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By directing its victims' sex lives, the bacterial parasite **WOLBACHIA** may be helping to produce new species

GENDER-BASED GENOCIDE: The bacterium *Wolbachia* eliminates male *Acraea encedon* butterflies because only females can serve as hosts for the parasite and pass its spawn on to the next generation.

arasitic SEX ppeteers

By Laurence D. Hurst and James P. Randerson

Don't bite the hand that feeds you.

The old adage sums up the approach parasites are expected to take with their victims. A freeloader that can spread only when its host reproduces ought not to be overly harmful: too much damage to its unwilling benefactor will affect the parasite's own chances to procreate. This scheme contrasts with the tactics of a pathogen that has a short infectious period, such as the flu virus. In that case, the virus has no long-term interest in the carrier's well-being, so a "get transmitted quick" strategy is favored almost regardless of the cost to the hapless host.

The widely distributed bacterium *Wolbachia* (a close relative of the

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gut bacterium *Escherichia coli*) is a boarder with a long-term interest in its invertebrate host. It lives within cells and is transmitted to the next generation by invading its host's eggs. Contrary to the old saying, however, the bacterium engages in various radical manipulations of its hosts, including killing male offspring, turning males into females and rendering some host matings infertile. If *Wolbachia*'s reproduction is so intimately tied up with that of its meal ticket, why does it create so much havoc?

The short answer is that the bacterium is not carried in sperm. This egg-only propagation method means that the parasite's reproductive interests lie firmly with female hosts. Males, as nontransmitters, are akin to evolutionary prison cells for the bacterium.

Over the course of time, however, Darwinian natural selection has been able to sidestep the parasite's custodial sentence in male hosts. Rather than being a dead end, the problem of nontransmission has been turned into an opportunity. *Wolbachia* may have played much more than a walk-on part in this evolutionary drama because its manipulations can have profound long-term effects on its victims. By interfering with host reproduction, *Wolbachia* may be setting the stage for new species to form.

Wolbachia is highly abundant and has rather catholic tastes in choosing its insect and invertebrate carrier species. When John H. Werren and his colleagues at the University of Rochester screened neotropical insects for the presence of *Wolbachia,* they found that 17 percent of 154 species harbored the bacteria.

The true frequency of infection may well be considerably higher, however. Greg Hurst and his co-workers at University College London and the University of Cambridge have found several well-studied species of *Wolbachia* in about a tenth of the individuals in a host population. Sampling only a few individuals from a given species, as the Rochester group did, is therefore likely to miss many incidences of infection. Further, Marjorie Hoy and Jay Jeyaprakash of the University of Florida have determined that the technique employed in the Rochester study often wrongly indicates an absence of parasitization. Using a more robust method, the Florida researchers discovered that more than three quarters of the species they tested were infected with the bacterium.

Nor is the parasite found only in insects; it appears in crustaceans (notably pill bugs and freshwater shrimps), mites and nematode worms as well. So far it has not been detected in a vertebrate animal. We estimate that more than 20 million species may harbor *Wolbachia,* suggesting that scientists have so far studied merely the tip of a huge and important iceberg.

Choosy Males

ONE WAY IN WHICH *Wolbachia* can profoundly influence its victims is by grossly distorting the normally balanced sex ratios of its hosts' populations. For instance, males are extremely rare in some groups of the African butterfly *Acraea encedon* and of the widespread pill bug *Armadillidium vulgare* (also called a wood louse).

COMPULSORY SEX CHANGES **and perhaps forced speciation can be traced to the *Wolbachia* bacterium. The tiny parasite transforms would-be male pill bugs (*above*) into females because it can reproduce only through the female's eggs. The split between two *Nasonia* wasp species (*an example is shown at right*) seems to have been caused by *Wolbachia*.**

THIERRY RIGAUD ET AL. *University of Poitiers*

In the butterfly, this phenomenon is caused by the pervasiveness of a male-killing strain of *Wolbachia* (more than 90 percent of *A. encedon* females support the parasite). The bacterium presumably kills off males to benefit its own relatives, which are infecting the slain males' sisters. This act of suicide by the individual makes sense because a *Wolbachia* bacterium in a male host is already a "dead man walking." Sperm do not carry the bacterium, so it has no chance of moving to another host anyway; hence, the parasite has nothing to lose. Moreover, its kin in the dead males' sisters benefit from this behavior because male killing occurs before the hosts have hatched. Consequently, a banquet of unhatched male offspring lies there ready to be devoured when the rest of the brood emerges. Such sibling cannibalism by parasitized females is thought to give them an important advantage over competitors.

Although this adaptive advantage has yet to be definitively demonstrated in *A. encedon* caterpillars, it has been established in *Adalia bipunctata,* commonly called the two-spot ladybug (or ladybird). The period between hatching and finding their first aphid meal is a particularly vulnerable time for the larvae of this ladybug. A free lunch provided by a deceased brother is therefore a great boon to them and consequently to the *Wolbachia* they carry.

In pill bugs, *Wolbachia* converts would-be males into females. By feminizing males, the parasite is changing a nontransmitting host into one that will pass the infection on to its offspring. To return to the prison analogy, this is rather like a convict receiving a hacksaw inside a cake.

In both the butterfly and the pill bug, the bacterium skews the populations' sex ratios massively toward females. Males therefore represent a valuable commodity. This scarcity reverses normal gender roles, because females are in demand in populations with an equal sex ratio. Females make a much larger contribution to the young in the form of large, nutritious eggs, whereas the males' investment of cheap sperm is much smaller. As the chief donors to their young, females are typically much fussier in their choice of mates, accepting only the fittest males.

The selection process is inverted in *Acraea* and *Armadillidium,* however, because males are so hard to come by. Indeed, work by Francis M. Jiggins and his colleagues at the University of Cambridge has revealed that in heavily affected populations of the *A. encedon* butterfly, the entire system of choosing mates has changed. Rather than spreading throughout the habitat, females form dense aggregations on small grassy plots. In one such cluster, 350 butterflies were found packed into a 200-square-meter area. In species with conventional sex roles, such as the sage grouse, these gatherings, known as leks, are where males congregate and females come to shop.

Could the aggregations in *Acraea* represent role-reversed leks to which males come to select a partner? The current evidence is inconclusive. By demonstrating that virgin females are more likely to inhabit the sites than mated females, Jiggins has shown that females are indeed gathering to find mates. The question remains, however, whether the males are doing the choosing.

Jiggins had originally found that mated females are more likely to lack the infection than virgins, implying that males may be selecting uninfected females as partners. Unfortunately, he and one of us (Randerson) have been unable to replicate this result, so the situation continues to be unclear.

In the example of the pill bug, Thierry Rigaud and his collaborators at the University of Poitiers in France have shown that males prefer not to mate with feminized males. Furthermore, if males do mate with transsexual individuals, they deliver relatively few sperm to them.

Our own work using mathematical techniques to model the evolution of choice in these situations has shown that natural selection for such an alteration in mate choice does occur if a host population contains sex-ratio distorters. This result raises the problem, though, of what happens to the parasite when infected females fail to find a mate. The theoretical models show that when males are able to distinguish perfectly between infected and uninfected females, the bacterium will simply be selected out of the population. If, however, the males occasionally make a mistake and mate with parasitized females, then this is enough to keep *Wolbachia* in the population.

JOHN H. WERREN

Sex Changes in Hosts

MALE MATE CHOICE may well be one response to the spread of a feminizing bacterium. An even more fascinating one may be the evolution of new mechanisms for genetically determining gender. Research by Rigaud and his associates has shown that *Wolbachia* can determine gender in *A. vulgare,* because male pill bugs have all the genes necessary to become females. All that is needed for an individual to become male is a shot of male-inducing hormone from the "male gland" early in development. Thus, if the parasitic bacterium can block development of this gland, its host will be female.

In some infected populations, the responsibility for gender determination has shifted from the pill bug to its parasite. Pill bugs have what is known as a WZ sex-determination system: males have ZZ chromosomes, and females have WZ. (This arrangement contrasts with the more familiar XY system, in which males are XY and females are XX.) Because only the few eggs that do not contain *Wolbachia* become males, infected WZ females give birth mostly to daughters: both WZ females and sex-changed males (ZZ females). These ZZ females in turn almost exclusively produce ZZ females. As a result, infected females of either type beget more daughters than normal females do. Thus, with each succeeding generation, the proportion of individuals with the normal female chromosome W drops and eventually nears zero. At that point, the parasite is left entirely in control of sex because everyone is ZZ—if the pill bug has the parasite, it's female; if not, it's male. In other infected pill bug groups, the hosts seem to have wrestled back control over their sex ratios by setting the proportion of offspring that receive the bacterium in some as yet undetermined manner.

Wolbachia's most common manipulation is to interfere in the success of the host's matings. Known as cytoplasmic incompatibility, this strategy renders all matings between infected males and uninfected females infertile because the bacterium releases toxins into the sperm's protoplasm [*see box on next page*]. Other pairings are left unaffected. Halting the procreation of uninfected females indirectly benefits females carrying the bacterium. As a result, these females contribute a larg-

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er proportion to the next generation, which allows their freeloading *Wolbachia* to spread more widely.

One consequence of this intervention is that the bacterium restricts gene flow between different groups of its host. This is most pronounced in bidirectional cytoplasmic incompatibility, in which two groups of hosts contain mutually incompatible strains of the bacterium. In that instance, all matings between hosts from the different groups are doomed by internal sabotage.

Barriers to free gene flow between populations are all-important in speciation, the origination of new species. In the classic example, biologists imagine that a physical barrier arises, perhaps the formation of a new mountain range or the creation of an island resulting from a rise in sea level. This obstacle splits a previously homogeneous population into two, preventing interbreeding between the new populations and allowing them to drift apart genetically. Over time the groups diverge to the point that they can no longer interbreed if they are later brought together. Because their parents' genetic systems are now incompatible, hybrid offspring either do not survive or are sterile.

On the Road to Speciation

BIOLOGISTS FAMILIAR with *Wolbachia* began to ask whether the impediments to gene flow created by cytoplasmic incompatibility could be enough to allow a population to bifurcate genetically without the existence of a physical obstruction. Were these bacterial freeloaders the agents of what might be called infectious speciation?

This question is difficult to answer because the process by which one species splits into two takes a long time to complete, much longer than the lifetimes of evolutionary biologists. The

Sexual Sabotage

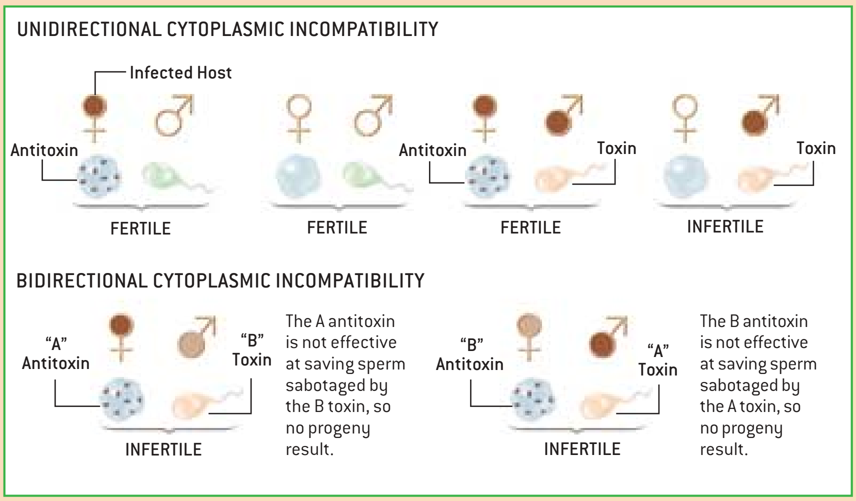
AN IMPORTANT ADAPTIVE tactic employed by some strains of the *Wolbachia* bacterial parasite is to interfere in the success of host matings by rendering certain pairings barren. By restricting the gene flow among its hosts, *Wolbachia* is thought to be contributing to the development of new species.

Cytoplasmic incompatibility, as the phenomenon is called, occurs when an infected male and an uninfected female attempt to mate. As an evolutionary strategy, it involves indirectly helping hosts that contain one's relatives by harming those that don't.

In males, some strains of *Wolbachia* add a toxin to the host's sperm, which probably alters the proteins that bind to DNA. The poisonous agent causes the male's chromosomes to condense abnormally on fusion with the egg and stops the newly formed zygote from developing. When the male mates with an infected female, however, no such condensation occurs. In this case, *Wolbachia* deploys an antitoxin in the egg that rescues the zygote.

This manipulation of the host by the bacterium has been interpreted as an act of evolutionary spite because it reduces the fertility of unparasitized females with no direct benefit to those bacteria that sabotage the sperm. The strategy is successful, however, because it indirectly benefits infected females and hence their resident *Wolbachia*. These females have no restrictions on their choice of mates and thus have an advantage over parasite-free females. As infected females beget more infected females, this strategy promotes the spread of the bacterium. Michael Turelli of the University of California at Davis and Ary A. Hoffmann of La Trobe University in Australia have uncovered good evidence for the success of this approach. In the eastern U.S., the so-called Riverside strain of *Wolbachia* that causes cytoplasmic incompatibility is spreading through the territory of its host population (the fruit fly *Drosophila simulans*) along a front traveling about 100 kilometers a year.

In unidirectional cytoplasmic incompatibility, just one strain of *Wolbachia* is involved, so matings in only one direction are sabotaged. Things get more interesting if there is more than one strain that produces cytoplasmic incompatibility, a situation known as bidirectional cytoplasmic incompatibility. For the sake of argument, imagine two bacterial strains, A and B. If the egg-saving antitoxin from strain A is ineffective against the toxin from strain B, and vice versa, then matings in either direction are incompatible. If all individuals in the population are infected with one strain or the other, the only successful matings are those between individuals harboring the same strain. Host matings between strains will always lead to incompatibility. The two *Wolbachia* strains have split the host population into two groups that cannot interbreed. *—L.D.H. and J.P.R.*



NINA FINKEL

UNWANTED HITCHHIKERS: *Wolbachia* bacteria reproduce only through their female hosts, making males superfluous. The parasites are shown here as lighter green spots on an insect egg (*top*) and as specks inside an infected cell (*bottom*).

MERIJN SALVERDA AND RICHARD STOUTHAMER (*top*); CLAUDIO BANDI AND LUCIANO SACCHI (*bottom*)

best that can be done is to look for *Wolbachia* hosts that exhibit cytoplasmic incompatibility and are on the pathway to speciation. By studying such suggestive examples, biologists hope to determine whether the parasite is likely to be a force in the evolution of new species.

Two species of parasitic wasp that appear to be near the end of such a journey are *Nasonia vitripennis* and *N. giraulti,* both of which are found in eastern North America. An investigation of these wasps by Johannes A. J. Breeuwer of the University of Amsterdam and Werren of the University of Rochester revealed that the barriers to successful mating between these species are not determined solely by differences between the wasps themselves—*Wolbachia* is involved. The researchers found that when both species are treated with antibiotics, matings between the two produce fertile young. Without the antibiotics, inviable offspring result because each wasp species contains a different strain of the parasite, leading to bidirectional cytoplasmic incompatibility between the hosts.

Wolbachia is not the only cause of current isolation between these two species of *Nasonia,* though. The second generation of hybrid wasps tends to have severe developmental problems, perhaps indicating that genetic changes in the wasps' own DNA are now sufficient to keep the two species apart. Whether the parasite was involved in promoting this divergence in the past has not been determined.

In the case of the reproductive separation between *N. giraulti* and *N. longicornis,* the story is different. These two parasitic wasps have been taxonomically determined to be different species—for example, they parasitize different host groups. But again, *Wolbachia* plays a key role—in this instance, by causing bidirectional cytoplasmic incompatibility. Werren and his University of Rochester colleagues Seth Bordenstein and Patrick O'Hara have recently shown that in the absence of the bacterium, the hybrids are normal and remain so in subsequent generations. Although genetic divergence of the wasps' DNA has yet to occur, *Wolbachia* appears to be on the cusp of forcing the evolution of new species.

In some cases, *Wolbachia* may contribute to the process of speciation without being the primary cause. A team of researchers, including D. DeWayne Shoemaker of Western Michigan University, Vaishali Katju of Indiana University and John Jaenike of the University of Rochester, has turned up evidence that even the incomplete obstacle to gene flow provided by unidirectional cytoplasmic incompatibility can contribute to reproductive isolation [*see box on opposite page*].

Shoemaker and his coworkers looked at two species of fruit fly, *Drosophila recens* and *D. subquinaria.* The first of these is infected with a *Wolbachia* strain that causes cytoplasmic incompatibility; the second is uninfected. As a result, matings between *D. subquinaria* females and *D. recens* males are infertile. If *Wolbachia* were the only factor at work, then gene flow between the fruit-fly species would still be possible, but it turns out that these matings do not occur successfully. Unlike *D. subquinaria, D. recens* females are quite choosy when selecting mates, hardly ever picking a male of the other species by mistake. Hence, the two barrier mechanisms complement each other. Gene flow in one direction is prevented by *Wolbachia* (even though matings occur), whereas the flow in the reverse direction is prevented by careful mate choice by *D. recens* females.

Despite these and other suggestive examples, the case for infectious speciation has yet to be proved. It is notable, however, that *Wolbachia* infections are especially prevalent in insects and mites, the most species-rich animal groups. Perhaps *Wolbachia* had a hand in nurturing new shoots on these bushy branches of the tree of life.

Who's Running the Show?

FAR FROM BEING a minor freeloader, the *Wolbachia* parasite is widespread in nature and manipulates the reproduction of a variety of host organisms in diverse ways. What is more, attempts by the bacterium's hosts to evade infection have sent their biology and evolution in unexpected directions. As scientists begin to explore more of *Wolbachia*'s still hidden biology, we expect this influential passenger to have other surprises in store. SA

MORE TO EXPLORE

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