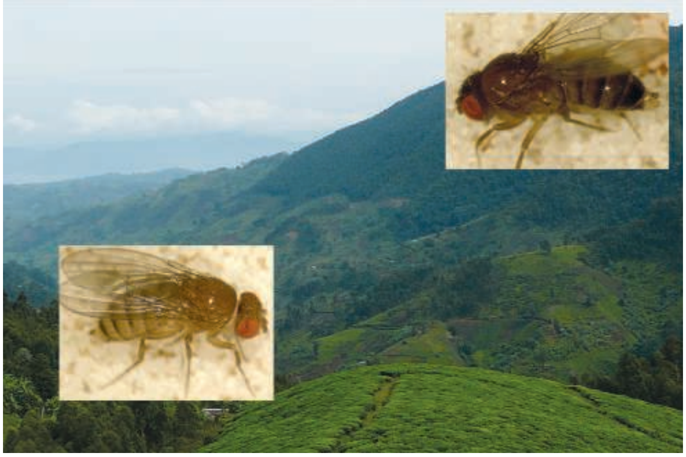
EVOLUTION

Spineless Fish and Dark Flies Prove Gene Regulation Crucial



Color coordinated. In Africa, lowland fruit flies are light-colored, whereas those at high altitudes are dark, all because of a change in gene regulation.

Almost 3 years ago, biologists got into a tussle over what drives morphological evolution: changes in the protein-coding portions of genes or changes in the DNA regions that regulate gene activity. At the time, some researchers felt there was little hard evidence to support the idea that regulatory changes were indeed important (*Science*, 8 August 2008, p. 760).

Now, on page 1663 and in last week's *Science* Express (www.sciencemag.org/cgi/content/abstract/science.1182213), two teams not only independently report that changes in regulatory DNA were responsible for an adaptation in natural populations of fish and insects, but each group has also pieced together details of the underlying genetic alterations in those animals. "They provide beautiful and convincing examples of how [certain] regulatory elements can be lost or modified to reduce [gene] expression, ultimately causing morphological change," says Hopi Hoekstra, an evolutionary biologist at Harvard University and one of the chief skeptics.

In one case, the same piece of regulatory DNA was lost in different freshwater fish populations, each time causing the loss of pelvic spines. In the other case, the darkening of a fruit fly took place through an accumulation of small mutations in regulatory DNA. Taken together with other discoveries of noncoding regions involved in evolution, "there is broad support now" that changes in regulatory DNA can generate morphological variation, says Günter Wagner, an evolutionary developmental biologist at Yale University.

The fish study took place at Stanford University, where David Kingsley has spent the past decade tracking down the genetic basis for why three-spined sticklebacks (*Gasterosteus aculeatus*) that live in salt water have spines jutting from their belly whereas some of their freshwater counterparts do not. In salt water, the pelvic spines help make the stickleback difficult for a predator to swallow, but the protuberances become a hindrance in fresh water as they could serve as handholds onto which dragonflies and other carnivorous insects grab.

In 2004, Kingsley and his colleagues tracked the disappearance of the pelvis in multiple populations of freshwater sticklebacks primarily to a gene called *Pitx1*. The gene is expressed during development in many places in both fish types. But it's active in the pelvic region of the saltwater animals and not in the freshwater ones, Kingsley's team found. Yet the gene's coding region is virtually unchanged between fresh- and saltwater fish, suggesting regulatory DNA is the difference.

The new work confirms that suspicion. When Kingsley, his graduate student Yingguang Frank Chan, now at the Max Planck Institute for Evolutionary Biology in Plön, Germany, and their colleagues studied fish with and without a pelvis, they determined that DNA upstream of *Pitx1* was responsible for silencing the gene. They broke that DNA into fragments and determined exactly which piece, an enhancer called *Pel*, turned *Pitx1* on in the pelvis. When the researchers compared the sequence of *Pel* in saltwater sticklebacks and freshwater ones from nine different lakes, they found the latter populations each had various amounts of missing DNA bases in the enhancer region, including an apparently key 488-base region that is absent in most of the lake fish.

"The fact that multiple alleles, with unique mutations, were observed is surprising and emphasizes the tinkering nature of the evolutionary process," says Patricia Wittkopp, an evolutionary biologist at the University of Michigan, Ann Arbor.

To confirm *Pel*'s importance, the researchers added a saltwater stickleback's version of the enhancer and *Pitx1* to the fertilized eggs of freshwater sticklebacks that don't normally sport pelvic spines. The resulting transgenic freshwater fry developed the structures.

For the insect study, evolutionary biologists Sean Carroll and Mark Rebeiz of the University of Wisconsin, Madison, and their colleagues followed up on work into why the normally yellow abdomens of fruit flies (*Drosophila melanogaster*) have become dark in some African populations living at high elevations. Earlier work done by collaborators John Pool of the University of California, Berkeley, and Charles Aquadro of Cornell University suggested that this color change was due to a change in the activity of a gene called *ebony*.

The new work narrows down the cause to an enhancer upstream of the gene. By dissecting the function of this region in different *Drosophila* populations, ones with either dark or light abdomens, the researchers identified five mutations that reduced *ebony* expression to varying degrees.

Three of those mutations are present in *Drosophila* with light abdomens, but the dark flies from high altitudes also have two newer mutations. These two have the biggest effects on squelching *ebony* expression, but all five mutations combine to create the full color change.

The work "leads to two very important conclusions about classic evolutionary genetic questions," says William Cresko, an evolutionary biologist at the University of Oregon, Eugene. One is that a "big" evolutionary step—a color change—actually can come about because of several little steps: multiple mutations in the enhancer. Secondly, it shows that adaptive mutations sometimes exist unnoticed in a population.

Others are also in hot pursuit of evolutionary changes based on regulatory elements. Wittkopp is homing in on mutations in a regulatory region that change body color between two closely related species of fruit flies (*Science*, 23 October, p. 540). And Hoekstra's group has a regulatory region in its sights that seems to underlie mouse color changes (*Science*, 28 August, p. 1095). But developing a broad understanding of the relative roles of shifts in gene regulation versus changes in the proteins encoded by genes "will require many more case studies from across a wide array of taxa," cautions Cresko. Given how much work the two new studies took, he adds, it may require a "quantum leap" in technology for that to be possible.

–ELIZABETH PENNISI

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