

NOVEMBER 15, 2005

Development Genes Evolve New Functions

For an animal to acquire a new form during evolution, the proteins that control its physical development sometimes take on new or altered functions through changes to the genes that encode them. But these proteins often carry out many essential roles that must be preserved for the animal to survive, and the function of most developmental proteins has been conserved throughout evolution. Now HHMI researchers have shown how those proteins can evolve new functions while retaining their old ones—enabling new animal forms to arise.

HHMI investigator Sean Carroll and HHMI predoctoral fellow Chris Todd Hittinger report their findings in the December 1, 2005, issue of the journal *Development*, published early online in November. Hittinger is first author on the paper. Carroll, his mentor at the University of Wisconsin-Madison, is the senior author.

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Findings from the growing field of evolutionary developmental biology, sometimes called "evo-devo," have been surprising because the genetic sequences controlling development are not as diverse as expected, given the diversity of the organisms themselves. In fact, the ability to conserve function is almost frightening in its precision, Carroll observed. Previous studies have shown that *Hox* genes swapped between species with seemingly little in common are able to maintain their function. Having long focused on the surprising genetic similarities between organisms, scientists such as Hittinger and Carroll are now tackling the underlying mechanisms that cause the differences.

Among the most highly conserved of the developmental genes are the *Hox* genes—a large family of genes best known for their role in controlling the pattern of body development. Like many developmental regulators, the

proteins produced by *Hox* genes control the activity of a diverse assortment of target genes. Due to their broad range of cellular responsibilities, even subtle changes to these proteins' functions may be detrimental to the organism, limiting the opportunity for evolution. Indeed, the function of Hox proteins—which are found in all higher animals—has remained virtually unchanged through time.

Nevertheless, there are rare examples of Hox proteins that have adopted new functions through evolution. To better understand what it takes for these highly conserved genes to evolve, the researchers analyzed a specific segment within a Hox protein known as Ultrabithorax (Ubx). In insects, Ubx prevents the development of limbs along the abdomen—but the same protein in other organisms lacks this function.

Fossil records show that insects' forebears had many legs, much like a centipede. Over time, however, insects lost their abdominal legs, retaining only the six located on the thorax. In modern insects, the repression of abdominal legs is partially attributed to a specific segment of the Ubx protein that scientists refer to as QA.

Using the fruit fly (*Drosophila melanogaster*), Hittinger, Carroll, and co-author David Stern at Princeton University deleted the portion of the *Ubx* gene that encodes QA—effectively reversing evolution by deleting this developmentally important protein sequence. “By physically altering the genome and removing a small part of Ubx implicated in limb repression, we've created the first insects in 300 million years that don't have this piece of protein,” said Hittinger.

Simply deleting QA did not cause the flies to grow abdominal legs. However, when the scientists further manipulated the flies' genes to reduce the expression of both the QA-deleted version of Ubx and another Hox protein, rudimentary abdominal limbs did form. This demonstrated that QA is no longer strictly required for leg repression in modern insects but is one of many regions of Ubx and the other Hox proteins now involved in leg repression.

The study showed that subtle changes in some of the proteins produced by the genes that regulate development, such as Hox, enable other proteins to evolve new functions.

“Hox proteins are central to the evolution of animal form, and this work offers us insights into how small changes in these proteins are used to fine-tune their activities in different kinds of animals,” said Carroll. His research has shown that the evolution of body parts more commonly occurs through changes in how development genes are regulated than through the evolution of new genes.

Deleting or “knocking out” a gene is more straightforward and such mutants have long been known for Ubx, but researchers are taking the next step to understanding evolutionary pathways. “It's much easier to knock out a gene, but here we actually remove and study the part of the gene that is

insect-specific and arose during evolution,” Hittinger said.

The redundancy found in the protein sequences that contribute to leg repression makes evolutionary sense, he added. “Backup systems may prevent catastrophic developmental failures from occurring when the embryo is stressed,” Hittinger explained. In fact, redundancy may provide development with a robustness that matters more in the wild, where conditions vary more than they do in laboratory-controlled conditions.

“The most interesting questions remaining for evolutionary geneticists are whether certain evolutionary paths are favored and what conditions cause them to be favored,” said Hittinger. He noted that their work shows that many small peptide sequences exist whose functions are poorly understood.