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RESEARCH NEWS

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Researchers Trace Evolution to Relatively Simple Genetic Changes

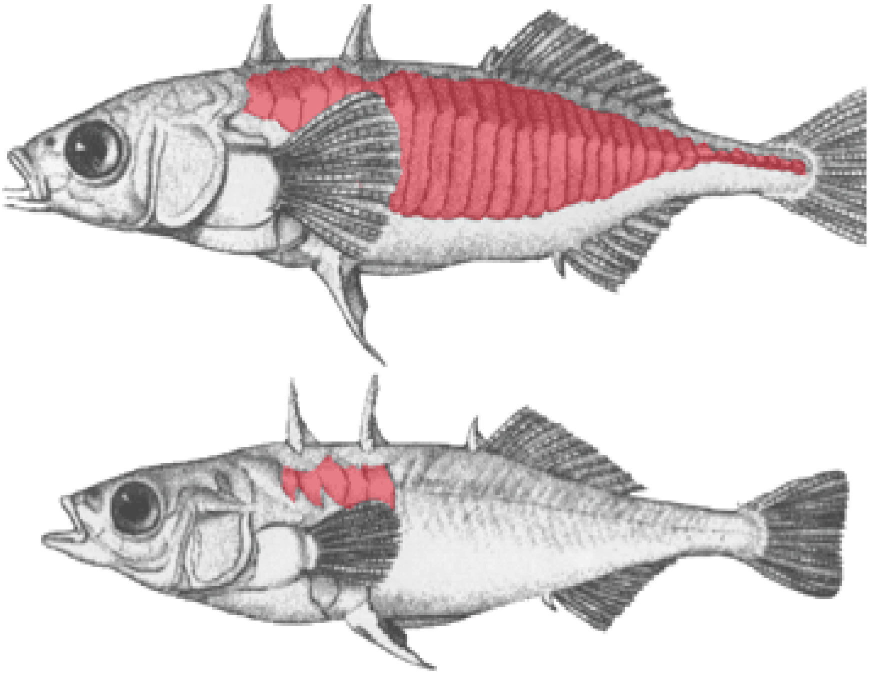

Image Title: Wild populations of stickleback fish have evolved major changes in bony armor styles (shaded) in marine and freshwater environments. New research shows that this evolutionary shift occurs over and over again by increasing the frequency of a rare genetic variant in a single gene. - David Kingsley, HHMI at Stanford University, modified from Cuvier (1829).

In a stunning example of evolution at work, scientists have now found that changes in a single gene can produce major changes in the skeletal armor of fish living in the wild.

The surprising results, announced in the March 25, 2005, issue of journal *Science*, bring new data to long-standing debates about how evolution occurs in natural habitats.

“Our motivation is to try to understand how new animal types evolve in nature,” said molecular geneticist David M. Kingsley, a Howard Hughes Medical Institute investigator at the Stanford University School of Medicine. “People have been interested in whether a few genes are involved, or whether changes in many different genes are required to produce major changes in wild populations.”

> "This is one of the first cases in vertebrates where it's been possible to track down the genetic mechanism that controls a dramatic change in skeletal pattern, a change that occurs naturally in the wild."
> **- David M. Kingsley**

The answer, based on new research, is that evolution can occur quickly, with just a few genes changing slightly, allowing newcomers to adapt and populate new and different environments.

In collaboration with zoologist Dolph Schluter, at the University of British Columbia, Vancouver, Canada, and Rick Myers and colleagues at Stanford, Kingsley and graduate student Pamela F. Colosimo focused on a well-studied little fish called the stickleback. The fish—with three bony spines poking up from their backs—live both in the seas and in coastal fresh water habitats all around the northern hemisphere.

Sticklebacks are enormously varied, so much so that in the 19th century naturalists had counted about 50 different species. But since then, biologists have realized most populations are recent descendants of marine sticklebacks. Marine fish colonized new freshwater lakes and streams when the last ice age ended 10,000 to 15,000 years ago. Then they evolved along separate paths, each adapting to the unique environments created by large scale climate change.

“There are really dramatic morphological and physiological adaptations” to the new environments, Kingsley said.

For example, “sticklebacks vary in size and color, reproductive behavior, in skeletal morphology, in jaws and teeth, in the ability to tolerate salt and different temperatures at different latitudes,” he said.

Kingsley, Schluter and their co-workers picked one trait—the fish's armor plating—on which to focus intense research, using the armor as a marker to see how evolution occurred. Sticklebacks that still live in the oceans are virtually covered, from head to tail, with bony plates that offer protection. In contrast, some freshwater sticklebacks have evolved to have almost no body armor.

“It's rather like a military decision, to be either heavily armored and slow, or to be lightly armored and fast,” Kingsley said. “Now, in countless lakes and streams around the world these low-armored types have evolved over and over again. It's one of the oldest and most characteristic differences between stickleback forms. It's a dramatic change: a row of 35 armor plates turning into a small handful of plates - or even no plates at all.”

Using genetic crosses between armored and unarmored fish from wild populations, the research team found that one gene is what makes the difference.

“Now, for the first time, we've been able to identify the actual gene that is controlling this trait,” the armor-plating on the stickleback, Kingsley said

The gene they identified is called *Eda*, originally named after a human genetic disorder associated with the ectodysplasin pathway, an important part of the embryonic development process. The human disorder, one of the earliest ones studied, is called ectodermal dysplasia.

“It's a famous old syndrome,” Kingsley said. “Charles Darwin talked about it. It's a simple Mendelian trait that controls formation of hair, teeth and sweat glands. Darwin talked about `the toothless men of Sind,' a pedigree (in India) that was striking because many of the men were missing their hair, had very few teeth, and couldn't sweat in hot weather. It's a very unusual constellation of symptoms, and is passed as a unit through families.”

Research had already shown that the *Eda* gene makes a protein, a signaling molecule called ectodermal dysplasin. This molecule is expressed in ectodermal tissue during development and instructs certain cells to form teeth, hair and sweat glands. It also seems to control the shape of - bones in the forehead and nose.

Now, Kingsley said, “it turns out that armor plate patterns in the fish are controlled by the same gene that creates this clinical disease in humans. And this finding is related to the old argument whether Nature can use the same genes and create other traits in other animals.”

Ordinarily, “you wouldn't look at that gene and say it's an obvious candidate for dramatically changing skeletal structures in wild animals that end up completely viable and healthy,' he said. "*Eda* gene mutations cause a disease in humans, but not in the fish. So this is the first time mutations have been found in this gene that are not associated with a clinical syndrome. Instead, they cause evolution of a new phenotype in natural populations.”

The research with the wild fish also shows that the same gene is used whenever the low armor trait evolves. “We used sequencing studies to compare the molecular basis of this trait across the northern hemisphere,” said Kingsley. “It doesn't matter where we look, on the Pacific coast, the East coast, in Iceland, everywhere. When these fish evolve this low-armored state they are using the same genetic mechanism. It's happening over and over again. It makes them more fit in all these different locations.”

Because this trait evolves so rapidly after ocean fish colonize new environments, he added, “we wondered whether the genetic variant (the mutant gene) that controls this trait might still exist in the ocean fish. So we collected large numbers of ocean fish with complete armor, and we found a very low level of this genetic variant in the marine population.”

So, he said, “the marine fish actually carry the genes for this alternative state, but at such a low level it is never seen;” all the ocean fish remain well-armored. “But they do have this silent gene that allows this alternative form to emerge if the fish colonize a new freshwater location.”

Also, comparing what happens to the ectodysplasin signaling molecule when its gene is mutated in humans, and in fish, shows a major difference. The human protein suffers "a huge amount of molecular lesions, including deletions, mutations, many types of lesions that would inactivate the protein," Kingsley said.

But in contrast, “in the fish we don't see any mutations that would clearly destroy the protein.” There are some very minor changes in many populations, but these changes do not affect key parts of the molecule. In addition, one population in Japan used the same gene to evolve low armor, but has no changes at all in the protein coding region. Instead, Kingsley said, “the mutations that we have found are, we think, in the (gene's) control regions, which turns the gene on and off on cue.” So it seems that evolution of the fish is based on how the *Eda* gene is used; how, when and where it is activated during embryonic growth.

Also, to be sure they're working with the correct gene, the research team used genetic engineering techniques to insert the armor-controlling gene into fish “that are normally missing their armor plates. And that puts the plates back on the sides of the fish,” Kingsley said.

“So, this is one of the first cases in vertebrates where it's been possible to track down the genetic mechanism that controls a dramatic change in skeletal pattern, a change that occurs naturally in the wild,” he noted.

“And it turns out that the mechanisms are surprisingly simple. Instead of killing the protein (with mutations), you merely adjust the way it is normally regulated. That allows you to make a major change in a particular body region - and produces a new type of body armor without otherwise harming the fish.”