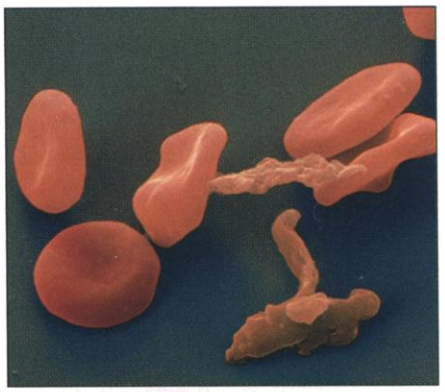


Hominid malaria? The earliest hominids in Africa were probably infected with *Plasmodium falciparum*, which causes the deadly form of malaria.

needle use has made transmission easier for the virus and encouraged its diversification.

The most recently discovered hepatitis virus, hepatitis G, also seems to have an old heritage. Discovered in 1995, it infects between 5% and 15% of the world's population but causes no detectable disease. It is distantly related to hepatitis C, yet unlike that virus, many versions have been found in primates. The family tree of hepatitis G nicely mirrors the evolutionary tree of its primate hosts. The deepest split between both the viruses and their hosts is between New World and Old World forms. In an upcoming issue of the *Journal of General Virology*, Peter Simmonds of the University of Edinburgh, U.K., argues that the virus infected a primate tens of millions of years ago and has since speciated along with its host.

Ananias Escalante of the Venezuelan Institute for Scientific Investigation in Caracas is probing the history of malaria with a practical bent; he hopes it can point the way to vaccines. He and others have shown that *Plasmodium*, the parasite that causes malaria, invaded our species in much the same way that HIV has, with several introductions of related species. *P. vivax*, which causes mild disease, jumped from a primate into hominids in Southeast Asia perhaps 1 million years ago, he suspects, whereas *P. falciparum*, the deadliest parasite, was probably infecting the earliest hominids in Africa.

But the history of *P. falciparum* after it invaded humans is more controversial. In 1998, Stephen Rich, now at Tufts University in Grafton, Massachusetts, and his colleagues argued that although *P. falciparum* was an ancient human disease, all living strains emerged from a bottleneck that might have occurred as recently as 5000 years ago. A study of human genes involved in fighting malaria also points to a recent explosion (*Science*, 27 April, p. 627). But over the past 3 years, other teams have analyzed a broader selection of the parasite's DNA and found evidence for a much older expansion. In an upcoming issue of *Molecular and Biochemical Parasitology*, Escalante and his colleagues compare various forms of the *AMA-1* gene in *P. falciparum* from Kenya, Venezuela, Thailand, and India and conclude that they descended from a common ancestor that existed 500,000 years ago. Escalante is now tracking the evolution of certain genes along the many branches of the *Plasmodium* tree. Genes that have been very mutable may not be useful targets for a malaria vaccine, he suggests, because they may continue to change rapidly. More attractive are genes that have remained relatively constant over millions of years of evolution in primates, rodents, and birds. "If we can find something that has been conserved and creates a nice immune response, that might be a good thing to put into a vaccine," says Escalante.

If what's past is indeed prologue, researchers who study the evolutionary history of diseases may someday be able to predict the emergence of new diseases. "We tend to wait until infections get going in humans before we worry about them," says Holmes. "What I think we need to be doing is going out into wild species and seeing what's there, what are potentially emerging viruses." By figuring out their place on the tree of life, researchers may be able to determine which are most likely to make the leap into humans and become the plagues of the future.

–CARL ZIMMER

Carl Zimmer is the author of *Parasite Rex*; his column, "The Evolutionary Front," appears regularly in *Natural History*.

NEWS

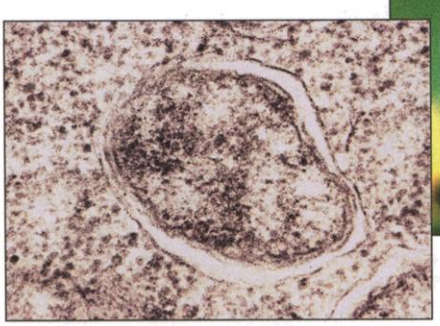
Wolbachia: A Tale of Sex and Survival

By manipulating the sex lives of its hosts, this ubiquitous bacterium boosts its reproductive success

On certain afternoons in Uganda, bright orange butterflies with black-and-white wings gather together on small patches of low grass, sometimes in the hundreds. Such congregations are nothing unusual in the animal kingdom; normally, males convene to try to win the attention of females. But the swarms—known as leks—that *Acraea encendana* form are bizarre: 94% of the butterflies are females, and they jostle for the attention of the few males, who seem reluctant suitors. "You wouldn't expect males to be surrounded by all these virgin females and not wanting to mate," says Francis Jiggins of Cambridge University. Even more bizarre is the cause of their sexual skew: They are plagued with a strain of bacteria known as *Wolbachia*, which kills males but spares females.

Wolbachia's powers would be remarkable enough if they only drove Ugandan butterflies into female-dominated leks. But this sexist microbe may be the most common infectious bacterium on Earth. Although no vertebrates (humans included) are known to carry *Wolbachia*, infection is rampant in the invertebrate world, showing up in everything from fruit flies to shrimp, spiders, and even parasitic worms.

In case after case, researchers are finding that *Wolbachia* don't leave their survival to chance. To maximize their numbers, the bacteria manipulate the sex life of many of their hosts, using some of the most baroque strategies known in evolution. That's one reason why *Wolbachia*, discovered in 1924, have just recently become the darlings of evolu-



Sexist microbe. *Wolbachia* favor females, like this Ugandan butterfly, because they will carry on the lineage.

CREDITS: (TOP TO BOTTOM) MECKES/OTTAWA/PHOTO RESEARCHERS; FRANCIS JIGGINS/CAMBRIDGE UNIVERSITY; COURTESY OF BACCHI AND BANDI

tionary biologists. Last summer the first international *Wolbachia* conference was held in Crete. The first *Wolbachia* genome project should be finished this year by Scott O'Neill of Yale University and his colleagues. And whereas humans merited a single genome project, six other *Wolbachia* genome projects are under way. "The whole field is just exploding," says O'Neill.

And rightly so, say *Wolbachia* fans. There are tantalizing hints that *Wolbachia*'s extraordinary ability to manipulate their hosts for their own evolutionary benefit can help turn a population of hosts into a new species. And some researchers think that *Wolbachia* can be used as a weapon against pests and parasites that cause diseases such as malaria and river blindness.

Favoring females

Researchers did not begin to fathom the remarkable ways in which *Wolbachia* ensure their own success until the 1970s. *Wolbachia* can only live inside the cells of their hosts. If they live in a female, they can infect her eggs and be passed down to her offspring. But if they live in a male, they hit a dead end; as his sex cells divide into tiny sperm, the bacteria are squeezed out. That means only infected females can keep a lineage of *Wolbachia* alive. To ensure a steady stream of progeny, researchers discovered, *Wolbachia* sometimes boost their own reproductive success by increasing that of infected female hosts.

Researchers discovered in the 1970s that, through a process known as cytoplasmic incompatibility, *Wolbachia* make it difficult for uninfected females to reproduce. Their strategy works like this: If a healthy female mates with a male carrying *Wolbachia*, some or all of her fertilized eggs will die. But a female carrying *Wolbachia* can mate with either infected or uninfected males and produce viable eggs—all of which have *Wolbachia* in them. As a result, the infected females outcompete parasite-free ones, and the overall proportion of *Wolbachia* carriers increases in a population.

The nuts and bolts of this phenomenon remain a matter of speculation. "That's still a big open question," says John Werren, a *Wolbachia* expert at the University of Rochester in New York. The evidence so far suggests that the bacteria that end up in males produce a toxin that alters their host's sperm. When these males mate with uninfected females, the tainted sperm do a lousy job of fertilizing their eggs. Meanwhile, *Wolbachia* living in females produce an antidote that somehow restores the sperm to their full viability.

On the run

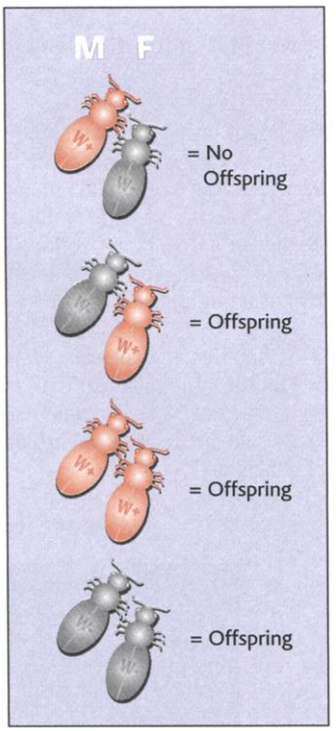
Despite these startling discoveries, few microbiologists had even heard of *Wolbachia* through the 1980s. "Basically, *Wolbachia* was thought to be an obscure bunch of bacteria that lived in just a few insects," says Werren. That obscurity, it turned out, was simply due to the fact that *Wolbachia* are not easily cultured outside a host and thus escape detection through traditional means. But with the advent of the polymerase chain reaction in the early 1990s, researchers were at last able to fish through animal cells for *Wolbachia* genes. They caught a huge harvest.

Surveying insects in Panama, England, and the United States, Werren found that about 20% in all three countries were infected. "Twenty percent is definitely a minimum, if for no other reason that I only sampled one or two individuals per species," says Werren. Indeed, other researchers have found infection rates as high as 76%. All told, *Wolbachia* may infect well over 1 million species of insects, and the bacteria are not limited to insect hosts: Researchers have been finding them in such disparate groups of invertebrates as millipedes, crustaceans, and mites.

When *Wolbachia* enter a new population, they race through it. In the 1980s, Michael Turelli of the University of California, Davis, and Ary Hoffman, now at La Trobe University in Australia, discovered a new strain of *Wolbachia* in fruit flies in Southern California. To their amazement, they found that the microbe was expanding across the state at a whopping 100 kilometers a year. Since then it has swept across the country and much of the world.

Wolbachia spread so quickly, researchers realized, because they take control of their hosts' reproduction. And in the past decade, they've discovered that cytoplasmic incompatibility is only one of many tricks the bacteria use to do so. In some species of wasps, for example, *Wolbachia* completely alter the host's sex life, manipulating the host to give birth only to females which then no longer need to mate with males to reproduce. In other species, they allow males to be born but alter their hormones to feminize them and make them produce eggs.

Rapid speciation? Infected with different strains of *Wolbachia*, populations of *Nasonia* wasps cannot interbreed, possibly creating new species.



Baroque strategy. Infected females are the clear winners in this reproductive game.

A fourth way *Wolbachia* can boost their reproductive success is to destroy their male hosts (and, paradoxically, themselves in the process). In a number of hosts, *Wolbachia* kill all of the male eggs that they infect. When the female hosts hatch, they don't have to compete with their brothers for food—in fact, their brothers are their food. By cannibalizing the male eggs, the *Wolbachia*-infected females increase their chances of survival.

With so many of their brethren killed off, the few males that remain can enjoy remarkable reproductive success. A species that might normally be split 50–50 between males and females may become permanently skewed to females, as in the case of the Ugandan butterfly Jiggins studies. And because these females have only a few males to mate with, there's more reproductive payoff in being a male than a female butterfly. This situation, Jiggins suspects, may radically alter the behavior of the butterflies, driving males to be very choosy in their mates, preferring healthy females to *Wolbachia*-infected ones. Indeed, "uninfected females are more likely to mate," Jiggins points out. If a male chooses an infected mate, he may father few sons or none at all, thereby reducing his chances of having grandchildren.

Bizarre speciation

Wolbachia may even provide clues into how species originate. New species arise when populations become isolated. Gradually, each population acquires new genes, and, if their isolation lasts long enough, those new genes make them unable to mate with other members of their species. In the 8 February issue of

CREDIT: (TOP) JOHN H. WERREN

Nature, Werren and Seth Bordenstein of the University of Rochester demonstrated that *Wolbachia* may be able to create just this sort of isolation, as has long been suspected.

The biologists looked at two closely related species of wasps—*Nasonia giraulti* and *Nasonia longicornis*—that carry two different strains of *Wolbachia*. Normally these two species cannot mate. But when Werren and Bordenstein cured the wasps of their *Wolbachia* infection, the wasps could produce healthy hybrids that could in turn produce healthy offspring of their own. The wasps are divided into two species, Werren argues, only because they carry different strains of *Wolbachia*. Each species carries a strain that prevents its males from fathering wasps with females of the other species. The bacteria thus create a reproductive wall between them.

Although some evolutionary biologists have suspected for more than 40 years that *Wolbachia* may be agents of speciation, not everyone agrees, and only recently have researchers such as Werren and Bordenstein begun to test the possibility carefully. "Every time I look further into this topic, I'm coming away with data that say it is important," says Werren. If the work holds up, Werren concedes, they will have stumbled upon a very unconventional path to speciation. Whereas geographically splitting a species in two can create new species over the course of thousands of years, *Wolbachia* might be able to push their host apart in a few generations.

Yet other researchers argue that the *Nature* paper does not close the case. Although the paper is "interesting," *Wolbachia* expert Hoffman says that "the research does not demonstrate that *Wolbachia* causes speciation." He points out that the two wasp species do not live side by side in nature; they might have acquired their incompatible *Wolbachia* strains after they were isolated. What's more, Hoffman adds, if two strains of *Wolbachia* invade a host species, mathematical models suggest that one of them will often drive the other out of existence.

Wolbachia as weapon

Given the breakneck pace at which *Wolbachia* sweep through the invertebrate world, researchers might be able to use them to fight pests and the diseases they carry, speculate O'Neill and others (*Science*, 20 October 2000, p. 440). To fight malaria, for example, researchers might be able to introduce a gene encoding resistance to *Plasmodium* (the protozoan that causes the disease) into *Wolbachia*'s genome. Researchers might then infect mosquitoes with the altered *Wolbachia*, which could theoretically produce antibodies that block the transmission of the parasite through the insect's body. With *Wolbachia*'s wide reach, entire populations of the insects might become resistant, says O'Neill. Other insects that might be candidates for *Wolbachia* infection include tsetse flies (which spread sleeping sickness) and leaf hoppers (which spread viral diseases between rice plants). At this stage, however, such strategies remain speculative. It may not be possible, for example, to find a suitable antibody gene, or it may not do its job properly when expressed in bacteria.

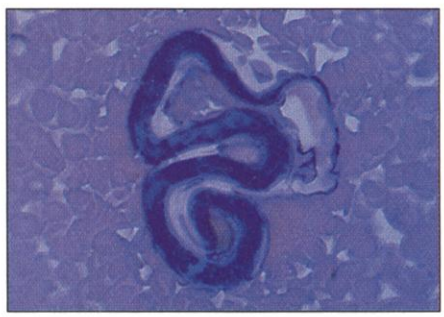
Taking a different strategy to fighting malaria, O'Neill and his colleagues are investigating a virulent strain of *Wolbachia* that infects *Drosophila melanogaster* and cuts their life-span by up to 50%. By killing insects before they get too old, *Wolbachia* could be devastating to parasites they carry, because they need time to develop inside their hosts before they can infect humans. "Under certain conditions, we should be able to see 80% to 100% reductions in disease transmissions," asserts O'Neill. He and his colleagues have already succeeded in infecting a different species of *Drosophila* with the virulent strain of *Wolbachia* in the lab—it cuts their life-span as well—and they're now investigating whether they can establish it in mosquitoes.

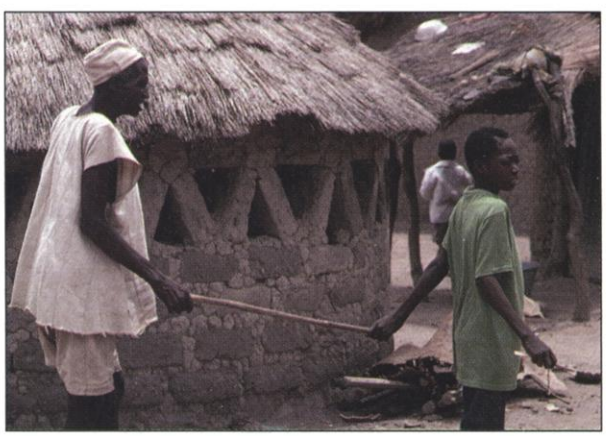
A quicker approach would be to insert the virulence-producing genes directly into the *Wolbachia* that live in mosquitoes—if researchers could find the genes. That's one reason O'Neill's team is sequencing the approximately 1-million-base genome of the virulent strain that infects *D. melanogaster*; they plan to finish it this year. Because *Wolbachia* strains have evolved so many adaptations for manipulating their hosts, other researchers have started genome projects on six more.

Researchers also hope to use *Wolbachia* to battle river blindness and elephantiasis. These diseases are caused by parasitic worms (called filarial nematodes) carried by flies and mosquitoes. But unlike insect-borne diseases such as malaria and sleeping sickness, these worms carry *Wolbachia* and depend on the bacteria for their well-being.

As early as the mid-1970s, researchers knew that some sort of bacteria were living inside the worms. In 1995, researchers sequencing the genome of one filarial nematode stumbled across *Wolbachia* genes (*Science*, 19 February 1999, p. 1105). *Wolbachia* have now been found in almost every other species of filarial nematode.

Although *Wolbachia* are parasites in most invertebrates, researchers suspect that they live mutualistically with nematodes. Perhaps the clearest sign that the worms derive some benefit from an infection is the fact that they suffer if their *Wolbachia* are wiped out by antibiotics. *Onchocerca ochengi*, a filarial nematode in cattle, for example, die when their bacteria are destroyed. In other species, the females simply become sterile.





Treating river blindness. By wiping out *Wolbachia* from its filarial worm hosts (right, the nematode that causes elephantiasis), researchers hope to treat that disease and river blindness.

Researchers don't yet know what sort of service *Wolbachia* provide the worms, but they are already investigating whether they can fight filarial diseases by killing the bacteria. German researchers reported in the 8 April 2000 issue of *The Lancet* that when they gave the antibiotic doxycycline to people suffering from river blindness in Ghana, the worms' embryogenesis stopped. Antibiotics might prove superior to ivermectin, the drug now used to fight river blindness, say the researchers. Ivermectin kills young parasitic worms but has to be taken every 6 months, whereas one dose of antibiotics may be able to stop the worms from producing any offspring.

Whether as a mutualist or a parasite, *Wolbachia* are proving to be among the most versatile microbes ever found. As O'Neill says, "the discoveries are accelerating so much it's hard to predict where we're going." Some new directions are likely to emerge from the forthcoming genome sequences of these master manipulators.

–CARL ZIMMER

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