitx1* protein, comparative expression studies showed that the gene was no longer expressed properly at some locations in the freshwater fish, including the place where hindfins would normally develop.

“*Pitx1* is actually involved in a number of different processes, including pituitary development and craniofacial development,” said Kingsley. “So, any changes in the protein itself would affect all of those structures. We think that this finding shows how evolution has been able to make use of exactly the same major developmental regulator that is lethal if eliminated—and yet avoids lethality by tweaking the regulatory region of the gene. Therefore, such changes only affect the gene's expression in a very specific developmental site. It allows this very important gene to produce a morphological transformation, without producing effects that would reduce viability.”

The team's findings in natural populations of fish may add important insights into the evolutionary process, said Kingsley. “There are many theoretical predictions about whether evolution is controlled by many genes of little effect or few genes of major effect,” he said. “There are relatively few examples of natural populations in which genetic studies can actually let the organism tell you how complicated the genetics of their traits really are. I consider sticklebacks a great system for that kind of study.”

Further research will focus on identifying the specific DNA alterations responsible for causing changes in the regulation of *Pitx1*. This kind of analysis will be particularly challenging, Kingsley said, because the nature and function of these genetic regulatory regions is not well understood in either fish or other animals. However, knowing that such regions may be the basis for major evolutionary change adds new impetus to characterizing them more fully, and studying how they have changed in animals that have adapted to a range of different environments.