Evolving With a Little Help From Our Friends

By Carrie Arnold

Symbiotic microbes seem to play a major role in preventing three related species of Nasonia wasp from interbreeding. The species vary in wing size: N. vitripennis has the smallest wing, N. giraulti has the largest, and N. longicornis is somewhere in between.

You could call it Seth Bordenstein's "Frankenstein" moment. A little over a year ago, Bordenstein, a biologist at Vanderbilt University in Nashville, Tenn., and his then-graduate student, Robert Brucker, mated two incompatible species of wasp in the lab, creating a hardy hybrid that lived when most others died.

Normally, when members of two related species of parasitic wasps in the genus Nasonia, N. giraulti and N. longicornis, mate with their more distant relative N. vitripennis, the hybrid offspring die. Until recently, no one could figure out exactly why, but it was clear that this was one of the major barriers dividing the species. But when Bordenstein and Brucker treated the wasps with antibiotics, eliminating the millions of microbes that lived on their bodies, they found that many of the hybrid offspring unexpectedly could survive and thrive. By stripping off the wasps' microbiomes — the microbial community inhabiting the insects — Bordenstein and Brucker had brought a totally new hybrid wasp to life.

Seth Bordenstein, a biologist at Vanderbilt University, is studying how symbiotic microbes keep different species of Nasonia wasps from interbreeding.

The findings, published in Science in July 2013, highlight a surprising idea in biology: that symbiosis — a long-term, stable and often beneficial interaction between organisms — could drive two populations apart, the first step in the development of new species. Although the idea has been floating around for nearly a century, it has only recently begun to gain traction in biology. This idea contrasts sharply with the traditional picture of evolution, in which new species emerge either from geological isolation or from a relentless struggle for food and mates. According to this new hypothesis, a host organism's microbes might trigger changes in mating and reproduction that begin to define two different populations.

Almost all animals, including humans, are an amalgam of host and microbe, with thousands of microorganisms occupying every available niche. In exchange for a home, these microbes perform vital functions for their hosts, protecting them from pathogens, processing food, and even altering social behavior. New, sophisticated methods for tracking microbial communities have helped scientists appreciate just how important microbes are. Bordenstein's work is part of a new offshoot in the study of symbiosis, exploring how different collections of microbes might shape the evolution of new species.

"This work is changing our idea of what a species is and how it might form," said Nicole Webster, a marine microbiologist at the Australian Institute of Marine Science. Beginning in the mid-1990s, some scientists, including Bordenstein, began to define a species not just as a single organism, but as the organism plus its microbiome.

Other scientists have joined Bordenstein on his quest, discovering evidence that microbes might play a role in the development of other species as well. Gut microbes in fruit flies can rapidly change the insects' mating behaviors, often a precursor to the development of new species. In hyenas, bacteria synthesize many of the chemical compounds animals use to communicate, and changes to the microbes might have helped to push populations apart.

Bordenstein's team is now trying to figure out exactly how microbes can turn one host species into two by destroying their ability to produce healthy hybrid offspring. Preliminary findings suggest that the presence of certain symbiotic microbes alters the activity of genes that regulate the immune system. Bordenstein theorizes that when a wasp, such as a hybrid, plays host to the wrong microbes, the wasp's immune system may attack and kill itself instead of foreign pathogens. Conversely, the immune system can also affect the types and numbers of microbes that colonize the wasp. If this work holds up, Bordenstein may have added a new dimension to how scientists think about evolution.

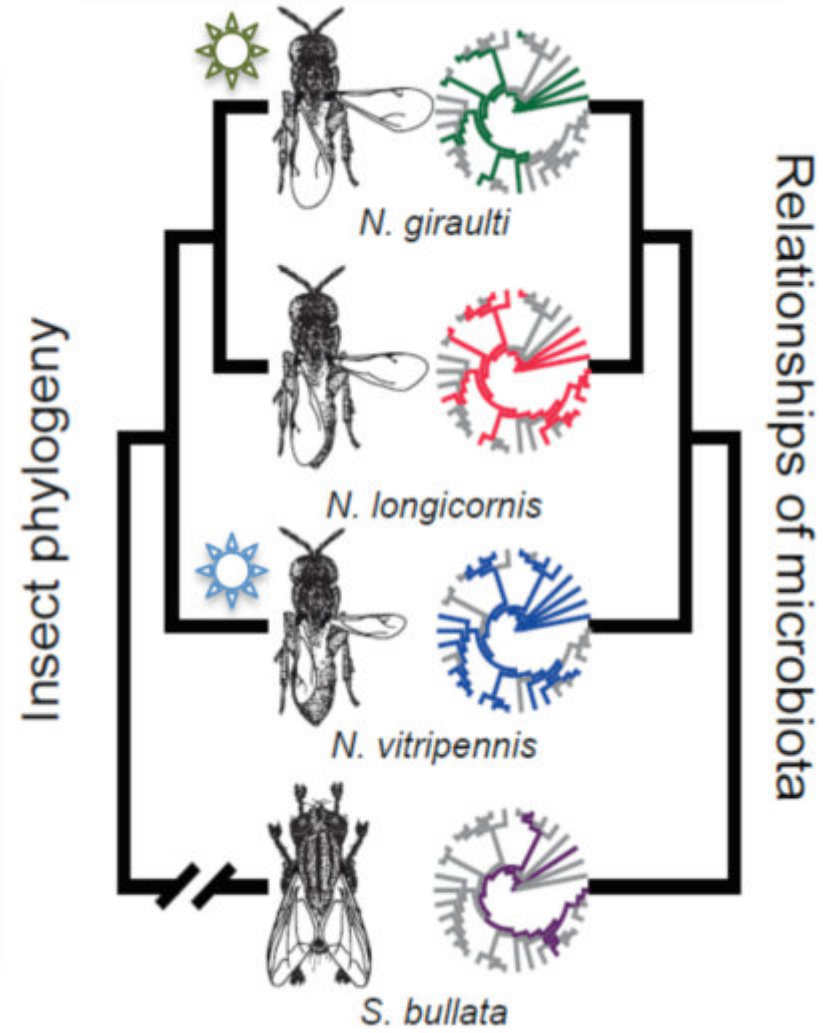
"It's now very clear that symbiosis is the rule, and not the exception, and that it plays a much more important role in evolution than anyone thought," Bordenstein said.

The idea that cooperation could be a driving force in evolution was first proposed more than a century ago by Peter Kropotkin, a Russian naturalist. In his 1902 book "Mutual Aid: A Factor of Evolution," Kropotkin writes, "There is an immense amount of warfare and extermination going on amidst various species … there is, at the same time, as much, or perhaps even more, of mutual support, mutual aid, and mutual defense. … Sociability is as much a law of nature as mutual struggle."

Kropotkin, however, was best known as an anarchist, not as a biologist. "Mutual Aid" was big on ideas but short on the kind of experimental proof needed to convince scientists that symbiosis and cooperation are important factors in natural selection.

Over sixty years after the publication of "Mutual Aid," Lynn Margulis, then a young biologist at the Boston University, took up the cause of symbiosis. In 1966, Margulis provided evidence that mitochondria, molecular machines that help cells produce energy, and chloroplasts, which help plant cells turn sunlight into sugar, originated from symbiotic bacteria. According to the theory, billions of years ago, one microbe engulfed another, one of the first major steps toward the complex plant and animal cells we see today.

Margulis' idea was met with skepticism. It took 20 years of intense debate for scientists to somewhat reluctantly accept it, and they believed it to be a freak occurrence.



Removing the microbes from three species of Nasonia wasps allows them to produce viable offspring, suggesting that their microbes play a crucial role in keeping the species apart.

In 2001, a study of Nasonia wasps conducted by Bordenstein a decade before his breakthrough with Brucker suggested that microbes influence the evolution of their hosts more often than scientists had expected. He knew that two closely related species, N. giraulti and N. longicornis, diverged from a common ancestor only about 400,000 years ago — “the blink of an eye in evolutionary time,” Bordenstein said. Historically, he said, researchers would have looked for genetic changes in Nasonia DNA to help explain how the species diverged. Bordenstein believed that a symbiotic bacterium called Wolbachia might be keeping the two species apart. Previous work had shown that if members of the same species carry different types of Wolbachia, they can’t produce healthy offspring. But scientists didn’t yet know if Wolbachia strains were the major difference between the two species. Bordenstein theorized that different types of Wolbachia divided the parent species into two groups that were unable to interbreed — the traditional definition of a new species.

By removing Wolbachia from these wasps, the researchers showed that the Wolbachia infections were the wasps’ major barrier to interbreeding. “It was as if they were no longer two separate species,” Bordenstein said. “This was some of the first evidence that a symbiotic microbe could wedge two species apart.”

Far from being a rare, one-off event, Bordenstein’s findings suggested that the microbiome has played a larger than expected role in the evolution of new species. Thousands of insect species are infected with Wolbachia, making symbiosis a potentially major player in the development of these species.

Bordenstein's study generated a lot of interest and some skepticism. Many evolutionary biologists had historically not paid much attention to the role of symbiosis in evolution and thus lacked a context in which to understand the work, said Bradford Harris, a doctoral candidate in the history of science at Stanford University. "People still think of evolution as the 'survival of the fittest,'" Harris said. "It's a nice, convenient one-liner to explain a very complicated phenomenon." But this simplistic explanation makes it difficult to grasp the less individualistic aspects of evolution.

In Bordenstein's most recent study, the researchers found that it's not just Wolbachia that's important. Eradicating the full complement of the wasps' microbes allowed hybrids of N. vitripennis and its two distant relatives to survive. The findings are significant because they suggest the phenomenon might not be limited to insects. Only insects carry Wolbachia, but all animals have a range of symbiotic microbes.

"These experiments can't tell us whether the microbes were actually causing speciation, but they were certainly a major contributor, adding to the reproductive barrier that was already in place," Brucker said. Scientists still don't know whether the microbes can create the reproductive barrier in the first place, widen an already existing barrier, or even do both.

Joshua Gibson, an evolutionary biologist at Purdue University in West Lafayette, Indiana, and a Nasonia expert, thinks it's too soon to say conclusively that the microbiome can cause speciation based on Bordenstein's work. "It's not clear whether the microbiome led to the genetic changes or whether the genetic changes caused the microbiome to shift," he said.

Even if the microbiome did cause a new species of Nasonia to evolve, that doesn't mean it is happening everywhere across the animal kingdom, Gibson added. The factors affecting each species will be different, as will the importance of the microbiome, he said.

Preliminary evidence does suggest that microbes might help to keep two animal species separate. The spotted hyena and the striped hyena live side by side on the African savanna. Both species communicate information on sex and reproductive status via a complex combination of volatile chemicals found in a foul-smelling, orange-brown paste extruded from scent glands near the anus.


Kevin Theis, an evolutionary ecologist at Michigan State University holding a spotted hyena.

“They basically rub their hindquarters on the long grass, which spreads the paste,” said Kevin Theis, an evolutionary ecologist at Michigan State University in East Lansing, Michigan, who studies the animals. “Hyenas paste at the highest rates at the borders of their territory and at communal dens.”

Previous work speculated that microbes are the actual producers of the chemical compounds used in mammalian communication, and Theis now had the genetic tools to test this hypothesis. He swabbed the paste laid down by members of both species and identified the microbes found in each sample. Theis’ results, published in 2013 in the Proceedings of the National Academy of Sciences, showed that the microbes found in the paste were highly correlated to the type of chemicals present.

Theis’ most recent work, presented at the annual meeting of the American Association for the Advancement of Science in February, showed that not only did the two species have different microbiomes, they also had different social behaviors. He is currently studying whether the two might be linked. He also found that microbes differed between males and females. Because females pick mates in part based on the scent of a male’s paste, it’s possible that a major changes to a male’s scent, perhaps rendering him unrecognizable, could drastically change who mates with whom. It’s not yet clear whether symbiotic microbes led to the split between striped and spotted hyenas, but Theis said scientists now better understand the broad influence that microbes can have. “Microbes can play a role in evolution because we are showing that they affect behavior, and behavior is a primary target of selection,” Theis said.

What might change an organism’s microbiome in the first place? Animals acquire their symbiotic microbes in a variety of ways, by touching things or from the very process of being born. Diet,

however, remains one of the main avenues through which symbiotic microbes populate an organism. Changes in diet, as Webster points out, can also favor one population of microbes over another, which scientists have found leads to changes in mating behavior.

In 1989, Diane Dodd, a biologist then working at Yale University, wanted to find out how dietary changes could contribute to speciation. She fed one group of flies a starch diet while another group got maltose, a simple sugar. After 25 generations, Dodd found that the starch-eating flies would no longer mate with the maltose eaters. The two populations had started down the path to becoming separate species. But exactly how this happened remained unclear for more than 20 years.

Looking at Dodd's study in 2009, Gil Sharon thought he knew why. A graduate student in microbiology at Tel Aviv University in Israel at the time, Sharon thought that the flies' constellation of symbiotic microbes might be the cause of their change in behavior. So Sharon repeated Dodds' experiment and verified that the change in diet caused a change in behavior. Then he dosed the flies with antibiotics and found that their mating preferences disappeared. When Sharon then provided the germ-free flies with bacteria-laden food, he found that these preferences returned in just one generation. In a 2010 paper in PNAS, Sharon concluded that symbiotic bacteria could alter mating preferences.

Biologists believe that different symbiotic microbes may have helped separate two closely related species in Africa, the spotted (shown here) and striped hyenas.

"If the flies have different food sources, they have different bacteria and different odors. Those odors affect their mate choice," Theis said. "So if two populations are separated by a difference in food sources, and you give them a certain amount of time and isolation, they might not be able to reunite later on because they have different mating preferences." This would be a major step toward speciation.

Because changes to microbes appear to happen far more quickly than changes to the actual genome, these kinds of changes might accelerate the pace at which different populations are pushed apart, setting the stage for the evolution of new species.

The idea is still controversial, however. While microbiologists have been fairly open to it, other biologists have been harder to convince. "The jury is still out," said Angela Douglas, an entomologist and microbiologist at Cornell University. "Clearly, microbes are an integral part of what it means to be an animal, but researchers need to repeat these experiments in more ecologically relevant conditions."

Biologists have nearly all accepted the importance of the microbiome to the overall health and well-being of an organism. What troubles Jerry Coyne, an evolutionary biologist at the University of Chicago, is that they can't rule out other factors for speciation. "There are no studies as of yet that prove definitively that the microbiome causes speciation," Coyne said. "It's theoretically possible, but we don't know for sure yet."

Meanwhile, Bordenstein and other scientists are working to determine exactly how symbiotic microbes can help split one species into two. Bordenstein is now examining whether certain microbial species are more important than others and how the presence of a foreign microbiome in a hybrid species alters its immune system.

In order to fully grasp the complexities of how symbiotic microbes might affect evolution, Bordenstein said, the fields of microbiology and evolutionary biology will have to form a symbiotic relationship of their own. "I see it as a chance to define the future of biology," he said. "Will we have a genomic-centric view of life, in which the nuclear genome is the only way we look at evolution, or will we have a more unified view, in which the definition of an animal changes to include both the genome and the microbiome?"

This article was reprinted on Wired.com.