



Deciphering the Genetics of Evolution

Powerful personalities lock horns over how the genome changes to set the stage for evolution

Sitting quietly in the back of the seminar room, Hopi Hoekstra doesn't stand out as a rabble-rouser. But last year, this young Harvard University evolutionary geneticist struck a nerve when she teamed up with evolutionary biologist Jerry Coyne of the University of Chicago in Illinois to challenge a fashionable idea about the molecular mechanisms that underlie evolutionary change. Egos were bruised. Tempers flared. Journal clubs, coffee breaks at meetings, and blogs are still all abuzz.

For decades, the conventional wisdom has been that mutations in genes—in particular in their coding regions—provide the grist for natural selection. But some 30 years ago, a few mavericks suggested that shifts in how genes are regulated, rather than alterations in the genes themselves, were key to evolution. This idea has gained momentum in the past decade with the rise of “evo-devo” (*Science*, 4 July 1997, p. 34), a field born when developmental biologists began to take aim at evolutionary questions. They have proposed that mutations in regulatory DNA called cis elements underlie many morphological innovations—changes in body plans from bat's wings to butterfly

spots—that allow evolution to proceed. The idea has gained support from evidence that DNA outside genes—at least some of which are cis-regulatory elements—can be crucial to an organism's ability to survive and thrive over the long term.



Urging caution. Harvard's Hopi Hoekstra argues that genetic changes must be adaptive to count as important in evolution.

The zeal with which some biologists have embraced this so-called cis-regulatory hypothesis rubbed Hoekstra and Coyne the wrong way. In a 2007 commentary in *Evolution*, they urged caution, arguing that the idea was far from proven. The article sparked a sharp debate, with accusations from both sides that the other was misrepresenting and misinterpreting the literature. “What really got people upset is the tone of the paper,” says Gregory Wray, an evolutionary biologist at Duke University in Durham, North Carolina. A year later, fists are still flying—the latest skirmish took place in May on the *Scientific American* Web site—and several papers prompted by the debate have just been published.

Although both sides would agree that cis-regulatory changes and mutations in coding regions of genes themselves probably both play a role in evolutionary change, the debate has become so intense that the middle ground is sometimes lost. Those on the sidelines are calling for patience. “There are strong winds from both directions,” says evolutionary biologist David Kingsley of Stanford University in Palo Alto, California. “There are a handful of tantalizing examples of both coding and regulatory change, but the solution will come when lots of examples are worked out and worked out fully.”

The heat has fueled more careful looks at the evidence and

Diversity of form. Changes in regulatory DNA are implicated, but not always proven, in the evolution of morphological traits from a variety of organisms.

a push to find more examples of cis-regulatory changes behind evolutionary modifications. It has also stimulated discussions of related ideas about how evolution proceeds in a genome: the role of transcription factors, for example, and whether evolution is predictable, with certain types of changes being caused by mutations within genes and others by alterations in nearby DNA. "I think we are on the threshold of a very exciting time," says Wray.

Regulation and evolution

Early suggestions that gene regulation could be important to evolution came in the 1970s from work by bacterial geneticists showing a link between gene expression and enzyme activity in bacteria. About the same time, Allan Wilson and Mary-Claire King of the University of California, Berkeley, concluded that genes and proteins of chimps and humans are so similar that our bipedal, hairless existence must be the product of changes in when, where, and to what degree those genes and proteins come into play. They had drawn similar conclusions from studies of other mammals, as well as birds and frogs. But the tools to track down the molecular controls on gene expression and protein production didn't yet exist.

More than 2 decades later, David Stern, a Princeton University evolutionary biologist, was probing the genetic changes that result in hairless fruit fly larvae. Typically, *Drosophila melanogaster* larvae are covered with microscopic cuticular hairs called trichomes, but not those of a relative called *D. sechellia*. In 2000, Stern found that mutations in genes were not involved and that changes in the regulation of a gene called *shavenbaby* were the cause. Sean Carroll of the University of Wisconsin (UW), Madison, saw a similar pattern in his group's studies of pigmentation patterns in fruit flies and in 2005 wrote an influential paper in *PLoS Biology* that helped convince the field that cis-regulatory changes were central to morphological evolution.

Carroll argued that mutations in cis regions were a way to soft-pedal evolutionary change. Genes involved in establishing body plans and patterns have such a broad reach—affecting a variety of tissues at multiple stages of development—that mutations in their coding regions can be catastrophic. In contrast, changes in cis elements, several of which typically work in concert to control a particular

gene's activity, are likely to have a much more limited effect. Each element serves as a docking site for a particular transcription factor, some of which stimulate gene expression and others inhibit it. This modularity makes possible an infinite number of cis-element combinations that finely tune gene activity in time, space, and degree, and any one sequence change is unlikely to be broadly disruptive.

Data have been accumulating that suggest such regulatory changes are important in evolution. Take sticklebacks. In this fish, marine species have body armor and spines, but freshwater species don't. Four years ago, researchers tracked some of the difference to altered expression patterns in a gene called *Pitx1* but found no coding differences in the *Pitx1* gene of the two species (*Science*, 18 June 2004, p. 1736). "There's no doubt there's been a regulatory change," says Carroll.

Carroll, his postdoc Benjamin Prud'homme, and their colleagues discovered that closely related fruit flies vary in the pattern of wing spots used in courtship, and they have traced these changes to the regulation of a gene called *yellow* at the sites of the spots. Multiple cis-element changes—adding a few bases or losing others—have caused spots to disappear and reappear as *Drosophila* evolved and diversified, they reported in the 20 April 2006 issue of *Nature*.

Similarly, Carroll's group reported in the 7 March issue of *Cell* that various alterations in a cis element controlling a *Drosophila* gene called *tan*—which plays a role in pigmentation and vision—underlie the loss of abdominal stripes in a fruit fly called *D. santomea*. This species diverged from a dark sister species once it settled onto an island off the



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OF WISCONSIN, MADISON

west coast of Africa less than 500,000 years ago.

Bat wings, too, may have arisen in part from a change in a cis element regulating a gene, *Prx1*, involved in limb elongation. Chris Cretekos, now at Idaho State University, Pocatello, and Richard Behringer of the University of Texas M. D. Anderson Cancer Center in Houston isolated this cis element in the short-tailed fruit bat and then substituted it for the mouse version of this regulatory DNA in developing mice. The resulting mice had a different expression pattern of the gene and longer forelimbs than usual, Cretekos, Behringer, and their colleagues reported in January in *Genes and Development*. The mouse and bat *Prx1* protein differs by just two amino acids, which don't seem to affect its function, they note.

And there are several cases in plants where cis elements have proved important. Teosinte, the ancestor of domesticated corn, sends up multiple stalks, whereas corn grows via a single prominent one. In 2006, John Doebley and his colleagues at UW Madison linked this change to a difference in DNA several thousand bases from a gene called *teosinte branched 1*, indicating a role for non-coding cis elements in the evolution of corn.

"When you think about the sort of evolution we're interested in—why is a dog different from a fish—that

has to depend on changes in gene regulation," insists Eric Davidson, a developmental biologist at the California Institute of Technology in Pasadena.

Where's the beef?

But Hoekstra and Coyne say this enthusiasm doesn't rest on solid evidence. In their *Evolution* article, they picked apart these examples



and the rationale behind them. They pulled quotes from Carroll's work to criticize his fervor and berated the evo-devo community for charging full speed ahead with the cis-regulatory hypothesis. "Evo devo's enthusiasm for *cis*-regulatory changes is unfounded and premature," they wrote. Changes in gene regulation are important, says Hoekstra, but they are not necessarily caused by mutations in cis elements. "They do not have one case where it's really nailed down," she says.

Coyne and Hoekstra accept only cases in which a mutation in a cis element has been demonstrated to modify a particular trait, not just to be correlated with a difference. That's "the big challenge," says Hoekstra. In the stickleback case, for example, the fact that the marine species expresses *Pitx1* where spines develop and the lake species does not—although both have the same unmodified gene—doesn't prove that a cis element is responsible for the difference, Hoekstra and Coyne argue. Even Kingsley, who works on this gene in sticklebacks, agrees that the case isn't airtight. "We still need to find the particular sequence changes responsible for the loss of *Pitx1* expression," he says.

Furthermore, the duo insist that the modified trait must be shown to be beneficial in the long run. Thus, they dismiss the *shavenbaby* example not only because causative changes in cis-regulatory elements haven't yet been identified but also because no one really knows whether the fine hairs on fruit fly larvae confer a selective advantage. "I'm distressed that Sean Carroll is preaching to the general public that we know how evolution

works based on such thin evidence," Coyne told *Science*.

Coyne and Hoekstra also take issue with the notion that morphological changes are unlikely to be caused by mutations in the genes for body plans because those genes play such broad and crucial roles. Similar constraints apply across all genes, they argue. Processes such as gene and genome duplication and alternative splicing can provide room for evolutionary changes by enabling genes to take on new roles while still doing their original jobs, they note.

They point instead to a large body of evi-



Mice camouflage. Changes in the coding regions of genes underlie the coat color differences between a light, beach-dwelling subspecies of mouse and the brown mainland one.



Fruit fly fashions. Mutations in regulatory DNA help explain species differences, such as abdominal stripes and no stripes (*left*) and wings with and without spots (*above*).

dence indicating that so-called structural changes in protein-coding genes play a central role in evolution. They list 35 examples of such changes—including a mutation in a transcription factor—in a variety of species to bolster their case. They also point out that the small differences between the chimp and human genomes, which led Wilson and King to question whether mutations in coding regions can account for the differences between the species, still add up to plenty of meaningful gene changes—an estimated 60,000. "Adaptation and speciation probably proceed through a combination of *cis*-regulatory and structural mutations, with a substantial contribution of the latter," they wrote.

Beyond the debate

Almost as soon as their article appeared, lines were drawn and rebuttals planned. Carroll thought he was misrepresented. "I am not trying to say that regulatory sequence is the most important thing in evolution," he told *Science*. But when it comes to what's known about the genetic underpinnings of morphological evolution, "it's a shutout" in favor of cis elements, he asserts. By not accepting that body-plan genes are a special case, Hoekstra and Coyne "muddied clear distinctions that are based on good and growing data," he charges. Carroll also doesn't buy into the requirement that the new form needs to be shown to result in a selective advantage.

Günter Wagner, an evolutionary developmental biologist at Yale University, is also critical. "There clearly are well-worked-out examples where microevolutionary changes

can be traced back to cis-regulatory changes,” he says. Coyne and Hoekstra were “too harsh.” Other evolutionary biologists grumbled that because the article was an invited perspective it didn’t undergo official peer review.

On the other hand, William Cresko of the University of Oregon, Eugene, thinks it was high time for a reality check. Some researchers, he said, had become “complacent about the data.” Katie Peichel of the Fred Hutchinson Cancer Research Center in Seattle, Washington, agrees: The cis-regulatory hypothesis got “taken up without [researchers] realizing there are nuances. We haven’t solved morphological evolution.”

In spite of the intense rhetoric, the debate has had at least some humorous moments. At the IGERT Symposium on Evolution, Development, and Genomics in Eugene, Oregon, in April, Wray—who concluded in a March 2007 *Nature Reviews Genetics* piece that cis regulation was, for certain genes, more important than structural changes—and Coyne shared center stage as the keynote speakers. Coyne’s title was “Give me just one cis-regulatory mutation and I’ll shut up,” and he wore a T-shirt that said “I’m no CIS-sy.” Wray’s T-shirt said “Exon, schmexon!” suggesting that coding regions, or exons, didn’t matter all that much. (Carroll couldn’t make it to the meeting.) Yet in May, Carroll and “I’m no CIS-sy” faced off online on the *Scientific American* comments page.

On the positive side, the dispute has stimulated some new research. Rather than ask which type of change is more important, for example, Wray is examining

whether there are any patterns in the types of mutations that are associated with different types of genes. He has scanned the human, chimp, and macaque genomes for regions that are positively selected in each species, looking for stretches conserved in two of the species but much changed in the third. He kept track of whether the region is coding or noncoding and determined which genes are involved. This computer study gives a sense of what kinds of mutations are important in the evolution of various types of genes but does not tie specific sequence changes to particular altered traits. At the IGERT meeting, he reported that genes related to immune responses and basic cell signaling have evolved primarily through mutations in coding

regions. In contrast, changes in noncoding, regulatory DNA predominated for genes important for development and metabolism.

Stern has gone a step further. After looking at Hoekstra and Coyne’s paper, he and Virginie Orgogozo of the Université Pierre et Marie Curie in Paris did a comprehensive literature survey to ferret out any evolutionarily important mutations, dividing them according to whether they affected physiology (building muscle cells or mediating nerve cell transmissions, for example) or morphology—affecting body plan development. Unlike Hoekstra and Coyne, they included data on domesticated species and didn’t demand that the change be clearly adaptive. Overall, cis-regulatory changes represented 22% of the 331 mutations cataloged. However, in comparisons between species, cis-regulatory mutations caused about 75% of the morphological evolution,



Friendly fight. Keynote speakers Greg Wray (left) and Jerry Coyne promoted their take on the genetic basis of evolution with custom T-shirts.

they report in an article in press in *Evolution*. The data indicate that both types of changes affect both types of traits, with cis-regulatory ones being more likely for morphological trait changes between species, Stern says.

Yet even these data are inconclusive, Stern warns. Because developmental biologists focus on expression patterns, and physiologists on the proteins themselves, the former tend to find regulatory changes and the latter, coding-region alterations, potentially biasing which trait depends on which type of mutation.

Also, coding changes are more likely to be identified than changes in regulatory regions in part because once a gene is linked to a trait

it is easy to assay for mutations there. “It’s like shooting fish in a barrel,” says Carroll. In contrast, regulatory DNA is harder to pin down. It can be close to or far from the gene itself, and a given gene could have several regulatory elements, any one of which might have the causal mutation. Thus the numbers may be misleading, a point also made by Hoekstra and Coyne. “It’s really difficult to say that one’s going to be more important than the other,” says Stern. But it’s clear that cis regulation is important, he adds. “I really want to emphasize that evo-devo [researchers] haven’t come to this way of thinking simply through storytelling. We came to it through the data.”

To complicate matters further, mutations in coding regions can themselves alter gene regulation. As part of their take on the debate, Wagner and Yale colleague Vincent Lynch make the case in an article published online on 22 May in *Trends in Ecology & Evolution* that mutations in transcription factors can lead to evolutionarily relevant modifications in gene expression. For example, variations in a repetitive region of the gene *Alx-4*—which codes for a transcription factor important for toe development—can alter expression patterns and change body plan in dogs. Great Pyrenees are missing 17 amino acids in this region compared with other dog breeds, and these 45-kilogram pooches have an extra toe that other breeds lack. “This is an important part of gene regulatory evolution,” says Wagner.

Researchers are also trying to figure out where noncoding RNAs fit in, how gene duplications make way for change, and what roles even transposons and other repetitive DNA may play.

“The important question is about finding out whether there are principles that will allow us to predict the most likely paths of change for a specific trait or situation,” says Patricia Wittkopp of the University of Michigan, Ann Arbor.

With so much unknown, “we don’t want to spend our time bickering,” says Wray. He and others worry that Hoekstra, Coyne, and Carroll have taken too hard a line and backed themselves into opposite corners. Coyne doesn’t seem to mind the fuss, but Hoekstra is more circumspect about their *Evolution* paper. “I stand by the science absolutely,” she says. “But if I did it over again, I would probably tone down the language.”

—ELIZABETH PENNISI