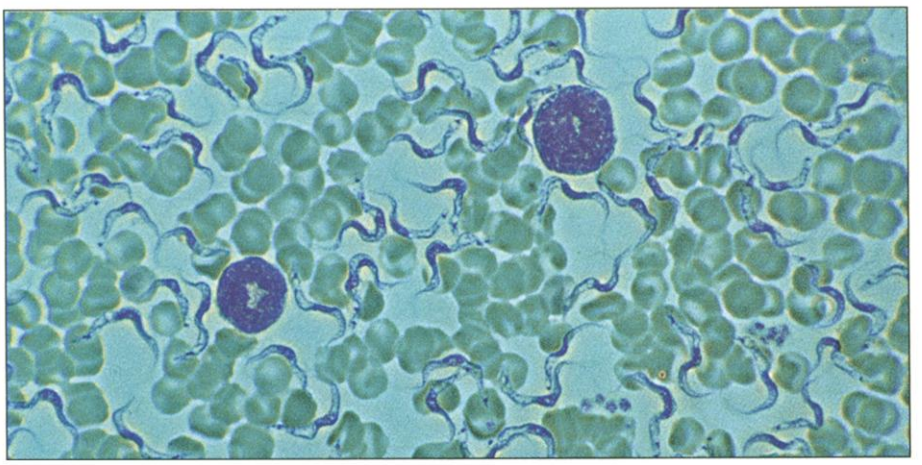
Genetic Trees Reveal Disease Origins

By analyzing genetic variation and constructing evolutionary trees of infectious organisms, researchers are turning conventional wisdom about when some diseases arose on its head



Before the split. The trypanosomes that cause sleeping sickness (above) in Africa and Chagas' disease in South America may have descended from a common ancestor 100 million years ago, when Africa, South America, and Australia were joined.

Wendy Gibson is a paleontologist without fossils. A microbiologist at the University of Bristol, U.K., Gibson studies trypanosomes, single-celled parasites that cause sleeping sickness and related diseases. Although trypanosomes infect millions of people and countless mammals, they are as evanescent as they are common. No fossil of a trypanosome exists, and, as Gibson notes, "we can't replay history." Nevertheless, she and her colleagues have been able to reconstruct the past 100 million years of trypanosome evolution, as continents have split them apart and their hosts have evolved into new forms, including humans.

Until the 1980s, the only way to study the rise of diseases was to plumb the past for ancient clues. Historians parsed tantalizing passages in ancient texts such as the Bible or *The History of the Peloponnesian War*. Archaeologists inspected skeletons for lesions and other signs of diseases. Since then, researchers have figured out how to isolate ancient pathogens; recently, they retrieved pathogens from Egyptian mummies and victims of the 1918 Spanish flu epidemic, to cite just two examples. And last November, French researchers reported that they had recovered DNA from *Yersinia pestis*, the bacterium that caused the Black Death in the 14th century, lurking inside the teeth of two people who died during the outbreak.

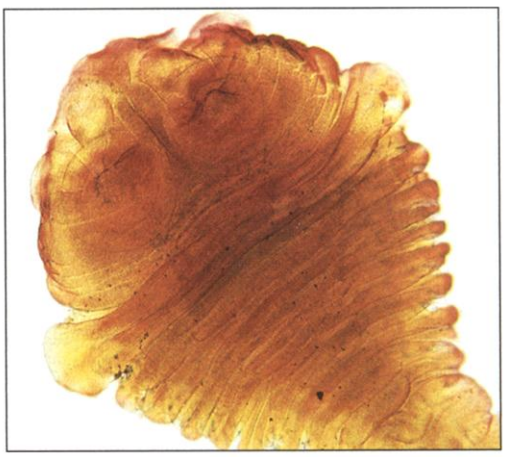
But ancient DNA from pathogens is so rare that it can provide only limited information. A much richer lode can be mined from the genes of living pathogens, Gibson and others say—if you know how to interpret the data. Armed with new tools, researchers are now building evolutionary trees of disease-causing organisms and, in the process, overturning some conventional wisdom about how diseases arose. These phylogenies enable them to determine which strains of a pathogen are more ancient or more recently evolved. More clues come from the geographical distribution of each strain, which can show where it originated and how it subsequently spread. And by discovering closely related pathogens in animal hosts, researchers are gaining new insights into when they first jumped into the human race. Such genetic analyses have only become feasible in the past 10 years or so, Gibson explains, as researchers have been able to gather long DNA sequences from a broad range of pathogens and analyze them with powerful computers: "It's a new way of getting answers to problems where there is no other evidence."

Research into the genetic history of diseases is in its infancy. It is still common, for example, for researchers to find that their data produce two or more evolutionary trees that are equally likely to be correct. Even so, this method is shedding light on the origins of all kinds of parasites, from tapeworms to bacteria to viruses. "In 1990, people didn't think of looking at viral sequences through the eyes of evolution that often," notes Edward Holmes of Oxford University. "Now, thinking about evolutionary relationships is the norm."

Sleeping sickness in Gondwana

With these tools in hand, researchers can trace the evolution of human diseases from millions of years ago, well before the origin of our species. Take trypanosomes. *Trypanosoma brucei* causes sleeping sickness, which kills an estimated 300,000 people in Africa each year. In South America, the closely related *T. cruzi* causes Chagas' disease, which affects 20 million people each year. In the 2001 *Advances in Parasitology*, Gibson and her Bristol colleagues report that by analyzing DNA sequences from 62 different species of the genus *Trypanosoma*, they have found evidence of a common ancestor for *T. cruzi* and *T. brucei*—100 million years ago. At that time, Africa, South America, and Australia were joined in the supercontinent Gondwana. Africa split off first, and its drift is reflected in the evolutionary tree of trypanosomes: *T. brucei* and almost all other African trypanosomes belong to a single branch. By contrast, certain trypanosomes that infect kangaroos in Australia show a kinship with South American forms such as *T. cruzi*.

African trypanosomes probably coevolved with human ancestors for millions of years; like baboons and several other African primates, humans carry an antitrypanosome factor in their blood that prevents many African species of trypanosomes from infecting them. Gibson speculates that the ancestors of both baboons and humans were apes that lived on the African plains, where they



Unexpected hosts. Research suggests that tapeworms first infected our hominid ancestors as they scavenged grazing mammals on the African plains.

CREDITS: (TOP TO BOTTOM) WENDY GIBSON/UNIVERSITY OF BRISTOL; ALFRED PASIEKA/PHOTO RESEARCHERS

Can Genes Solve The Syphilis Mystery?

Whether Columbus brought syphilis to the New World—or to the Old World—has been the subject of conjecture for at least 500 years. Over the past 5 decades, paleopathologists have been scouring skeletons for clues. The bones, however, tell an ambiguous story. Some seem to clearly implicate Columbus, or at least his crew. Bones of precontact Native Americans bear scars that are consistent with syphilis, and the first records of syphilis in Europe turned up shortly after Columbus returned from the New World.

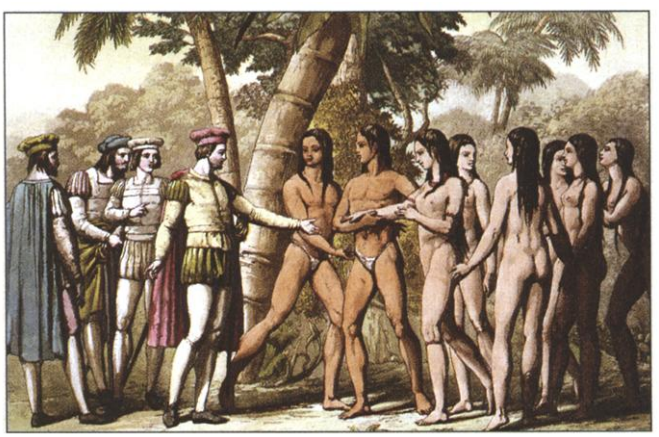
But in June 2000, a new report challenged that idea. Researchers at the University of Bradford, U.K., who have been excavating skeletons from an English monastery in the town of Hull, claimed that the skeletons show signs of syphilis. British television producers commissioned a study of the age of one of the affected skeletons and announced last summer that it dated back between 1300 and 1450. If the monks did in fact have syphilis, they couldn't have gotten it from Columbus's voyage, which was still years in the future. That doesn't necessarily mean that their infections couldn't have come from the New World—perhaps the Vikings brought syphilis home instead (*Science*, 4 August 2000, p. 723).

Since that report, however, the story has gotten murkier. According to Anthea Boylston, who leads the excavation, the preliminary date may need to be recalibrated because the residents of Hull ate a lot of fish, which can skew radiocarbon results. And it's possible, some critics say, that the residents of Hull didn't have syphilis at all.

Bruce Rothschild, a New World–origin advocate at Northeastern Ohio Universities College of Medicine in Rootstown, questions the diagnosis of the monastery skeletons. Only a small fraction of victims typically develop the characteristic bone lesions and deformities of syphilis. Yet 30% of the bones at the monastery reportedly show evidence of syphilis, implying that the entire population of both monks and villagers had the disease. Rothschild suggests that the people buried at Hull contracted yaws, a closely related skin disease that typically leaves its mark on a higher fraction of its hosts. He has also offered evidence that syphilis was present not only in the New World when Columbus arrived, but at the very place he landed. In the October 2000 issue of *Clinical Infectious Diseases*, Rothschild describes signs of syphilis on bones dating back between 1200 and 500 years ago found in the Dominican Republic. "That's the smoking gun," he says.

Some resolution may come from George Weinstock of Baylor College of Medicine in Houston and his colleagues. In 1998 they sequenced the genome of *Treponema pallidum pallidum*, the bacterium that causes syphilis. Since then they've compared parts of its genome to that of *T. p. pertenue*, the bacterium associated with yaws. "They're remarkably similar," says Weinstock. "We've found only four areas with noticeable differences." Weinstock hopes to analyze those areas on strains of syphilis and yaws and construct a phylogeny. Another possibility is to use them to isolate the bacterial DNA from bones Rothschild and others have studied and determine which form they are. "We should be able to scan a large number of syphilis isolates from around the world. That's one of the things on our list to do, for sure."

–C.Z.



Dangerous liaisons. Did Columbus bring syphilis back to Europe, or was the disease already endemic?

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were plagued by the tsetse fly that carries trypanosomes, although she concedes that on that score "we need more hard evidence."

Sleeping sickness is one of a number of diseases known to have been with humans for hundreds of thousands (or even millions) of years, including some diseases that have only recently come to light. *Helicobacter pylori*, the stomach bacterium that causes ulcers, was just discovered in 1982; since then, it has been found in stomachs around the world. That global distribution, combined with its genetic variability, hints at an ancient origin. *H. pylori* tends to be transmitted within families, and Mark Achtman of the Max Planck Institute for Infection Biology in Berlin says it's possible that parents have been passing it down to children since they emerged from Africa. He and his colleagues have constructed an evolutionary tree based on genetic variation among *H. pylori* strains. It has two deep branches, one of which infects Europeans and one which infects East Asians. That split might have occurred when emigrants from Africa first parted ways to settle the two continents. In fact, thanks to the genetic diversity of the bacteria, they may prove to be a tool for reconstructing ancient human migrations. "They may turn out to be a better tool than human genomes," says Achtman.

Rethinking the domestication link

Until recently, scientists have assumed that relatively few human diseases have such ancient pedigrees. The rest were thought to have colonized our bodies with the rise of civilization 10,000 years ago. With the advent of agriculture, people began living close together, making it easy for pathogens to jump from host to host. At the same time, people acquired some of the parasites that had infected their livestock, the logic went. But new phylogenies are suggesting that some supposedly recent diseases are surprisingly old.

Today human tapeworms cycle between pigs or cows, their intermediate hosts, and humans. Because of that life cycle, researchers in the 1940s proposed that the three species that infect humans today descend from tapeworms that pioneered our guts when cattle and pigs were first domesticated. But when Eric Hoberg of the U.S. Department of Agriculture and his colleagues recently constructed a phylogeny of human tapeworms and other species of the genus *Taenia*, they found no support for this idea. As they described in the 22 April issue of the *Proceedings of the Royal Society of London*, the closest relatives of human tapeworms did not colonize either cows or pigs. Instead, they lived inside East African herbivores such as antelopes, with the lions and hyenas that kill them as their final hosts. "Once we found these host relationships, we knew we had found something interesting," says Hoberg.

The researchers then analyzed the amount of genetic variation among different species of tapeworms. If the agricultural hypothesis were correct, that variation should

have pointed to a common ancestor 10,000 years ago. But Hoberg's team concluded that this common ancestor could have lived as long as 1 million years ago. "What we think happened is that as hominids made the shift from herbivory to carnivory, they were exposed to these tapeworms," says Hoberg. By scavenging or hunting on the East African savannas, our ancestors became an attractive new host for tapeworms, and species evolved that were specialized to live inside humans. Only hundreds of thousands of years later did they make cows and pigs their intermediate hosts.

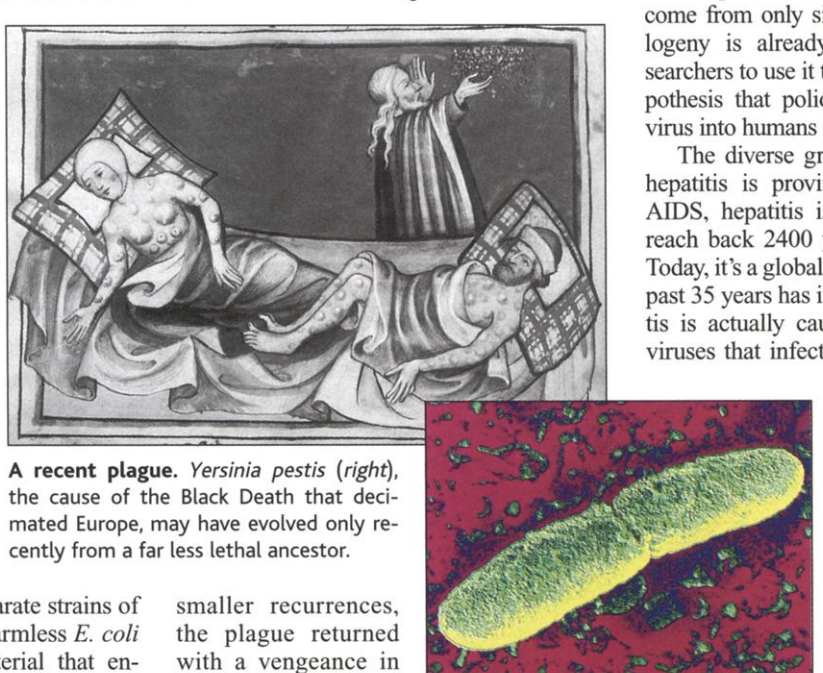
Dysentery's roots may also go farther back than scientists have suspected. Caused by bacteria that burrow into the mucosal membrane of the intestines, the disease brings with it bloody diarrhea that can ultimately kill a host. Today dysentery is widespread in poor countries where dense populations depend on contaminated water supplies. Unknown in any other host than humans, dysentery fits the classic model of a disease that could not have existed before the dawn of civilization.

The dysentery-causing bacteria were originally thought to belong to a genus known as *Shigella*, closely related to the common (and generally harmless) bacteria *Escherichia coli*. In the 12 September 2000 issue of the *Proceedings of the National Academy of Sciences*, microbiologist Peter Reeves and his colleagues at the University of Sydney in Australia showed that *Shigella* is not a genus in itself; rather, it consists of eight separate strains of *E. coli*. "*Shigella*" arose as harmless *E. coli* strains acquired genetic material that enabled them to invade intestinal cells, Reeves speculates—a view that is now widely accepted, although experts are divided over when this occurred.

To date the microbe, Reeves's group analyzed the genetic variation among the three main branches of *Shigella*. Two of them appear to be between 50,000 and 270,000 years old; the third is slightly younger, at 35,000 to 170,000 years. If so, dysentery existed long before the rise of civilization. But as Reeves himself notes, "we're on thin ice here." His team based these dates on a molecular clock derived from the mutation rate of *E. coli*. Compared to normal *E. coli* strains, *Shigella* may replicate—and evolve—much faster, he says, because it spends little time outside its host. But if future research bears out Reeves's preliminary estimate, "we'd have to rethink how it [the bug] could survive" unless its hosts lived in close quarters and transmitted the bacterium between them.

Achtman calls the new work one of the "first breaks in the dam." He believes that more genetic analyses will show that many bacterial diseases predate the rise of civilization. Additional research, although less definitive, hints at precivilization origins for other supposed diseases of civilization, such as tuberculosis and anthrax. Even the notorious *E. coli* O157:H7, which was first linked to food poisoning in the 1980s, appears to have existed for millions of years before it started making hamburgers fatal.

In other cases, molecular research is revealing that some diseases are relatively young. Bubonic plague made a dramatic entry into the historical records in A.D. 542, when it swept through the Roman Empire, wiping out a million people, including 40% of the population of Constantinople. After several smaller recurrences, the plague returned with a vengeance in 1347, creating the Black Death, which killed up to a third of all Europeans.



A recent plague. *Yersinia pestis* (*right*), the cause of the Black Death that decimated Europe, may have evolved only recently from a far less lethal ancestor.

How long had *Y. pestis* been circulating among humans before it first made its debut in historical records? According to Achtman and his colleagues, not very long. They have reconstructed a molecular history of the plague by comparing the genes of different strains of *Y. pestis*. The organism turns out to be closely related to *Y. pseudotuberculosis*, a bacterium that is shed in rodent feces and causes a mild disease. Achtman estimates that *Y. pestis* evolved from a strain of *Y. pseudotuberculosis* only 1500 to 20,500 years ago.

HIV, hepatitis, and malaria

The same methods that let scientists trace human diseases back hundreds of thousands of years are also revealing how some diseases have evolved and spread in this century. The best documented example of these arrivistes is HIV. Vanessa Hirsch of Georgetown University in Washington, D.C., for example, showed in 1989 that HIV-2, one major form of the virus that causes AIDS, is closely related to a virus that infects sooty mangabeys in West Africa. The monkey virus, it appears, jumped into humans from sooty mangabeys kept as pets or hunted for food.

More recently, Beatrice Hahn of the University of Alabama, Birmingham, and her colleagues constructed a phylogeny of HIV-1, the far more common form of the virus, indicating that it crossed the species barrier from West African chimpanzees to humans around 1930 (*Science*, 28 January 2000, p. 607). Although researchers have generally accepted these results, they remain tentative because the sample sizes are so small. (Hahn's results come from only six chimps.) But HIV phylogeny is already robust enough for researchers to use it to reject a controversial hypothesis that polio vaccines introduced the virus into humans (*Science*, 27 April, p. 615).

The diverse group of viruses that cause hepatitis is proving more elusive. Unlike AIDS, hepatitis is an old foe; its records reach back 2400 years ago to Hippocrates. Today, it's a global scourge. But only over the past 35 years has it become clear that hepatitis is actually caused by several unrelated viruses that infect and inflame the liver. To date, researchers have identified at least six hepatitis viruses, each of which is identified with a single-letter suffix.

Although just discovered in 1989, hepatitis C infects 170 million people worldwide (*Science*, 2 July 1999, p. 26). It is in the Flaviviridae family, a group of viruses made of single strands of RNA that includes dengue and yellow fever. Researchers can't find any particular kinship between any other flavivirus and hepatitis C, which prevents them from figuring out which species served as host to the ancestors of today's viruses. "We don't know where it comes from, but it's way more diverse than HIV, and so it must have been around a lot longer," says Holmes of Oxford. Holmes has measured the rate at which new branches have budded off the hepatitis C tree. For most of its history, the rate was relatively slow until it exploded 50 to 80 years ago, says Holmes. "Here's my scenario. It's probably endemic in parts of the world, but the real explosion in the West corresponds to blood products and needle sharing during drug use"—in other words,

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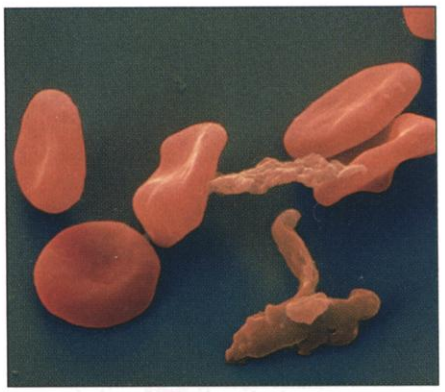


Hominid malaria? The earliest hominids in Africa were probably infected with *Plasmodium falciparum*, which causes the deadly form of malaria.

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

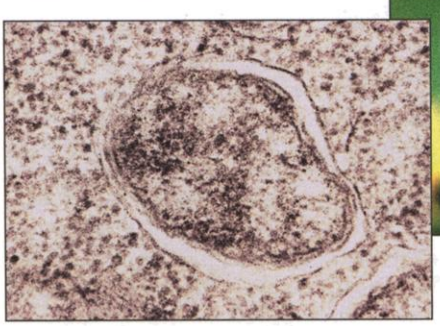
Wolbachia: A Tale of Sex and Survival

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.

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