
FEATURES

Two captive *Heliconius* species mate, illustrating the rampant hybridization among plants and animals in nature.

SHAKING UP THE TREE OF LIFE

Species were once thought to keep to themselves. Now, hybrids are turning up everywhere, challenging evolutionary theory

By **Elizabeth Pennisi**

In 2010, a comparison between the genomes of a Neandertal and people today settled what anthropologists and geneticists had debated for decades: Our ancestors had indeed mated with their archaic cousins, producing hybrid children. They, in turn, had mated with other modern humans, leaving their distant descendants—us—with a permanent Neandertal legacy. Not long afterward, DNA from another archaic human population, the Denisovans, also showed up in the modern human genome, telling a similar story (*Science*, 23 December 2011, p. 1629).

For researchers and the public alike, this evidence of interbreeding among distinct human populations—so different some still argue there was more than one species—created a shock wave. Suddenly hybridization "just captured our imagination," says Michael Arnold, an evolutionary biologist at the University of Georgia in Athens. "That genomic information overturned the assumption that everyone had."

The techniques that revealed the Neandertal and Denisovan legacy in our own genome are now making it possible to peer into the genomic histories of many organisms to check for interbreeding. The result: "Almost every genome study where people use sensitive techniques for detecting hybridization, we find [it]—we are finding hybridization events where no one expected them," says Loren Reiseberg, an evolutionary biologist at the University of British Columbia in Vancouver, Canada.

PHOTO: RAUL GONZÁLEZ ALEGRÍA

"Big Bird," a hybrid Darwin's finch, could be on its way to becoming a new species.

A shortcut to a species

By **Elizabeth Pennisi**

A hefty finch with an outsized head is the poster child for a recently recognized source of new species: hybridization. For decades, biologists have explored how cross-species matings can accelerate evolution by introducing genetic novelty into the parent lineages (see main story, p. 817). But they now realize that the hybrid offspring themselves can thrive and set off on their own evolutionary path. "Big Bird," one of Darwin's finches on the Galápagos Islands, may be the best documented animal example.

Peter and Rosemary Grant, evolutionary biologists at Princeton University, and their graduate student Trevor Price noticed the unusual male in 1981, after he arrived on the Galápagos island of Daphne Major. Weighing 28 grams instead of the typical 18 grams for male finches, sporting a big head, and singing an unusual song, Big Bird was probably born on neighboring Santa Cruz Island from a mating between a cactus finch and medium ground finch. At first, the immigrant and its young consorted with Daphne Major's medium ground finches. But after a severe drought from 2003 to 2005 killed 90% of the island's avian inhabitants, the two Big Bird descendants that survived and their 26 offspring crowded into one corner of the island and have kept to themselves ever since.

This group breeds just among their own kind, and even though male Big Bird finches are very territorial, they react aggressively only when other Big Bird males infringe, suggesting they don't see those other male finches as reproductive rivals. They also have a distinctive food source: Thanks to their intermediate-sized beaks, they are able to crack certain seed cases that other birds can't. "It's been going on like that for a few generations," Peter Grant says.

Until recently, biologists insisted that a species has to be reproductively isolated—unable to produce viable offspring when it mates with another species. The Big Birds are far from meeting that definition, but since many scientists no longer insist on it, "It's provocative to call [these birds] a new species," Peter Grant says. Still, the Grants are unwilling make that final call on the Big Birds.

DNA analysis is turning up more examples of hybrid species. Between 4% and 10% of plant species appear to have arisen this way. And researchers are finding new examples among birds, insects, fish, and marine mammals. The clymene dolphin (*Stenella clymene*), found in the Atlantic Ocean, emerged from dalliances between the striped and spinner dolphins, and at least two of five cichlid species—a group known for being very diverse—in Africa's Lake Victoria originated from hybrids.

Among birds, the Italian sparrow is recognized as a distinct species—it's a hybrid of the Spanish and house sparrows. The Hawaiian duck is descended from a hybridization event 10,000 years ago between the mallard (*Anas platyrhynchos*) and Laysan ducks (*A. laysanensis*). And the root-knot nematode, a deadly plant pest, adds another layer of complexity: One of its parent species was itself a hybrid species.

As evolutionary biologist Scott Taylor of the University of Colorado in Boulder puts it, hybridization is "making it seem that speciation is more complex than we thought." ■

All these data belie the common idea that animal species can't hybridize or, if they do, will produce inferior or infertile offspring—think mules. Such reproductive isolation is part of the classic definition of a species. But many animals, it is now clear, violate that rule: Not only do they mate with related species, but hybrid descendants are fertile enough to contribute DNA back to a parental species—a process called introgression.

None of this came as a surprise to James Mallet, a Harvard University evolutionary biologist who decades ago began quantifying hybridization among butterflies. He, Arnold, and a few other biologists have long argued that hybridization is pervasive and plays an important role in evolution. In addition to research on plants, where hybridization has long been documented, they pointed to studies of animals including Darwin's finches in the Galápagos Islands, tropical butterflies, and mosquitoes. Yet most other evolutionary biologists regarded these organisms as anomalies. "We thought that hybridization was kind of rare and therefore not that significant," says Carole Smadja, an evolutionary biologist at the National Center for Scientific Research's Institute of Evolutionary Biology based at the University of Montpellier in France. Now, says Stuart Baird, an evolutionary biologist at the Academy of Sciences of the Czech Republic in Studenec, "You can't deny it because it's in our own family tree."

Biologists long ago accepted that microbes can swap DNA, and they are now coming to terms with rampant gene flow among more complex creatures. "A large percent of the genome is free to move around," notes Chris Jiggins, an evolutionary biologist at the University of Cambridge in the United Kingdom. This "really challenges our concept of what a species is." As a result, where biologists once envisioned a tree of life, its branches forever distinct, many now see an interconnected web.

Hybridization, says Mallet, "has become big news and there's no escaping it."

IN 1949, botanist Edgar Anderson suggested that plants could take on genes from other species through hybridization and back crosses, where the hybrid mates with the parent species. He based this then-radical proposal on genetic crosses and morphological studies of flowering plants and ferns suggesting mixtures of genes from different species in individual genomes. Five years later, with fellow botanist G. Ledyard Stebbins, he argued such gene exchange could lead to new plant species. Their ideas quickly hit home with other plant researchers, but not with zoologists. "There was a very different conventional view in botany

PHOTO: PETER GRANT

than in zoology," Rieseberg says.

Most of those who studied animals had instead bought into the argument by the famous mid-20th century evolutionary biologist Ernst Mayr that the formation of a new species requires reproductive isolation. Mayr and his contemporaries thought that the offspring of any hybrids would be less fit or even infertile, and would not persist. To be sure, captive animals could be interbred: Breeders crossed the African serval cat with domestic cats to produce the Savannah cat, and the Asian leopard cat with domestic breeds to produce the Bengal cat. There's even a "liger," the result of a zoo mating of a tiger and a lion. But like male mules, male ligers are sterile, supporting the notion that in nature, hybridization is mostly a dead end.

By the early 1990s, however, these ideas "weren't reflective of all the information out there," recalls Arnold, who has championed the importance of hybridization in animals in four scientific books. Support for his view had already begun rolling in from some of evolution's most iconic creatures, the Galápagos finches that Darwin observed as he developed his ground-breaking ideas. Princeton University evolutionary biologists Peter and Rosemary Grant were making annual visits to a small Galápagos island called Daphne Major and observing the Darwin's finches (*Geospiza*) there. They recorded matings, how many young survived, and what the birds ate. Early on, they noticed that 1% to 3% of the pairs consisted of a male of one species and a female of another. Such hybridization surprised them, Rosemary Grant recalls. "Our mind frame was that it wasn't happening."

The hybrid offspring varied quite a bit, in traits including beak size and shape. That variation came in handy when drought or floods destroyed the bird's usual food plants, leaving behind seeds ill suited to the parents' original beak size. Far from being less fit, the hybrids with their intermediate-sized beaks thrived. There were even hints that hybridization was leading to new species of finches (see sidebar, p. 818). In 1992, the Grants surveyed the avian literature and found that Darwin's finches were far from unique. Almost 10% of all bird species failed to respect species boundaries, they reported. Ducks and geese were the most promiscuous, with reports of half the species exchanging genes. Woodpeckers, hawks, grouse, partridges, and hummingbirds also interbred. "That flew in the face of the current wisdom," Peter Grant says.

What the Grants saw in birds, Mallet saw in butterflies. Tropical butterflies belonging to the genus *Heliconius* have wing patterns that generally vary widely from species to species, but entomologists long ago noticed some striking exceptions, in which certain species and subspecies closely resemble one another. Mostly scientists chalked up the similarities to independent, but parallel, evolution. But 50 years ago, Larry Gilbert threw together multiple species of Costa Rican *Heliconius* in an insectary at the University of Texas in Austin and found that they interbred, swapping wing patterns and sometimes generating entirely new ones. Mallet and Jiggins, his graduate student at the time, found the same to be true a decade ago when they let *Heliconius* from Ecuador interbreed.

Studies of sunflowers have driven home the importance of hybridization. Matings between the top two species yielded a new one, *Helianthus anomalus* (bottom).

PHOTOS: (TOP TO BOTTOM) KEITH WHEELER/SCIENCE SOURCE; DAVID HOSKING/FLPA/SCIENCE SOURC E; W. D. AND DOPHIA BRANSFORD/LADY BIRD JOHNSON WILDFLOWER CENTER

Intrigued, Mallet surveyed all the examples of *Heliconius* he could find in museum collections around the world and calculated, based on wing patterns, that more than 30% of the *Heliconius* species had formed hybrids. His writings persuaded some animal biologists that hybridization isn't just important for plants. "Jim is recognized as an evolutionary zoologist, so when he wrote about it, [others] sat up and said, 'Wow, that's cool, it's about animals,' while there'd already been a few of us who have been saying this for 20 years," Arnold says. "He's been very influential in a good way."

NOW, cheaper DNA sequencing is enabling researchers to track the genetic impact of all this crisscrossing. In 2012, the Grants teamed up with Leif Andersson at Uppsala University in Sweden, sending him 120 blood samples from all Darwin's finches and two close relatives for DNA sequencing. The Swedish team "showed the genetic underpinning" of the Galápagos field observations, says Rosemary Grant, proving that many of the 18 species interbred with one another. The mixing was so great that, genetically speaking, two different finch species on any particular island were more similar to each other than to the same species on a different island.

Moreover, the Grants' many years of field data enabled them to show that just a few introgressed genes could have a very strong effect. In their surveys, they took multiple measurements of each bird, as if fitting it for a suit, noting features such as the size and shape of the beak. Just by looking for variants of a gene called *ALX1* in the DNA from each individual, the Uppsala team was able to sort the birds into two groups that perfectly matched the two major beak shapes. Birds that had acquired two different variants of the gene, from ancestors belonging to distinct species, had an intermediate beak shape, suggesting a tight connection between genetics and morphology. The finding, reported last year in *Nature*, showed that the hybridization the Grants had observed over the decades had taken place throughout the finches' million-year history.

Genome analytical tools originally developed to gauge how much DNA passed between humans and Neandertals have enabled researchers to track gene flow between various species in more detail. Working with butterfly genomes sequenced by a consortium including Mallet, Jiggins, and Kanchon Dasmahapatra, now at the University of York in the United Kingdom, showed significant gene flow on 11 of 21 chromosomes in two butterfly subspecies living in the same place in Peru. Butterfly gene exchange is "promiscuous," the consortium reported in a 2012 *Nature* paper.

Similar genome analyses have since documented significant gene flow in many groups of mammals, including cats, says William Murphy, a geneticist at Texas A&M University in College Station. By surveying the nuclear and mitochondrial DNA of 38 cat species, his team found that at least three neotropical cat species had interbred over their 3-million-year history, they reported last year in *Genome Research*. In North America, they found bobcats and lynx have frequently hybridized and continue to do so today along the U.S.-Canada border.

The blurring of species lines can complicate conservation. A recent genomic study showed that the red wolf, an endangered species, and the eastern wolf are both relatively recent hybrids of coyotes and gray wolves. The result, reported in July in *Science Advances* by Robert Wayne of the University of California, Los Angeles, and his colleagues, could suggest that the two wolf varieties don't qualify for protection under the Endangered Species Act. Then there are the "pizzlies" and "grolars," as hybrids between polar and brown bears are known. Extensive genomic studies of the two bear species indicate they have long had dalliances. But as polar bears are driven southward by climate change, such unions are producing more offspring. In the United States, hunting polar bears is illegal, except for Native Alaskans, but a hybrid of the bears is not protected.

AS EXAMPLES of hybridization have multiplied, so has evidence that, at least in nature, swapping DNA has its advantages. When one toxic butterfly species acquires a gene for warning coloration from another toxic species, both species benefit, as a single encounter with either species is now enough to teach predators to avoid both. Among canids, interbreeding with domestic dogs has given wolves in North America a variant of the gene for an immune protein called β-defensin. The variant gives wolf-dog hybrids and their descendants a distinctive black pelt and better resistance to canine distemper, Wayne says. In Asia, wolf-dog matings may have helped Tibetan mastiffs cope with the thin air at high altitudes. And interspecies gene flow has apparently allowed insecticide resistance to spread among malaria-carrying mosquitoes and the black flies that transmit river blindness.

In each case, the beneficial genetic changes unfolded faster than they would have by the normal process of mutation, which often changes DNA just one base at a time. Given the ability of hybridization and introgression to speed adaptive changes, says Baird, "closing that door [with reproductive isolation] is not necessarily going to be a good thing for your longterm survival."

A fair number of biologists remain uneasy with this picture of unchecked gene swapping. "Some people are going way overboard in saying that everything is hybridizing with everything else," says Joel Cracraft of the American Museum of Natural History in New York City. "It's a little hyperbol[ic] to say it's wiping away species boundaries."

The Grants think the divide over the importance of hybridization and its effect on speciation is partly cultural. They and other field biologists accept that a species can be legitimate even if it can sometimes mate with others. In contrast, says Rosemary Grant, "Some people in museums only [recognize] a species when it's absolutely certain there's complete genetic incompatibility." A species may have to evolve for tens of millions of years before it can meet that definition—if it happens at all.

"We always like to put things in boxes. We are removing people's boxes," Baird adds. What's more, widespread gene swapping threatens a standard approach to building the tree of life: comparing DNA from different species. "That would [work] if the truth were trees, but that's not the truth," Baird says. Because of hybridization, disparate species can share similar sequences. As Baird puts it, evolving species "don't form trees, they form nets."

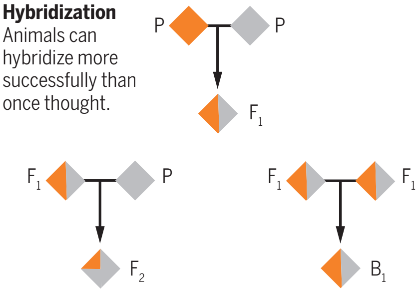
The malaria-carrying *Anopheles gambiae* mosquito and its relatives drive home how challenging it can be to build a phylogenetic tree when hybridization runs amok. Last year in *Science*, evolutionary biologists Nora Besansky of the University of Notre Dame in Indiana, Matthew Hahn from Indiana University in Bloomington, and their colleagues reported that gene flow among six mosquito species was so extensive that only 2% of the insects' genomes—mostly in the X chromosome—reflected those mos-

Hybridization shapes evolution

When two species mate and produce fertile offspring, evolution can take new turns: Backcrossing introduces new genes into the parent species' genomes (introgression) or hybrids start to breed only with other hybrids, forming new species.

Two species comingling

Hybridization
Animals can hybridize more successfully than once thought.

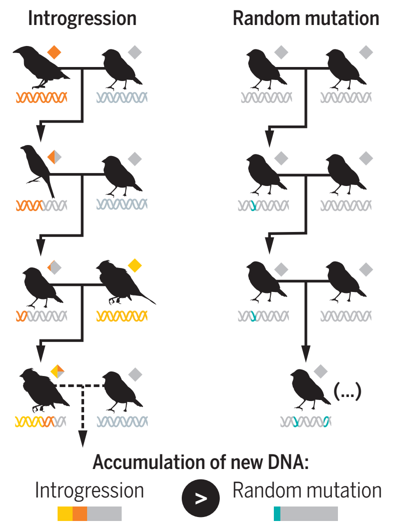


Introgression
New DNA joins the parent's (P) genome if a hybrid (F_1) mates back with a parent.

Speciation
Hybrids mating among themselves can lead to a new species (B_1).

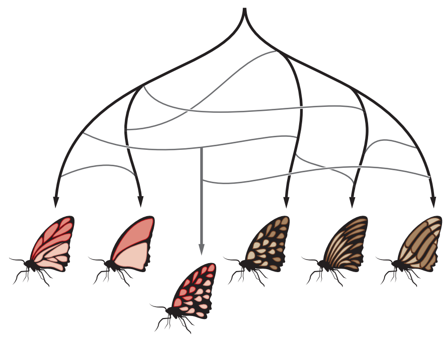
Accelerating genetic change

Introgression quickly introduces much more genetic variation than can random mutations and can help organisms adapt better to changing environments.



From tree of life to a web

Hybridization creates new connections between branches on the tree of life and can lead to new species.



Hybrids are everywhere on the tree of life. (Clockwise from top left): liger (lion/tiger), barred salamander hybrid (Western/California barred salamanders), *Heliconius heurippa (H. cydno/H. melpomene*); Hawaiian duck (mallard/Laysan duck), pizzlies (polar/brown bear).

PHOTOS: (CLOCKWISE FROM TOP LEFT) REUTERS/CHRISTIAN CHARISIUS/ALAMY STOCK PHOTO; GARY NAFIS; JUAN GILLERMO MONTAÑÉS AND MAURICIO LINARES; EPA EUROPEAN PRESSPHOTO AGENCY B.V./ALAMY STOCK PHOTO; KEVIN SCHAFER/MINDEN PICTURES

quitoes' true history. "It is really a beautiful story that no doubt has Ernst Mayr hyperventilating in his grave," Besansky says.

The resulting "net" for the mosquitoes showed that the two most important human malaria vectors split off early in the evolution of this group, and later likely shared through hybridization the genes that make them suitable hosts for the malarial parasite and, consequently, so dangerous to people. Such introgression could also explain why it's so difficult to sort out the exact history of the early explosion of birds or of mammals millions of years ago.

The Grants believe that complete reproductive isolation is outdated as a definition of a species. They have speculated that when a species is no longer capable of exchanging genes with any other species, it loses evolutionary potential and may become more prone to extinction.

This idea has yet to be proven, and even Mallet concedes that biologists don't fully understand how hybridization and introgression drive evolution—or how to reconcile these processes with the traditional picture of species diversifying and diverging over time. Yet for him and for others, these are heady times. "It's the world of hybrids," Rieseberg says. "And that's wonderful." ■

Shaking up the Tree of Life

Elizabeth Pennisi (November 17, 2016)

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Editor's Summary

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