EVOLUTION

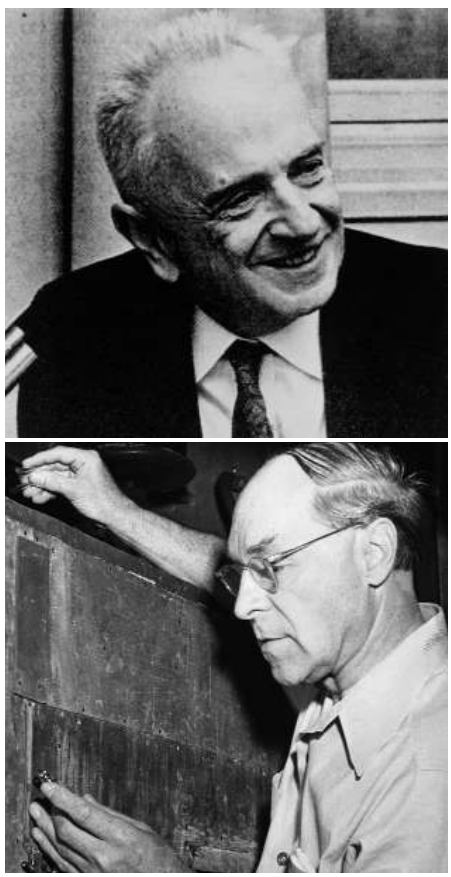
Two Rapidly Evolving Genes Spell Trouble for Hybrids

Evolutionary geneticists are pinning down pairs of genes that help promote speciation; these genes are rapidly evolving, but not in response to ecological pressures

New species arise when populations become separated and evolve along different paths until, eventually, their members can no longer breed successfully with each other. That was Darwin's revolutionary insight, and it has shaped our understanding of the natural world. But the underlying mechanism has been hard to pin down. Why, for example, do even closely related species have difficulty producing viable offspring? Hybrids, if they survive at all, tend to be less fit than their parents. And therein lies the crux of speciation.

Now, one group has nailed down a 70-year-old theory about why hybrids are usually doomed to failure. On page 1292, Daniel Barbash, a geneticist at Cornell University, and his colleagues report the identification of a pair of genes that are key to making two closely related fruit fly species reproductively incompatible. Other groups are closing in on genes that cause problems for hybrids in monkeyflowers and marine invertebrates called copepods. In each case, the genes appear to be evolving rapidly, implying that they are under selective pressure. It's the "beginning of a new phase in speciation research, where we can get at both the specific genetic mechanisms and [the] interactions underlying one of the most fundamental questions in evolutionary biology," says Mohammed Noor of Duke University in Durham, North Carolina.

This work supports a theory first proposed in 1937 by Theodosius Dobzhansky and independently a few years later by Hermann Joseph Muller. They suggested that the root cause of hybrid failure is that pairs of genes whose proteins interact with each other—for instance, an enzyme and the protein it breaks down—evolve along different paths after populations split. In each population, the gene pairs evolve in concert so that their protein products continue to work together. But, said Dobzhansky and Muller, eventually the proteins in the individuals in one population will have changed so much that they no longer work properly with their former partners in the other population. When mixed back together in hybrids, these proteins are incompatible—an enzyme from one population will no longer break down the target protein from the other, for example—and potentially lethal problems arise: Hybrids may be sterile or may not survive at all.



Hybrid hypothesis. Independently, Theodosius Dobzhansky (*top*) and Hermann Joseph Muller proposed that incompatible genes could kill hybrids, speeding speciation.

The Dobzhansky-Muller model gained wide acceptance. "It's really our best general model of how mutations can accumulate to cause reproductive isolation," says Hopi Hoekstra, an evolutionary biologist at the University of California, San Diego. Confirming the details, however, has been challenging. "The problem is really, really hard because what you are trying to do is genetics between species," says H. Allen Orr, an evolutionary geneticist at the University of Rochester in New York.

Over the years, researchers have found evidence supporting parts of the Dobzhansky-Muller model but not all of it. Typically, researchers find one gene but not its putative partner. For example, for decades, researchers have known that crossing two aquarium fish—a platyfish and a swordtail—has dire consequences. The offspring develop large black spots, and crossing the hybrid back to a parent often results in lethal skin tumors. Cancer researcher Manfred Schartl of the University of Würzburg, Germany, tracked down a causative gene, *Xmrk2*, on the X chromosome. He knows that it interacts with a "suppressor" gene that keeps *Xmrk2* in check and suspects that *Xmrk2* and the suppressor have diverged across the two species so they no longer interact effectively. However, to this day, the true identity of the suppressor remains unknown.

Drosophila researchers were also stumped for a long time. They could produce offspring by mating *D. melanogaster* with *D. simulans*, *D. mauritiana*, or *D. sechellia*, but too few offspring survived for researchers to carry out additional breeding experiments. Takao Watanabe came to the rescue in the 1970s when he discovered a mutant strain of *D. simulans* that could hybridize quite successfully with *D. melanogaster*. Watanabe, a geneticist at the National Institute of Genetics in Mishima, Japan, surmised that somewhere in its genome, the *D. simulans* strain carried a mutant gene that interacts successfully with a partner in *D. melanogaster*. He called the unidentified gene *lhr* for "lethal hybrid rescue." The finding "jump-started the field," says Barbash.

In the late 1980s, Michael Ashburner and Pierre Hutter of the University of Cambridge uncovered evidence for a similar gene in *D. melanogaster*, calling it *hmr* for "hybrid male rescue." They didn't know the exact location or identity of this gene, but crosses between *hmr* mutant strains and *D. simulans* worked just fine. With these strains in hand, researchers were able to produce viable hybrids, and they began modifying the genomes of the parents further to track down the specific genes involved in hybrid sterility and lethality.

On to the genes

Barbash picked up where Watanabe and Ashburner and their colleagues left off. In 2003, he and his colleagues pinpointed and sequenced the *D. melanogaster hmr* gene and discovered that it was a transcription factor. A year later, he and Philip Awadalla of North Carolina State University in Raleigh and colleagues demonstrated that the *hmr* genes had indeed diverged functionally between the two species. When they put an intact copy of *D. melanogaster hmr* into the *hmr* mutant strain, hybrids with *D. simulans* died as larvae. But when they repeated the experiment

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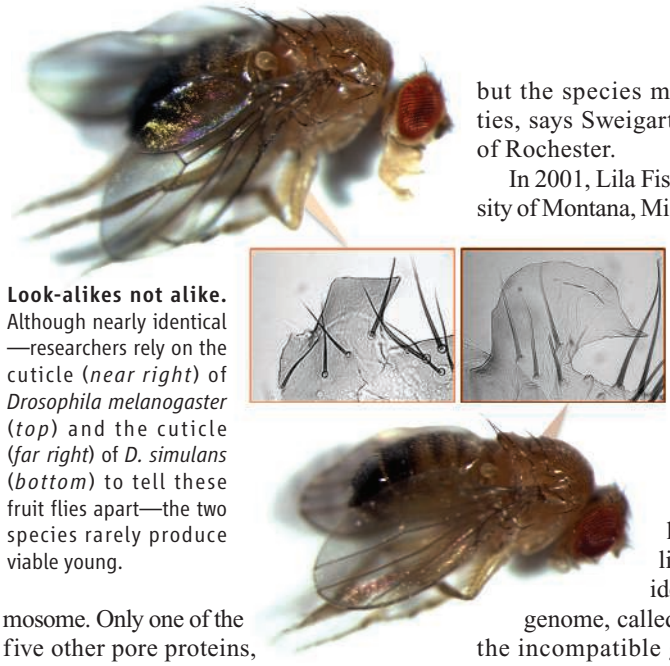
with an intact *hmr* from *D. simulans*, hybrids survived, Barbash reported. When they compared the differences in 250 genes between the two species, they found that *hmr* was one of the most rapidly evolving.

With one gene that fulfilled Dobzhansky and Muller's expectations in hand, Barbash began to chase down its partner. He focused on *lhr*, as several earlier studies suggested that *lhr* and *hmr* worked as a pair. The rough location of *lhr* was already known but not its identity. With the help of the newly generated genome sequence data for *D. simulans*, Nicholas Brideau, Jun Wang, and Heather Flores in Barbash's lab looked for genes whose sequence indicated that their proteins could interact with the hmr protein. They concentrated on one that had not only diverged quite a bit from its counterpart in *D. melanogaster* but is also mutated in Watanabe's *D. simulans* strain.

Brideau, Wang, and Flores designed an ingenious experiment to test whether they had the correct gene. They put the candidate *lhr* gene from *D. simulans* into *D. melanogaster* and mated the resulting fruit flies with Watanabe's *D. simulans* strain. If the candidate gene was indeed *lhr*, its presence in *D. melanogaster* should override the mutant *lhr* in *D. simulans* and result in dead hybrids. It did. Barbash's group has confirmed that the lhr and hmr proteins interact. "We don't understand the mechanistic or molecular basis of the interaction," Barbash says, "but both genes in combination are required to kill the hybrid."

Scores of other incompatible gene pairs have likely evolved over the millions of years that fruit flies have diverged. Daven Presgraves, an evolutionary geneticist at the University of Rochester, is well on his way to pinning down a second pair. In 2003, after devising a way to screen for hybrid lethality genes, he turned up with one called *Nup96*, which codes for a protein that is part of the nuclear pore in eukaryotes. To begin to track down *Nup96*'s partner, he and Wolfgang Stephan of the University of Munich, Germany, took a close look at five of the 30 other fruit fly pore proteins to see how they differed between *D. melanogaster* and *D. simulans*. To their surprise, all five are evolving quite fast, they reported online 20 October in *Molecular Biology and Evolution*. The screen Presgraves used to identify *Nup96* detects only those genes whose interacting partner is on the X chromosome. Only one of the five other pore proteins, called Nup153, has that genomic address. "We are certainly hot on the trail" of pinning down *Nup96*'s incompatible partner, says Presgraves.



Look-alikes not alike. Although nearly identical—researchers rely on the cuticle (*near right*) of *Drosophila melanogaster* (*top*) and the cuticle (*far right*) of *D. simulans* (*bottom*) to tell these fruit flies apart—the two species rarely produce viable young.

Although much of the progress in identifying Dobzhansky-Muller gene pairs comes from fruit fly studies, researchers are starting to track down these genes in other species. In the monkeyflower, for instance, they have narrowed the search to relatively small chromosomal regions. In other cases, such as copepods, two genes are in hand, but their relationship is known primarily through test-tube studies and not through genetic analyses.

While a graduate student with John Willis at Duke University, Andrea Sweigart tracked down the cause of hybrid sterility in two closely related species of monkeyflower. One, *Mimulus guttatus*, is pollinated by insects, while the other, *M. nasutus*, is self-fertilizing. Both species occur in western North America but tend to grow in different habitats. Hybrids do form where they coexist, but the species maintain distinct identities, says Sweigart, now at the University of Rochester.

In 2001, Lila Fishman, now at the University of Montana, Missoula, and Willis showed that second-generation hybrids suffer from male sterility, suggesting genetic incompatibilities were at work. From extensive breeding and genetic mapping studies between the two species and between hybrids and the parental lines, Sweigart and Willis identified two places in the genome, called hms1 and hms2, where the incompatible genes are located, they reported in the April issue of *Genetics*.

Ronald Burton of the Scripps Institution of Oceanography in San Diego, California, has found two interacting genes that may be helping to isolate different populations of copepods, a Californian intertidal invertebrate. He and his students have found that the gene for the protein cytochrome c, which is important for electron transport and energy generation, varies across copepod populations. Test-tube studies indicate that these variations affect the efficiency of the protein's reaction with cytochrome c oxidase, suggesting that these two could be genetically incompatible in hybrids.

Bad match. Sister species, the platyfish (*top left*) and the swordtail (*top right*) can interbreed, but hybrids (*bottom*) often develop deadly melanoma tumors.

Selective pressures

These new findings have thrown up some surprises. In particular, the genes behind hybrid lethality are evolving and adapting at an unusual pace compared to the rest of the genome. "Almost all these genes have a strong signature of natural selection," says Hoekstra. Yet the genes seem unlikely candidates for rapid evolution. The lhr protein is associated with heterochromatin, the parts of chromosomes containing lots of repetitive DNA, and nuclear pores are conserved from yeast to humans. "You just don't expect those genes to evolve rapidly," says Presgraves.

The fact that nucleoporin genes evolved quickly in species that are widely separated geographically suggests that ecological factors are not at the root of those gene changes, Presgraves adds. Indeed, notes Jerry Coyne, an evolutionary biologist at the University of Chicago in Illinois, "where the action is going to be is to [learn] what kind of natural selection is acting on these genes." The answer is unlikely to take another 70 years. **–ELIZABETH PENNISI**

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