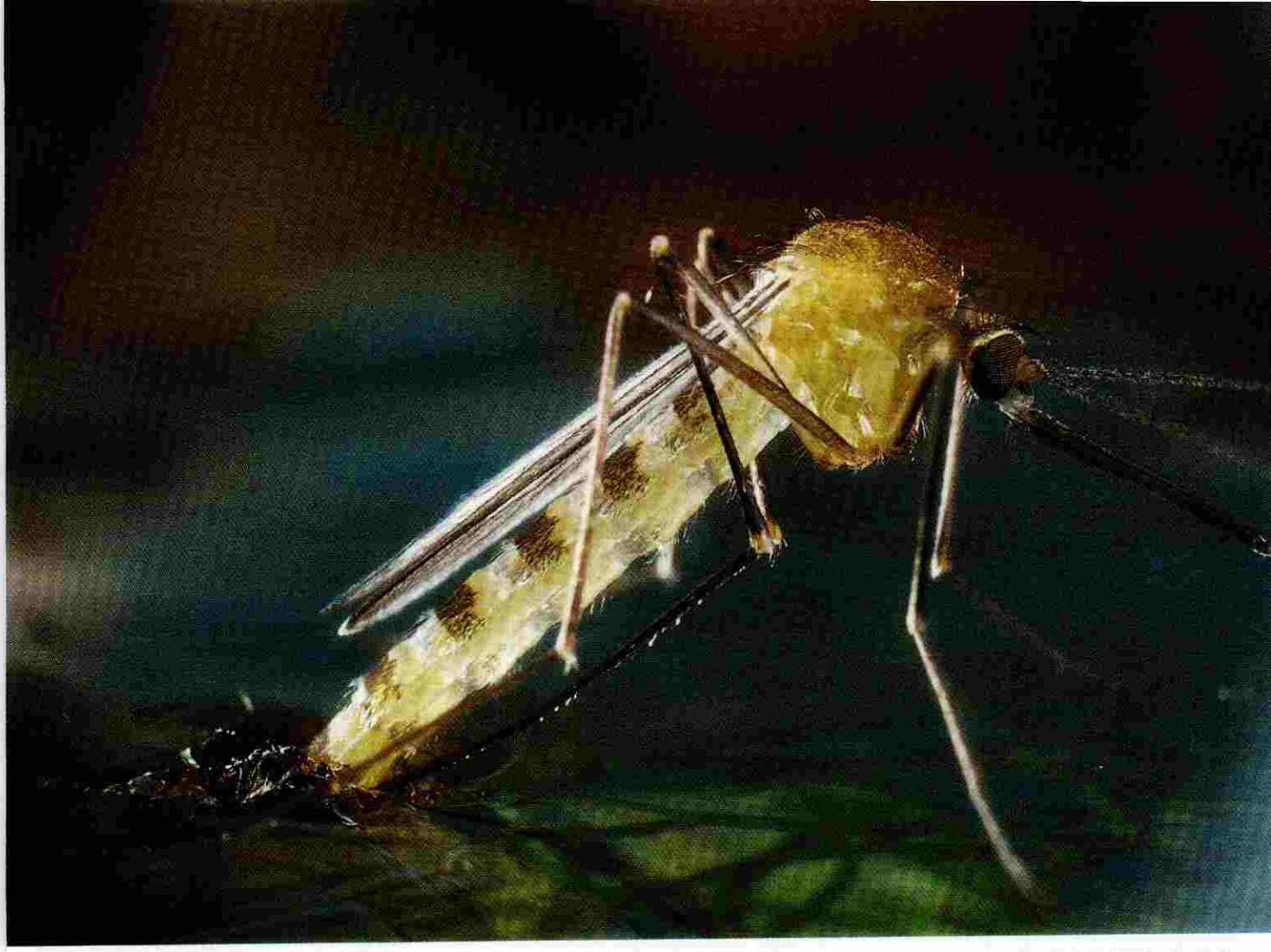


A Culex pipiens *mosquito, newly emerged from its pupal skin: Males of the species, if infected by* Wolbachia *bacteria, produce sperm that is incompatible with the eggs of uninfected females. Other* Culex *species have protozoan infections that are transmitted through the eggs and kill the male larvae but not the female ones.*

Invasion of the Gender Benders

By manipulating sex and reproduction in their hosts, many parasites improve their own odds of survival and may shape the evolution of sex itself.

By John H. Werren

Sex is fraught. Every teenager can attest to the havoc it wreaks—and to its unique power to change a life. Of course, that's one of life's lessons that survive far beyond the teenage years—and far beyond the human condition. To anyone who explores the ramifications of sex in other species, its permutations seem bottomless. In recent years, the study of evolution, of parasites, and even of disease has often led back to sex. Particularly fascinating are the ways in which some parasites manipulate sex and reproduction in their hosts—stories of exploitation and subterfuge that have amazed and astonished even life scientists long jaded by tales of biological intrigue.

Take the case of *Nosema granulosis,* a protozoan that often resides within the cells of *Gammarus duebeni,* a small shrimp that lives in intertidal pools along the coasts of Europe. When an infected mother shrimp reproduces, the protozoans hitch a ride in the cytoplasm of her eggs and thereby infect her offspring. But if the protozoans infect a male shrimp, they cannot readily infect his offspring by hitching a ride in his sperm, because sperm contain so little cytoplasm. As a result, *N. granulosis* is transmitted solely by female hosts, not by the males.

So what happens when the protozoan ends up in a baby male shrimp? That would seem to be the end of the line. What's a protozoan to do? To bypass this dead end, *N. granulosis* takes over the sex-determining mechanism of the shrimp and converts the male into a female. That bit of genetic magic assures the protozoan's passage to future generations—though how it accomplishes this, no one knows.

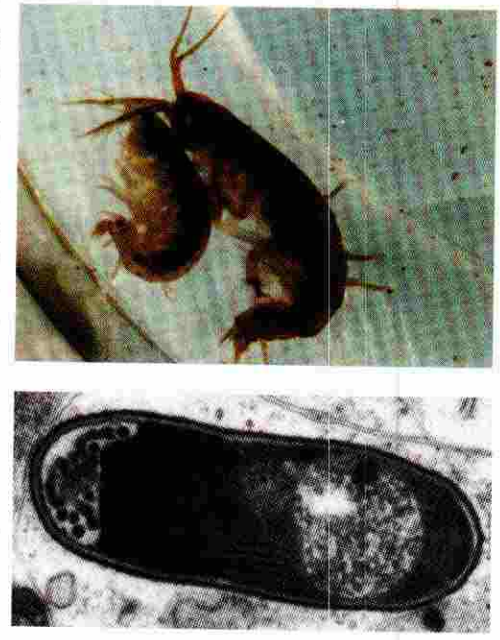
Naturally, if the protozoans were to become too common in host populations, they could drive the shrimp to extinction by causing a scarcity of males. Fortunately for the survival of both species, the protozoans are not transmitted to all the eggs of an infected mother; in the wild, in fact, they typically infect fewer than a fifth of the baby shrimp.

Parasites that manipulate the sex of their hosts are called reproductive parasites—and they are not as rare as one might like to think. Some, such as *N. granulosis,* convert males into females, but a widespread and diverse array of microorganisms simply kill the sons of their hosts; the daughters, which transmit the microorganisms, are allowed to live.

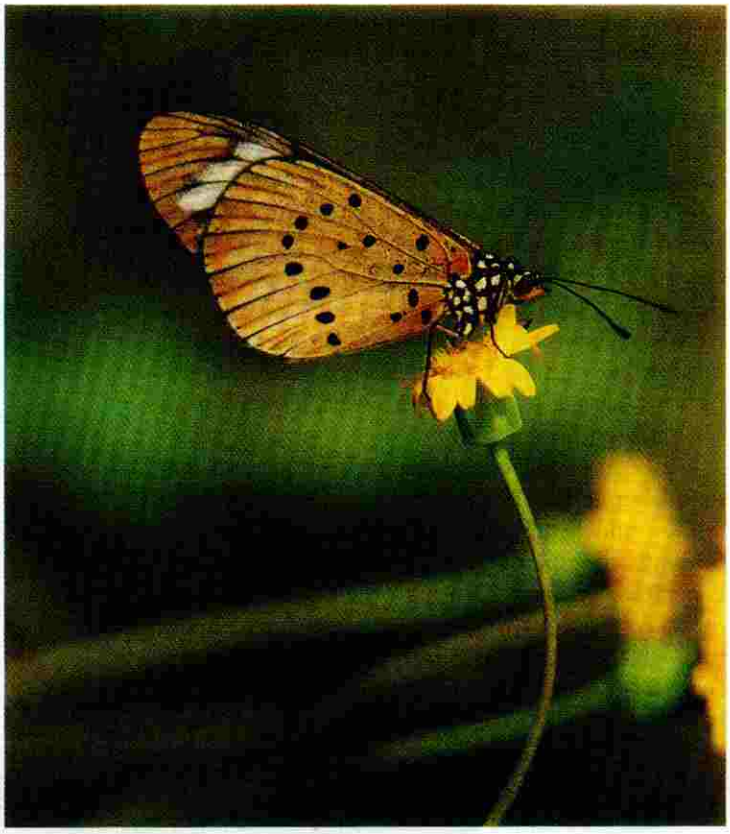
The protozoan *Amblyospora californica,* for instance, is transmitted through the eggs of infected female mosquitoes, but it kills the developing male larvae. Once again, that would seem to be a dead end for the protozoans in the males, but all is not lost. The protozoans in the males develop into specialized spores that cannot infect other mosquitoes but can infect small aquatic crustaceans called copepods. When a female copepod ingests the remains of a male mosquito larva killed by the protozoans, the copepod also ingests the spores. The protozoans then infect the female copepod and turn her ovaries into a "protozoan factory," generating the kind of spores that *can* infect mosquito larvae. When the mosquito larvae are filter feeding, they take in the spores from the water, and so complete the cycle. Thus the parasite has the best of both worlds: it exploits its female mosquitoes for transmission via eggs, and the male mosquitoes for infectious passage to new hosts. Pretty clever for an organism without a brain.

Other male-killers include various bacteria that make themselves at home in fruit flies, wasps, butterflies, and beetles. In those insects, though, the only way the microorganisms make it into the next generation of hosts is through the eggs of infected mothers. No sex-change operation on a male insect is possible; no suitable "third-party" species like the copepod is available to provide the parasites in males with an alternative host. For parasites that end up in a male, the options are limited. Killing the male insect has zero cost to the parasite, but what is the benefit?

In some cases it appears that killing off male hosts enhances the survival of the hosts' infected sisters. After all, without the males to compete with, the infected female insects have more resources for themselves. That alone, of course, doesn't help the parasites in the male insects. Unlike the *A. californica* protozoans, they gain nothing directly, because they die along with their hosts. They do gain indirectly, however, because the death of the male insects benefits the parasites' "family." All the parasites passed along by the infected mother insect are genetically identical to one another (that is, they are a clone). The parasites



Shrimp of the species Gammarus duebeni, *top, often harbor protozoans that are transmitted through the shrimp's eggs. In a step that ensures their own transmission, the protozoans can change a male host into a female. Bottom: The protozoan species in question,* Nosema granulosis.



Some populations of the white-barred Acraea, *above, are infected by* Wolbachia *bacteria that kill off most of the males. The female butterflies then assemble in courting areas, which attract the few remaining males.*

that happen to infect a daughter insect benefit from the additional resources available to her. So, by killing males the extended clone of parasites increases within host populations. For infected insect mothers, however, the infection is a disaster, because all of their sons are killed.

When male-killers become widespread, they can even affect the mating system of their hosts. Francis M. Jiggins, a biologist at the University of Cambridge, has detected male-killing microorganisms in high proportions of the individuals in some populations of African butterflies, and the highly skewed sex ratios that result lead to changes in the mating system. In *Acraea* butterflies males normally congregate at food plants, and matings take place there when the females arrive to lay their eggs. But in some populations of the white-barred *Acraea,* so many females are infected with male-killing bacteria (more than 95 percent in some cases) that males are extremely scarce. In those populations, females assemble in courting areas called leks to attract the few males that are flying about. These lucky males procure many matings, but there is still not enough sperm to go around, and many females remain uninseminated. Female leks are extremely rare in nature; in most species that form leks, it is the males that aggregate to attract the females. But under the pressure of male-killing bacteria, the white-barred *Acraea* appears to have evolved an unusual but adaptive mating system.

Biologists have just begun to document the diversity of male-killing bacteria in nature, and it is likely that a large percentage of invertebrate species play host to them. Vertebrates may also harbor male-killers, though none have yet been found. People need not worry, though: given the intense study of our own species, if we carried male-killing microorganisms, they would certainly have been discovered by now.

The white-barred *Acraea* is an extreme case; male-killers rarely infect a fraction of a population large enough to force a change in the mating system of a host species. Yet some biologists speculate that even a relatively small proportion of infected individuals (say, 5 percent) pushes the sex-determining genes of a host species to change in ways that enable it to escape or to suppress the male-killing effects. The cat-and-mouse game between male-killers and their hosts may be one of the motors contributing to the great diversity of sex-determining mechanisms that occur in nature.

The undisputed virtuosos of reproductive parasites are bacteria of the genus *Wolbachia,* which, like many of their brethren, are transmitted in the cytoplasm of eggs. These bacteria also infect across species boundaries, which has made them unusually widespread in invertebrates. *Wolbachia* bacteria infect many insects, arachnids (mites and spiders), crustaceans, and parasitic nematodes. At least 20 percent of all insect species harbor them, and the proportion could be as high as 70 percent—biologists are still trying to determine the number. Because most animal species are invertebrates, the abundance of *Wolbachia*'s hosts makes the genus among the most common parasitic bacteria on the planet. Analysis of its DNA indicates that the bacteria have lived in insects for at least 50 million years, and in invertebrates for at least 100 million. Only ten years ago *Wolbachia* was regarded as an obscure little group of bacteria, but the genus has come up in the world, at least in the eyes of biologists.

Its broad distribution is one of the major mysteries of *Wolbachia:* how can one genus of bacteria infect so many kinds of hosts? Some investigators speculate that species that are ecologically associated in some way (predators and prey, for instance, or competitors feeding on the same food resource) may occasionally exchange *Wolbachia.* But con-

vincing evidence has not yet surfaced to back up the speculation.

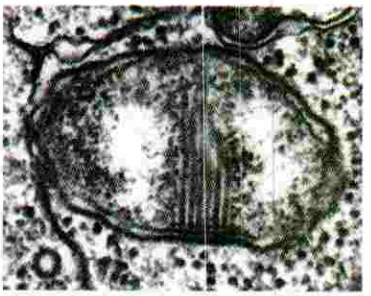
Wolbachia bacteria are masters at manipulating the reproductive and cell biology of invertebrates. Like other reproductive parasites, some members of the genus kill the male insects they infect, whereas others turn males into sexually functioning females. Some even induce parthenogenesis in their hosts—a mode of reproduction in which eggs develop into females without fertilization, thereby dispensing with males and their sperm. Parasitic parthenogenesis has been noted in more than three dozen species of insects, mainly wasps. The bacteria accomplish this trick by manipulating the basic processes of the cell in such a way that the single set of chromosomes in the egg is duplicated, and the unfertilized egg develops into a female.

When the bacteria in parthenogenetic insects are killed with common antibiotics such as tetracycline, the insects usually revert to sexual reproduction. Sometimes, however, the insect species have been parthenogenetic for so long that when the *Wolbachia* bacteria are eliminated, the insects can no longer reproduce sexually. In the small parasitic wasp *Encarsia formosa*, antibiotics lead to the production of males, but the males cannot mate: the genes needed for male courtship have been lost.

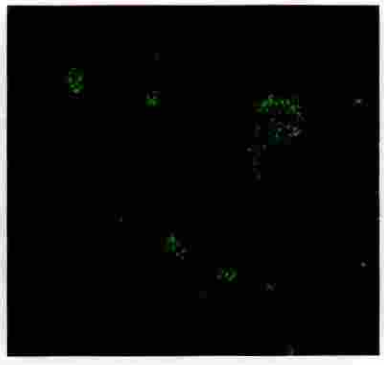
Parasites that are passed on through the eggs of their host species face a potential dead end if they find themselves in a son of their former host.

Other wasp species have similar stories to tell. In some, the females no longer respond to courtship; in others, the males no longer produce functional sperm. Given enough time, mutations accumulate in the genes for sexual characteristics, and the species can no longer revert to sexual reproduction. Their reproduction becomes completely dependent on the bacteria that live inside their cells.

But perhaps the most intriguing effect of *Wolbachia* is the ability of some strains to induce an incompatibility between host sperm and eggs, a process that may even implicate the microorganisms in the evolutionary divergence of insect species. The discovery of these capabilities has a long history. *Wolbachia* bacteria were



Above: A kind of bacterium living in this Encarsia *wasp causes it to reproduce parthenogenetically—that is, the wasp's eggs develop into females without the need for fertilization. Top right: The as-yet-unnamed bacterium, a relative of soil bacteria of the genus* Cytophaga.



A Brevipalpus phoenicis *mite, left, can be infected with the* Cytophaga*-like bacteria, above, which enable the mite to reproduce parthenogenetically. Other mite species carry* Wolbachia *bacteria that induce incompatibility between sperm and egg.*

first observed in the 1920s, when the pathologists Arthur Hertig and S. Burt Wolbach, working at Harvard Medical School, found them inside the eggs of *Culex* mosquitoes. Hertig later named the bacterial genus in honor of his colleague and mentor. In the 1950s the German biologist Hannes Laven discovered that when males from some strains of the mosquito *C. pipiens* were crossed with females of another strain, the offspring died as embryos. Laven subsequently showed that the effect was inherited through the mother's lineage. As he viewed it, the cytoplasm in the eggs of certain strains of insects was incompatible with the sperm from certain other strains. Laven was apparently unaware, however, that bacteria had earlier been discovered in the eggs of the insects.

It wasn't until the early 1970s that two other investigators, Janice H. Yen and A. Ralph Barr of the University of California, Los Angeles, made the connection. They showed that Laven's "cytoplasmic incompatibility" was caused by the bacteria. Antibiotic treatments that eliminated the bacteria also changed the compatibility relationships between males and females.

The basic pattern is that eggs from uninfected females are incompatible with sperm from infected males. The *Wolbachia* present in the testes of males biochemically "encrypt" the developing sperm, probably by altering proteins that bind to the sperm DNA. The same strain of *Wolbachia* must then be present in the egg to "decode" the encrypted sperm. Otherwise the chromosomes from the sperm are not properly processed in the fertilized egg, and the embryo dies. The actual mechanisms are still a mystery, but it is already clear to investigators that there are many different kinds of *Wolbachia,* which differ in their encryption systems.

The diversity of the encryption mechanisms raises the possibility that *Wolbachia* could play a role in the evolution of new insect species. If different populations of a species, or closely related species, are infected with different strains of *Wolbachia,* the bacteria could prevent the insects' gene pools from mixing. Just such a circumstance may have arisen in jewel wasps, a genus *(Nasonia)* of small parasitic wasps that kill fly pupae. There are three closely related species of jewel wasps, but each is infected with its own distinct *Wolbachia.* The bacteria render any matings between the different wasp species incompatible, thereby preventing the development of hybrids.

Biologists have also discovered that *Wolbachia* plays an essential developmental role in some host species. For example, if *Wolbachia* bacteria in the wasp *Asobara tabida* are eliminated with antibiotics, the female wasps fail to develop ovaries and so become sterile. Filarial nematodes—parasitic "worms" that cause such diseases as river blindness and elephantiasis in people and heartworm in dogs—also need the bacteria if their embryos are to develop properly. Antibiotic treatment of adult worms kills the embryos, rendering the adults sterile. This discovery has increased interest in the possibility that nematode diseases can be controlled with antibiotics [*see "The Worm and the Parasite," by T. V. Rajan, page 32*].

To study the details of *Wolbachia*'s capabilities, biologists have experimentally transferred the bacteria from one insect species to another. The method is similar to the microinjection techniques developed for in vitro fertilization: a needle containing the bacteria from one insect is injected into the egg of a different, uninfected species. Not surprisingly, perhaps, the "foreign" *Wolbachia* bacteria can have different effects in their new hosts. For example, in the adzuki bean borer moth *(Ostrinia scapulalis), Wolbachia* turns a male host into a female. When the same bacteria are injected into the common flour moth *Ephestia kuehniella,* however, they simply kill the males.

Alerted to the newly recognized importance of *Wolbachia* in manipulating invertebrate reproduction, investigators are now discovering an en-

Fifty million years from now, Wolbachia *bacteria may, like the mitochondria before them, have evolved into a new kind of cell organelle.*

tire pantheon of sex-manipulating microorganisms that are transmitted from females to their offspring through eggs. A recent finding is a relative (as yet unnamed) of soil bacteria in the genus *Cytophaga*. Biologists have shown that the unnamed bacterium induces parthenogenesis in hosts as varied as wasps and mites, and is likely to be widespread.

Others await discovery. The genus *Rickettsia*, which is a member of the same family as *Wolbachia*, includes a number of disease-causing bacteria spread by arthropods, such as the microorganisms responsible for Rocky Mountain spotted fever and typhus. Recently, *Rickettsia* bacteria that are transmitted through eggs and cause male-killing have been identified. I anticipate that once additional discoveries are made, it will be clear that most members of the genus are engaged in distorting sex in arthropods, and that causing disease in vertebrates is a relatively uncommon trait. The widespread occurrence of reproductive parasites illustrates a basic principle: whenever a microorganism is inherited through the eggs of its host, it will be selected for its capacity to manipulate the host's reproduction in ways that enhance the microorganism's transmission.

An even more remarkable story than that of *Wolbachia* and other reproductive parasites belongs to the "microbes" present in nearly all plants and animals—the mitochondria. Flourishing in the cytoplasm of nearly all nucleated cells, mitochondria are specialized organelles, with their own DNA. They are the cell's power stations, generating energy for cellular metabolism. There is now overwhelming evidence that mitochondria evolved from a symbiotic bacterium during the early evolution of nucleated cells. In fact, on the basis of similarities in their DNA, biologists now think mitochondria and *Wolbachia* may be distant relatives.

The small parasitic wasp Trichogramma kaykai *deposits its eggs within the eggs of butterflies. Inside the ovaries of this wasp are* Wolbachia *bacteria that induce parthenogenetic development of the wasp eggs.*

Like *Wolbachia*, mitochondria are inherited through the cytoplasm, and therefore from mothers but not from fathers. And, like *Wolbachia*, mitochondria that skew the sex ratio of their "host" organisms toward females can be favored by natural selection. Biologists have demonstrated that in many plants, such as corn and rye, mitochondrial variants cause an abortion of the male parts of the plant, the pollen-producing anthers. The effect is known as cytoplasmic male sterility, and it leads to an increased production of seeds, which transmit the mitochondria. A contest ensues: plant genes evolve that suppress the renegade mitochondria, and new mitochondrial variants arise that can escape the new control.

As far as anyone knows, animal mitochondria do not play such games. The reason may be simply that animal mitochondria have much smaller genomes than their counterparts in plants, and therefore may not be able to draw from as rich a grab bag of genetic trickery. Fortunately for animals and plants, most of the time mitochondria are quite well behaved.

The comparison with mitochondria raises one final, tantalizing question about bacteria of the genus *Wolbachia*. Given their ubiquity, their adopted homes within the cells of other organisms, and their heritability through the eggs of their hosts, why haven't they evolved into organelles like the mitochondria before them? Perhaps it's only a matter of time before they do. If bacteriologists take a peek in, say, 50 million years, they might well find that *Wolbachia* bacteria have been tamed by some invertebrate group and have evolved into a new kind of cell organelle. What service that organelle might perform is anyone's guess. □

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