Meet the Herod bug

A parasitic bacterium that uses an array of dastardly tricks to favour female hosts over males is holding evolutionary biologists in thrall. Jonathan Knight enters the strange world of *Wolbachia*.

Hitching a ride: *Wolbachia*, seen here as bright spots in an insect egg, pass from one generation of their host to the next.

RICHARD STOUTHAMER

According to the Bible, King Herod had the blood of thousands of male children on his hands. Yet his record for gender-biased genocide pales alongside that of *Wolbachia*, bacteria that infect a wide variety of invertebrates including insects, spiders, crustaceans and nematode worms.

The bacteria, which live inside the cells of its hosts' reproductive systems and other tissues, can only spread from one generation to the next by invading a female's eggs. Males, to *Wolbachia*, are useless. Accordingly, these bacteria remove males from the populations they infect with a chilling efficiency, using strategies that range from enforced sex changes to cold-blooded murder.

Wolbachia were long regarded as a biological quirk. But now it seems that they are much more prevalent than was originally thought, and evolutionary biologists are starting to view these bizarre parasites as a unique natural laboratory. Thanks to their male-hating habits, *Wolbachia* might help investigations of such key questions as how species are created and go extinct, and why different organisms determine the difference between the sexes in myriad ways. "It's clear that they are a major force in the biology of a lot of invertebrates," says Scott O'Neill, who studies the bacteria at Yale University in New Haven, Connecticut.

Wolbachia disrupt their hosts' biology in many ways. In mosquitoes, for instance, infected males can only reproduce successfully with females infected with the same strain of *Wolbachia*[1]. Although the bacteria themselves cannot hitch a ride in sperm, they alter the cells by an unknown mechanism so that the sperm can only fertilize eggs that contain the same *Wolbachia* strain. This 'cytoplasmic incompatibility' reduces reproduction among uninfected females, and so drives the infection through the population with each generation. *Wolbachia*-induced cytoplasmic incompatibility has also turned up in beetles[2], wasps[3], moths[4] and fruitflies[5].

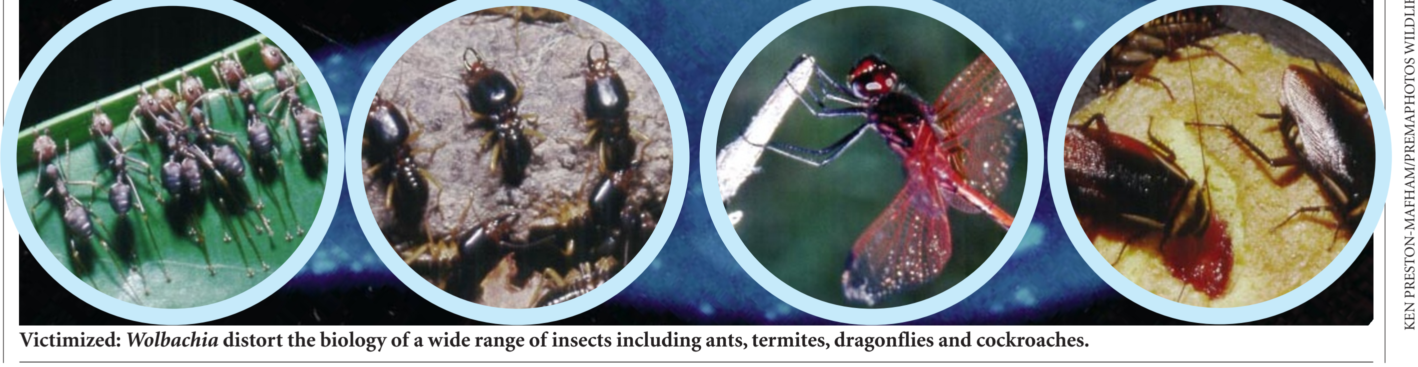
In other species, *Wolbachia* makes males dispensable. Some populations of parasitic wasp in the genus *Trichogramma* are entirely female, reproducing parthenogenetically — without fertilization. Parthenogenesis arises from time to time in animals ranging from insects to fish and lizards. But in 1990, Richard Stouthamer of the University of California, Riverside, found that antibiotics could 'cure' parthenogenesis in *Trichogramma*[6]. Later, he revealed that the culprit was a *Wolbachia* infection[7].

Wolbachia also convert male woodlice into females, apparently by suppressing a gland that produces a masculizing hormone[8]. In species including ladybirds, flour beetles and several species of fruitfly, *Wolbachia* simply kill males during early development[9–11]. By preventing these 'dead-end' hosts from hatching, they ensure better supplies of food for their infected daughters. "It's a bacterium that can do it all," says Stouthamer.

Out of the shadows

Wolbachia were relatively obscure until John Werren of the University of Rochester in New York looked for them in the wild — and found them everywhere. Using the polymerase chain reaction (PCR) to copy fragments of bacterial DNA from insect tissue samples, Werren discovered evidence of *Wolbachia* infection in about 17% of the species he surveyed in Panama and 20% of those in the United States and Canada[12].

Wolbachia might in fact be even more widespread than that. Last year, Marjorie



Victimized: *Wolbachia* distort the biology of a wide range of insects including ants, termites, dragonflies and cockroaches.

KEN PRESTON-MAFHAM/PREMAPHOTOS WILDLIFE

MARJORIE HOY

Jay Jeyaprakash (left) and Marjorie Hoy's studies of insects, including this ladybird, led them to conclude that *Wolbachia*'s prevalence is underestimated.

Hoy of the University of Florida in Gainesville was working with a species of spider mite that had a skewed sex ratio highly suggestive of a *Wolbachia* infection. She could not reproducibly detect the bacterium with standard PCR. Only 'long' PCR, which includes a second enzyme to correct errors in copying the DNA, gave consistent results.

This led Hoy to suspect that Werren's study might have underreported *Wolbachia*'s incidence. She teamed up with her Florida colleague Jay Jeyaprakash and collected specimens from 61 insect species and two species of spider from the field or local laboratory stocks. "We got some cockroaches and house flies and termites and dragonflies and ants — whatever was available," says Hoy. With long PCR, 76% of the species tested positive for *Wolbachia*[13].

Some researchers want to see further evidence before accepting that *Wolbachia* are so remarkably widespread. "I'd like to see that work replicated," says Francis Jiggins of the University of Cambridge. But Jiggins has his own evidence that Werren's estimate is low.

Werren only sampled one or two individuals from each species he tested. If *Wolbachia* only infects a small proportion of a population, he may have missed many species that do carry the microbe. Jiggins reported last month that, at least for butterflies, this is the case[14]. He surveyed a large number of individuals within a small number of African butterfly species, and found that typically only 15% of the individuals are infected. "We can certainly say that Werren's is a conservative estimate," Jiggins concludes.

If *Wolbachia* are as prevalent as these studies suggest, then the bacteria are likely to exert a powerful influence over the evolution of their hosts. One provocative proposal is that they might drive speciation — the emergence of new species. Speciation begins with the reproductive isolation of two populations of the same species. A mountain range, for instance, could separate populations for millennia until the accumulation of mutations in each prevents them from producing viable offspring if they ever do meet.

Cytoplasmic incompatibility could provide a similar reproductive barrier. If two groups within a population of insects are infected with two different types of *Wolbachia*, males from either group may be unable to fertilize females from the other. Werren believes such 'bi-directional' cytoplasmic incompatibility might allow speciation to occur. The best evidence so far comes from two species of wasp, *Nasonia longicornis* and *N. giraulti*. They look different — *N. giraulti* has much smaller wings — and do not interbreed. But they are closely related: DNA analyses suggest that their most recent common ancestor lived around 250,000 years ago[15].

In February, Werren and his graduate student Seth Bordenstein reported that the two species were infected with different strains of *Wolbachia* that were causing bi-directional cytoplasmic incompatibility[16]. Once cured with antibiotics, the wasps could produce fertile offspring. Infection with different strains of *Wolbachia* appeared to be one of the first reproductive barriers to have arisen between these recently divergent species.

Divide and conquer

But some experts question whether *Wolbachia*-induced cytoplasmic incompatibility is, by itself, enough to cause species to diverge. Scott Turelli, an evolutionary biologist at the University of California, Davis, points out that Werren's two wasp species are geographically separated: *N. giraulti* lives in eastern North America, whereas *N. longicornis* inhabits the western part of the continent. And Stouthamer notes that cytoplasmic incompatibility can break down, for instance where insects mate repeatedly[17]. He believes this may allow enough gene flow to prevent co-existing populations from becoming reproductively isolated. "My feeling is that cytoplasmic incompatibility can help once speciation has started, but it can't initiate speciation," he says.

John Jaenike, an evolutionary biologist at the University of Rochester, may have stumbled upon just such a case among fungus-eating flies. One species, *Drosophila recens*, collected from the eastern United States, is heavily infected with *Wolbachia*. Its males cannot fertilize females from another species, *D. subquinaria*, that lives in the Pacific northwest and is *Wolbachia*-free. The reverse cross — *D. subquinaria* males and *D. recens* females — does produce offspring. But in unpublished observations, Jaenike has found that *D. recens* females are reluctant to mate with their western relatives. He has yet to study the flies' behaviour in the zone in the central United States where their ranges overlap, but it is possible that female mating preference prevents hybridization in one direction whereas *Wolbachia*-induced cytoplasmic incompatibility stops it in the other.

Wolbachia are likely to exert a powerful influence over the evolution of their hosts.

Determined response

Speciation is not the only aspect of evolution in which *Wolbachia* may have a hand. Greg Hurst of University College London believes the bacteria may have helped to spawn the tremendous diversity in the way in which different invertebrate species determine whether to be male or female.

One method of sex determination, found in *Drosophila*, depends on the ratio of X chromosomes to autosomes (all the other chromosomes except the Y chromosome). Flies with two X chromosomes and two copies of each autosome will be female, whereas a 1:2 ratio produces a male. In *Trichogramma* wasps, fertilized eggs become female, and unfertilized eggs become male — unless a *Wolbachia* infection forces them to develop as females. There are many other mechanisms in different species.

To Hurst, this is an intriguing puzzle. In other respects, sex is relatively invariant — males produce sperm and females make eggs. So why should sex determination be different? "One hypothesis is that it's driven by parasites that affect sex," says Hurst. In other words, the struggle between the hosts' attempts to produce an even sex ratio, and *Wolbachia*'s desire for more females than males, has led hosts to evolve a variety of means to influence sex.

In March, for instance, Stouthamer's group reported the discovery of a 'parasitic' ▶

L. SACCHI & C. BANDI

Raising awareness: until John Werren looked for *Wolbachia* (inset inside a host cell) in the wild, they were seen as an obscure oddity.

Natural wonder: *Wolbachia*'s diverse effects on its hosts have impressed Richard Stouthamer.

chromosome in *Trichogramma* which causes females to produce only males[18]. This chromosome appears to restore some balance between the sexes to *Wolbachia*-infected wild populations of the wasp. 'Masculizing' genes have also been found in infected woodlice[19].

Decline and fall

For most species, a distorted sex ratio makes reproduction less efficient. But in theory, if the distortion becomes severe enough that females struggle to find mates, it could drive populations — and even species — to extinction. Jiggins and Hurst are investigating this possibility. Two years ago, they reported cases of male-killing by *Wolbachia* in ladybirds from Russia and butterflies from Uganda[20]. Both populations were more than 90% female, and nearly all individuals were infected.

Wolbachia has so skewed the gender ratio of the Ugandan butterfly, *Acraea encedon*, that a rare form of courtship has arisen. Usually, males compete for the attentions of choosy females. But in *A. encedon*, females wait in groups for a male to flutter by and then vie for a chance to mate with him. Many females never mate. "The question is whether they could ultimately go to extinction," says Hurst. Jiggins and Hurst are now separately studying similarly afflicted insect populations for evidence that some truly are on the brink.

A knock-on effect of male-culling might be to restrict where an insect species can live. For reasons that are not understood, *Wolbachia* do not transfer to the next generation as efficiently at higher temperatures[21]. At low temperatures, it may transmit so effectively that its male-killing habit can make populations extinct. So a population infected with *Wolbachia* might only survive in warmer climates. Jaenike is now testing this idea in *Drosophila innubila*, which lives in Arizona. He wants to know if male-killing *Wolbachia* are restricting the flies to life at warmer, lower elevations.

Intriguingly, just as *Wolbachia* are integral to the biology and evolution of their hosts, it seems that a virus known as the WO phage may be integral to the biology of *Wolbachia*. Shinji Masui and his colleagues at the University of Tokyo last year reported finding phage DNA sequences embedded in the chromosomes of several *Wolbachia* strains[22].

Phages can hitch a ride from their bacterial host for generations by inserting their DNA into the host's genome. Later the phage hops back out, gets packaged in its protein coat and bursts out of the cell to infect more bacteria. Sometimes, it takes a bit of bacterial DNA with it, and Masui suspects that this may allow the WO phage to shuttle genes from one *Wolbachia* strain to another. If so, it could explain why closely related strains of *Wolbachia* can cause radically different effects on their hosts. Genes involved in male killing, feminization and parthenogenesis might have travelled from one species to another many times, mixing up the *Wolbachia* evolutionary tree.

Preliminary evidence that genes are jumping between *Wolbachia* strains is now emerging from several labs that are sequencing the *Wolbachia* genome. O'Neill's Yale group has nearly finished one *Wolbachia* strain from a nematode worm, *Brugia malayi*, that lives in the human lymph system and blood, and another from *Drosophila melanogaster*. Although the sequences are still being analysed, there appear to be many mobile genetic elements such as phage DNA and transposons, 'jumping genes' that can hop in and out of the genome, inserting randomly.

Two become one

Wolbachia has a small genome — less than 1.5 million base pairs, or about a third of the size of the genome of the common gut bacterium *Escherichia coli*. Intracellular parasites often seem to develop slimmed-down genomes, as they lose genes that are only required by free-living bacteria[23]. And some biologists wonder if *Wolbachia* are on the evolutionary road towards becoming one with their hosts — like the mitochondria that generate energy inside our cells, which are thought to have once been parasites.

O'Neill, for one, is not convinced. Given the deleterious effects of *Wolbachia* on its insect hosts, he definitely views the bacteria as parasites: "The microbe has the upper hand." But in other invertebrates, who has the upper hand is less clear. The nematode worms that cause elephantiasis and river blindness in humans seem to need *Wolbachia* to survive — antibiotics kill the worms, apparently by killing their *Wolbachia*[24].

As interest in the bacteria explodes, strains of *Wolbachia* that are in the process of being incorporated by their hosts may be among the evolutionary treasures waiting to be discovered. The availability of the *Wolbachia* genome is also expected to aid research into the mechanisms by which the bacteria manipulate their hosts — which at present are not well understood. Fans of bizarre biology and afficionados of evolution should watch this space. ■

Jonathan Knight writes for *Nature* from San Francisco.

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Sexually harassed: *Wolbachia* kills male ladybirds, makes *Trichogramma* wasps (centre) reproduce asexually and has made populations of *Acraea encedon* butterflies 90% female.