zooplankton-feeders mated with small zooplankton-feeders, while the large insect-feeders chose other insect-feeders, and new species were formed.

A similar story seems to have unfolded a continent away, where the University of British Columbia's Schluter has been studying stickleback fish that speciated in tiny lakes carved out by glaciers just 13,000 years ago. Like the cichlids, the sticklebacks prefer different foods within the same lake and mate with sticklebacks of the same size—although they don't mate where they eat, so the assortative mating seems to be based on fish size and shape rather than habitat preference (but could be the indirect result of different food choice). And again, genetic studies by Eric Taylor of the University of British Columbia show that pairs of species found within the same lake are more closely related to each other than to any fish outside the lake, which indicates that the species formed in sympatry, says Schluter.

A frequent occurrence?

Just how often this kind of process creates new species is still a matter of dispute. "I think that ... after a major episode of extinction or other event that creates lots of empty niches, sympatric speciation is common," says Rice. Plant-feeding insects may be particularly adept at finding new opportunities to diverge from each other sympatrically when a new plant is introduced to their range, for example. And fish may go through similar bursts of speciation when a founder population finds a newly formed lake where there is little competition for resources. By contrast, when ecological niches are already full, most organisms may require geographic isolation to form new species.

Mayr, who thinks the cichlids in Cameroon are the most convincing case of sympatric speciation, is nonetheless skeptical that it occurs often: He thinks that sympatric speciation is responsible for "less than 1%" of all new species. Population genetics models show that it takes very little interbreeding to erase differences between two populations, such as the apple maggots and hawthorn flies.

But new studies of other creatures, ranging from ermine moths and *Heliconius* butterflies to aphids, sockeye salmon, and sea urchins, suggest that sympatric speciation has occurred more than a few times. These studies are also allowing biologists to refine ideas about the conditions under which sympatry might occur (see following story). While those refinements are being made, Bush now has the satisfaction of knowing that the debate is seldom over whether sympatry exists at all—but over how often and why it happens.

–Ann Gibbons

EVOLUTIONARY BIOLOGY

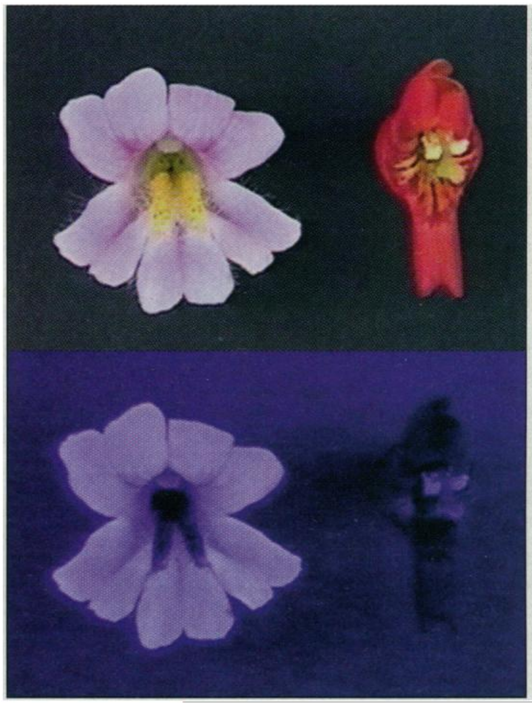
Starting Species With Third Parties and Sex Wars

It often takes a powerful barrier to make a new species. For some kinds of finches, isolated on islands, it was the open ocean that set them on separate evolutionary paths; for certain grasshoppers, it was mountain ranges. But three wasp species may have been pulled apart by nothing stronger than three distinct strains of bacteria living in their guts. Somehow, University of Rochester biologist John Werren has found, the bacteria in the females destroy male DNA from different species, keeping cross-species matings from producing offspring.

And perhaps the wasps aren't so unusual. Evolutionary biologists are fond of noting that in *On the Origin of Species*, Charles Darwin actually wrote very little about how species originate. To fill the theoretical vacuum, scientists have invoked geographic barriers as the usual way populations achieve reproductive isolation, the classic definition of a species. While this is still seen as a major mechanism, researchers now acknowledge that groups standing shoulder to shoulder can split apart as well, in a process known as sympatric speciation (see previous stories). And that process has more than one face, as the speciation conference in California this spring showed.

"The meeting clearly illustrated the diversity of ways that species can diverge," notes Guy Bush, an evolutionary biologist at Michigan State University, in whose honor the conference was held. Researchers described how such factors as bacterial agents, mimicry, changes in a single pigment gene or in the song of a bird, or the rapid evolution of a sperm surface protein can all trigger cascades of events leading to a new species, without any geographic barrier. Meeting participants also heard how another phenomenon that occurs within a single territory, the formation of hybrids—which have often been dismissed as the "mistakes" or "mules" of evolution—may play a much bigger role in the establishment of new taxa than has been previously believed. What has opened researchers' eyes to all these species-making processes, says ecologist Dolph Schluter of the University of British Columbia, are modern methods entailing molecular markers and genetic analysis, as well as time-honored, detailed observation. Researchers have been using these tools to take a much sharper look at populations in the wild, organisms' mating behavior, the basic biology affecting their fertility, and the flow of their genes. "That's what's been missing from speciation research—the whole natural history side of the organism," he says.

Third-party intervention. Often, this observational picture has to include a third party—another organism that helps create a barrier between two species. That is especially true of plants, many of which need an insect or bird pollinator to cross-fertilize. University of Washington evolutionary ecologist Doug Shemske and his colleague, plant geneticist Toby Bradshaw Jr., are investigating how pollinators can influence speciation in a study of two sister species of monkey-flowers (*Mimulus lewisii* and *M. cardinalis*). "It's clear that the pollinators contribute to their reproductive isolation," says Shemske, noting that in his lab the two plants easily produce fertile hybrids. In the monkey-flowers' native western mountain


D. SHEMSKE

Flower power. *(Top) Mimulus lewisii* and *M. cardinalis* are closely related. But bees are attracted to the contrasting colors in *M. lewisii (bottom left)*, so they pollinate it rather than *M. cardinalis (right)*, which helps keep the species apart.

habitat, however, botanists have yet to find such hybrids, even though in some regions, such as the Sierra Nevada, the plants' ranges overlap. It's the birds and the bees that keep the two monkey-flowers from mingling: *M. lewisii*'s pink blooms attract bumblebees, while *M. cardinalis*'s red blossoms call only to hummingbirds.

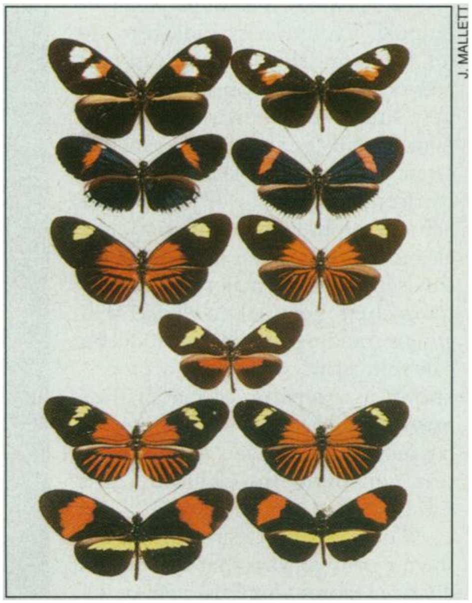
Yet at some point in the past, the two plants descended from a common ancestor, probably a bee-pollinated plant, speculates Shemske. How then did *M. cardinalis* arise? Something must have made an ancestral monkey-flower population more attractive to birds, and that something, Shemske says, required only a minor genetic rejiggering. "We want to get to that branching point," he explains, "and identify the genes that changed first." The researchers' previous work (*Nature*, 31 August 1995, p. 762) had pointed to three major contenders: sets of genes linked to a flower's color, size, and amount of nectar.

Shemske speculated that changes in just a few genes would be enough to isolate a population by attracting different pollinators, and his latest tests bear that out. Because bumblebees have poor color vision in the red end of the spectrum, they are not attracted to *M. cardinalis*. *M. lewisii*'s blossoms are a different matter. Their pale pink petals not only beckon to bees, but are even marked with yellow stripes, so-called nectar guides, which "look just like a runway to a bee," says Shemske. In orange- and reddish-colored hybrids, however, the yellow pigment gets distributed throughout the petal, obscuring the nectar guides—and reducing the number of visits by bees. "The yellow pigment is regulated by the single well-known gene, called *yup*," says Shemske, "and just by changing that one gene, we see a complete alteration in the bee's behavior." And that is exactly what you need for the first stage of speciation, he adds: "the evolution of a characteristic that reduces the gene flow between individuals in a population."

To try to determine which of the three traits may have changed first, this summer Shemske has placed potted second-generation hybrids in a Sierra Nevada test plot. These plants contain a mix of colors and flower shapes, allowing the researchers to see what might be especially attractive to bees or hummingbirds. Shemske is also breeding "mutants" of both plants that will swap distinctive characteristics. For example, some "mutants" will bear flowers identical to *M. lewisii* in shape and nectar, but colored red like those of *M. cardinalis*. "This should show us how much reproductive isolation is achieved by a single gene change," he explains, "and should get us very close to recreating the initial mutation."

Sometimes even smaller factors can block gene flow, as in the case of Werren's bacteria. He has found three different strains of *Wolbachia* bacteria in three species of the parasitic wasp *Nasonia*, where they reside in the females' eggs and in cells of the male reproductive tracts. The females pass the *Wolbachia* in their eggs from generation to generation, much as mitochondria are passed strictly from mother to offspring. And those bacteria, Werren has found, can destroy sperm that didn't develop in testes with the same bacterial strain. Werren isn't sure how this happens, or how the bacteria from the female recognize sperm with the "proper" infection—"perhaps the bacteria produce proteins that bind to the sperm's DNA," says Werren—but experiments demonstrate that when the bacteria do not match, the male genome is lost.

Unless, however, the wasps get some help from antibiotics. In the lab, the different species of insects all willingly mate—although to no avail—but after Werren and Hans Breuwer treated the wasps with antibiotics, the interbred species had no problem producing viable progeny. "In effect we found we could 'cure' speciation," Werren says.


J. MALLETT

New look. Although all are brightly colored to warn predators of their unpleasant taste, different races of *Heliconius* butterflies nonetheless have an extraordinary variety of patterns, which may eventually lead to new species.

Werren has now found similar types of symbiotic bacteria in four orders of insects from tropical and temperate zones, and he speculates that the parasites might explain part of the notable insect diversity. "They could be a factor in speciation," he says.

Competing sexes. Sometimes it doesn't take interference by a third party for former partners to miscue on their signals, setting them on the road to becoming separate species. In the case of *Echinometra mathaei*, the Pacific sea urchin species studied by Stephen R. Palumbi, an evolutionary biologist at Harvard University, that miscommunication happens when a female urchin's eggs fail to recognize the sperm from a would-be mate. The source of the confusion: the sperm attachment protein, called bindin, which comes in a number of varieties. "You'd think it [the bindin] would be invariant within a species" to assure fertilization, says Alan Templeton of Washington University in St. Louis. "And therefore this kind of variation is a big surprise."

The variation splits the sea urchin species into "mating guilds," explains Palumbi. In laboratory experiments, Palumbi and his team have shown that "sperm from one guild are better at fertilizing the eggs from that guild than they are at fertilizing the eggs of another," he says. They traced the eggs' choosiness to bindin by showing that males from the same guild carry sperm with nearly identical bindin, but in males from different guilds the protein differs by as many as 22 amino acids. Those differences speak volumes to the eggs' receptors: They simply don't respond to sperm lacking the right code. If nothing happens to restore gene flow between the guilds, Palumbi thinks they may be on the path to becoming separate species.

Palumbi speculates that the shifty bindin may actually have evolved because of actions by the females. Eggs may be changing the makeup of their receptors to protect themselves from being fertilized by more than one sperm—an event that spells the death of the zygote. Bindin thus may be varying its own makeup to keep up with these receptor changes. He is now investigating the egg's receptors to see if they too show signs of rapid variation.

If so, Palumbi notes, the sea urchins would be a prime example of the kind of evolutionary arms race between the sexes that William R. Rice, an evolutionary biologist at the University of California, Santa Cruz, proposed last May as a possible engine of speciation among fruit flies (*Nature*, 16 May, p. 232). After all, says James L. Patton, an evolutionary biologist at the University of California, Berkeley, something has to get groups moving in different directions: "You need some way of getting variation within a species, before you can get variation between species."

Hybrids—leaks in the barriers. Neither bindin nor bacteria are perfect barriers; sometimes fertilization does occur across group lines. The result is a hybrid. Current evolutionary theory views hybrids with mistrust, supposing most to be less fit than their parents and therefore doomed to failure. But that isn't always the case, argues Michael

The Species Problem

Everyone from politicians to population biologists uses the notion of a species. Geneticists compare different species to understand how genes evolve, ecologists use them to define the boundaries of ecosystems, and policy-makers use endangered species to argue for protecting lands. Yet biologists have not been able to agree on what a species is, exactly. And the definitional muddle has made it difficult, for instance, to classify organisms or show how a new species forms. "The definition of species is a constant thorn in the side of progress in speciation research," says University of British Columbia ecologist Dolph Schluter, who studies speciation in what may or may not be two species of stickleback fish.


T. HATFIELD

One species or two? These sticklebacks may be separate species—or not, depending on the species definition.

By the leading textbook definition, the sticklebacks probably don't count as two species. The two groups of fish interbreed occasionally and produce viable offspring, which disqualifies them from species status under a strict interpretation of the "biological species" concept, published by Harvard University biologist Ernst Mayr in 1942. Says Mayr, "I've always defined species as a reproductive community that is isolated ... from other such species" by "isolating" mechanisms. Those mechanisms are biological differences among individuals that prevent the populations from interbreeding. Even though individual pintail and mallard ducks, for example, can mate to produce offspring, the hybrids don't do well enough to establish a new group, so the two parent populations stay distinct and are usually recognized as separate species.

But other biologists say this rigorous standard isn't always realistic. "Reproductive isolation is a kind of mystical definition, in that you know it when it's absolutely complete, but actually there are plenty of examples of species that do hybridize in the wild," says evolutionary biologist James Mallet at University College in London. Coyotes interbreed with wolves and dogs, blue whales interbreed with fin whales, and many species of Protozoa, lower Metazoa, and plants do as well. "Are we going to say those aren't species?" asks Mallet.

The shortcomings of this concept have sent biologists looking for alternatives. Systematists, who classify organisms, often do so based on obvious differences in populations' morphology and genes. Mallet has tried to formalize this practice with his "genotypic cluster definition." It requires populations to fall into two distinct clusters based on differences in their genes or morphology if they are to qualify as separate species. His definition tolerates interbreeding as long as the genetic traits being studied stay distinct in two clusters.

But this is hardly the only alternative to Mayr's classical species concept. Ecologists often rely on an "ecological species" definition, for example, which defines species as groups that occupy different ecological niches—populations of identical-looking multicellular organisms called rotifers that live in different kinds of ponds or puddles, for example. Evolutionary biologists trying to trace the origins of species often use the "evolutionary species" concept, which says that a species is a single lineage of ancestral-descendant populations that remains separate from other such lineages in an evolutionary tree. Biologists using a classification scheme called cladistics identify these distinct branches on the evolutionary tree from unique "derived" characters: traits that appear in a descendant population but not among its ancestors or any contemporary relatives. The black-and-white markings on the tail of the Ethiopian wolf, for instance, separate it from the gray wolf, the species it is thought to have evolved from.

Others have tried to bring some of these ideas together under a conceptual big tent, such as the "cohesion species concept," proposed by geneticist Alan Templeton of Washington University in St. Louis. But scientists would still like to winnow the definitional diversity, so that when researchers such as Schluter publish on stickleback speciation, others won't voice doubts that he was looking at separate species in the first place. "Perhaps the best we can do is to agree to disagree in a rational manner" and agree on a limited set of concepts, says entomologist Stewart Berlocher of the University of Illinois, Urbana-Champaign.

–Ann Gibbons

Arnold, an evolutionary biologist at the University of Georgia. "[Harvard biologist] Ernst Mayr has said that hybridization isn't important in the speciation process; that hybrids will never get off the ground," he notes. "But, in fact, natural hybrids are produced that are very fit, and sometimes they lead to new evolutionary lineages."

Take the case of two species of finches on the island of Daphne in the Galápagos, which Princeton University biologists Peter and Rosemary Grant and their team have studied since the early 1970s. The cactus finch (*Geospiza scandens*) and the medium-beaked ground finch (*G. fortis*) "are kept apart by their songs. They are a barrier to gene flow," explains Rosemary Grant. The songs are passed down within a family, from father to son, as the Grants learned by recording the songs of fathers and sons in both species. Properly imprinted birds show no interest in the trills of the other species (and consequently do not mate with them).

But there are the occasional natural mishaps. The Grants observed cases when a *G. scandens* father died and his sons subsequently overheard a male *G. fortis* singing. They learned his song and ended up attracting and mating with *G. fortis* females. The *G. scandens* daughters also learned the wrong mating song and like their brothers chose the wrong mate.

Until 1983 such mishaps went nowhere, for the resulting hybrids always died, apparently because their beaks were unlike those of either parent and so weren't suitable for the available food supply. Because of their "intermediate bill size," the hybrids needed an "intermediate diet," says Rosemary Grant. But the small, soft seeds they required did not last much beyond the beginning of the island's dry season, and so the hybrids starved to death.

But in 1983, the island's weather changed, thanks to the arrival of a warm El Niño current. Suddenly, the parched landscape became "almost like a meadow," says Rosemary Grant, "with masses of annual plants." These choked out the cacti that formed the staple of the G. *scandens*'s diet, causing that species' population to plummet, until only 16 pairs remained. In the meantime, the hybrids' numbers increased, as they feasted on the huge supply of soft annual seeds. For 13 years, the weather was on their side, although today, now that the island is drying out, the hybrids may again have trouble. Yet without the hybrids and their back-crosses (a *scandens* mating with a *scandens/fortis* cross), Grant doubts that *scandens*'s genes could have survived. "*Scandens* was getting close to extinction," she says. The hybrids also increased the genetic variation in both species, she adds, preventing either one from becoming too inbred. Most significantly, adds Grant, "if this pattern had continued, I think that *fortis* and *scandens* would have fused into one species."

Arnold agrees. "That's exactly the kind of event that can, given enough time, produce a new species," he says. Nor should such hybrid vitality come as a complete surprise, he adds, noting that botanists estimate that as many as 70% of all flowering plants come from hybrids. He points out that studies of lizards, toads, and fish suggest that many animal species also have higher successful hybridization rates than many zoologists believe (*Trends in Ecology and Evolution*, February 1995, vol. 10, p. 67). "Evolution is driven by new mutations, so while the vast majority of hybrids will fail, you're going to occasionally have that rare success story—which is what Darwinian theory predicts," argues Arnold.

To try to determine what might make that success story possible, Arnold is studying the natural hybrids from two species of wild Louisiana irises, *Iris fulva* and *I. brevicaulis*, and comparing the fitness of each hybrid to that of the parental stock.

The hybrids can be divided into eight genotypes, he says, some of which are more fit, or reproduce more successfully, than others. For the most part, "the hybrids that are the least fit fall in between the two parental types morphologically," says Arnold, while the more fit plants are more like one parental stock or the other.

Yet there are also those hybrids that are even more successful than either parent. Apparently, he says, this is because they contain some mutation that enables them to venture into a new environment. The two parental species prefer distinct habitats: *I. brevicaulis* grows in dry, hardwood forests, while *I. fulva* is found in semiaquatic terrain. But some of their successful offspring have sprouted—and are thriving—in a different semiaquatic environment containing species of plants that are never found with *I. fulva*. This is the type of innovation that might kick off a speciation event, Arnold says.

Berkeley's Patton and others caution that before hybridization is given a starring role in speciation, Arnold needs "a finer way of distinguishing between 'successful' and 'unsuccessful' hybrids. Right now, his categories are just too broad, I think," Patton says. Still, Arnold thinks that hybrid "novelty can lead to new lineages."

The role of novelty. Novelty can also arise via chance mutations. Toss in some mimicry, genetic drift, and natural selection,


M. ARNOLD

Hybrid zone. Researchers are crossing *Iris fulva (left)* and *I. brevicaulis (right)* under different conditions to identify circumstances that make hybrids thrive.

and you've got a recipe for speciation, argues James Mallet, an evolutionary biologist at London's University College. Indeed, that combination may explain the bewildering variety of species and races seen in the South American passion-flower butterfly, *Heliconius erato*, says Mallet, who is studying them with Owen McMillan and Chris Jiggins.

H. erato is an extremely common butterfly, with at least 30 races, distinguished by different color patterns, living in tropical forests throughout South America. Part of the butterfly's success, explains Mallet, is probably due to the foul taste of all the varieties. They advertise their unpleasantness to would-be predatory birds via their brightly colored wing patterns. Successful warning signals are mimicked by other races and species of *Heliconius*—but in a curious manner: While species and races within any one region imitate each other, the pattern they are mimicking changes dramatically every 300 to 600 kilometers.

Thus there are regions of overlapping species or races that are patterned entirely differently from one another, creating opportunities for further divergence and speciation. "Wherever you've got two such entirely different forms—such as the Darwin finches with variable beak width—you've got strong pressure for divergence," says Mallet. Driving that pressure in the butterflies are the predatory birds, which learn which patterns taste bad. Patterns that are better at teaching this lesson are maintained or even spread. New patterns usually are doomed to failure, because the birds don't recognize them and attack. But occasionally, a "very good one will appear and given the right opportunity will spread."

That opportunity can happen in regions where there is a low density of one color pattern or where neither of two established patterns is dominant. Through genetic drift a butterfly bearing the new pattern may get lucky and pass a lot of its genes on to the next generation. And if the novel coloration continues to outperform the old, then—like a hot new fashion on Rodeo Drive—the newer model will spread.

Mallet believes an event like this underlies the divergence of *H. erato* and *H. himera*, a closely genetically related but differently patterned species. The latter, genetic studies indicate, began to diverge from *H. erato* 1 million years ago, perhaps initiated by a random color-gene change, says Mallet. That first step has been strengthened by the butterflies' preferences for two different habitats (*H. erato* likes a wetter forest), mate choice, and the birds: Even though the two produce viable hybrids, these are readily picked off because they don't bear a pattern that the birds have learned to avoid, thus pushing the species further apart.

"It's studies like these," says Patton, "where the organisms' natural history, biogeography, and genetics are known, that give us the best windows" on speciation. With such a diversity of organisms in the world, he and other biologists say, it should come as no surprise that there is a diversity of ways to produce them.

–Virginia Morell

Additional Reading

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Edward C. Metz and Stephen R. Palumbi, "Positive selection and sequence rearrangements generate extensive polymorphism in the gamete recognition protein bindin," *Molecular Biology and Evolution* **13** (2), 397 (1996).

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