Undesirable Sex Partners

Bacteria manipulate reproduction of insects and other species

By JOHN TRAVIS

It took a while before the medical community paid attention. The first known cases of what the tabloids gleefully called virgin births appeared, amusingly enough, in Las Vegas. Then physicians across the United States began documenting similar events. In each case, an unfertilized egg in a woman had spontaneously begun to develop, ultimately producing a healthy female baby.

One young researcher, who had analyzed the timing and locales of the virgin births, suggested a spreading infection might be causing the incidents. The Federal Centers for Disease Control and Prevention in Atlanta quickly dismissed the idea, calling it "ridiculous."

Several months later came a well-publicized report in the JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION concluding that the number of infertile couples was rising rapidly worldwide. The international uproar intensified when physicians began to observe another reproductive curiosity: Some newborns that were genetically male appeared to be female. One week, the NEW ENGLAND JOURNAL OF MEDICINE and the National Enquirer ran articles with the headline, "Is this the end of mankind, or just men?"

Science fiction? Definitely. For many insect species and other arthropods, however, the truth can be as strange as fiction when bacteria known as *Wolbachia* are around.

These microorganisms populate cells in the testes and ovaries of arthropods, often profoundly altering the reproduction of their hosts. In some species, infected males can generate offspring only if they mate with infected females. In others, infected females give birth without the need for the opposite sex. In one arthropod species, *Wolbachia* even transform embryos that would normally be males into females.

"These traits have all evolved because they increase the transmission of the microorganisms," says John H. Werren of the University of Rochester (N.Y.), who has documented the diversity of animals infected by *Wolbachia*.

There's no evidence that *Wolbachia* infects mammals, let alone humans, but that hasn't dulled biologists' fascination with them. "It's a very special group of bacteria," says Werren.

Scientists first identified the bacteria in the reproductive tissues of a mosquito species in 1924.

Yet it took a mystery and several decades before *Wolbachia* truly entered the limelight. The mystery emerged in the 1950s, when insect geneticists encountered problems while trying to cross different strains of mosquitoes.

"They started to find all these crossing abnormalities," says Scott L. O'Neill of Yale University Medical School. The most obvious one, dubbed cytoplasmic incompatibility, centered on the failure of certain strains to produce offspring when mating with other strains of the same mosquito species.

Scientists argued for 20 years over what caused cytoplasmic incompatibility, says O'Neill. Then, in 1971, Janice Yen and Ralph Barr, biologists at the University of California, Los Angeles, tabbed *Wolbachia* as the culprit.

Cytoplasmic incompatibility, the researchers found, occurs when males infected with *Wolbachia* mate with uninfected females. In such unions, no offspring, or just a few in some host species, result. This reproductive barrier can be eliminated with antibiotics that rid the mosquitoes of the bacteria.

Why does *Wolbachia* generate cytoplasmic incompatibility? To favor reproduction by infected females, says O'Neill. That helps the bacteria, which dwell in the cytoplasm of egg cells, pass on to future generations.

In species affected by cytoplasmic incompatibility, infected females have no trouble reproducing with infected males. Infected females also breed easily with uninfected males. Both kinds of unions transfer *Wolbachia* to offspring. Consequently, cytoplasmic incompatibility can spread *Wolbachia* rapidly through an uninfected population, says O'Neill, who organized a session on *Wolbachia* at the recent Symbiosis 96! Meeting in Bar Harbor, Maine.

Researchers are finding that *Wolbachia* infects a surprisingly large variety of species. Werren and Donald Windsor of the Smithsonian Tropical Research Institute in Panama reported last year that 16 percent of Panamanian insect species, including some in all of the major insect orders, harbor *Wolbachia*. Since the estimated number of insect species ranges from 10 million to 30 million, that means roughly 2 million to 5 million insect species play host to the bacteria.

"That's very much an underestimate," adds Werren, noting that researchers have time to test only a limited number of insects from each species.

Scientists have also found that *Wolbachia* infects a variety of isopods (a subgroup of crustaceans that includes wood lice), at least one species of mites, and perhaps even a worm species.

Wasps are among the favored hosts of *Wolbachia*. Take the jewel wasp, Werren's favorite research subject. *Wolbachia* infections in these insects produce an odd variation on cytoplasmic incompatibility: Uninfected female wasps mating with infected males can produce offspring, but their progeny are all male.

An explanation rests in the fact that wasps, like bees and ants, have an unusual mechanism for determining sex. In wasps, eggs fertilized by sperm contain a maternal and a paternal set of chromosomes and develop into females. Unfertilized eggs, with only a maternal chromosome set, develop into males.

When an infected male jewel wasp mates with an uninfected female, the paternal chromosomes from the sperm seem to fragment and fail to join the maternal set, says Werren. Consequently, only males result from such a mating. This indirectly aids the spread of *Wolbachia* by reducing the number of uninfected daughters produced by uninfected females, explains Werren.

Wolbachia sometimes takes a more feminist approach. In many parasitic wasps, which lay their eggs in developing insects that they have killed, *Wolbachia* infections eliminate the need for males. An infected female reproduces via an asexual process known as parthenogenesis. The unfertilized eggs simply duplicate their one set of chromosomes and develop into females.

These parthenogenetic wasps had

long been a biological curiosity until a few years ago, when Richard Stouthamer, working with Werren, showed that the phenomenon stemmed from *Wolbachia* infection. With antibiotics, "you can cure a line of its parthenogenesis and make it sexual," says Stouthamer, now at the Wageningen Agricultural University in the Netherlands.

The clear preference for females isn't limited to *Wolbachia* strains that infect wasps. At the Bar Harbor meeting, Thierry Rigaud of the University of Poitiers in France, reported finding the bacteria in the wood louse *Armadillidium vulgare*.

In these lice, *Wolbachia* frequently overrides genetic inheritance. The bacteria, says Rigaud, "feminize" an embryonic wood louse that is genetically male by disrupting the production or effects of masculinizing hormones during its development. The increased number of daughters allows *Wolbachia* to spread quickly.

While scientists continue to tally the animals that *Wolbachia* infects, as well as the outcomes of those infections, they are also trying to unravel the mechanisms by which *Wolbachia* distorts its host's reproduction. Are cytoplasmic incompatibility, parthenogenesis, and feminization distinct strategies pursued by *Wolbachia,* or are they merely reflections of different ways in which host species react to the bacteria?

As the best-known phenomenon, cytoplasmic incompatibility has received the most attention. An initial hypothesis to explain it was that proteins made by *Wolbachia* in the testes bind to the chromosomes inside sperm and disrupt their later union with maternal chromosomes.

That simple idea has a major flaw, however. Researchers haven't found any *Wolbachia* molecule incorporated into sperm. Nor are whole bacteria present in the sperm. As sperm mature, they lose most of their cytoplasm and squeeze out any *Wolbachia*.

The latest theory about cytoplasmic incompatibility comes from Timothy L. Karr of the University of Chicago. He argues that *Wolbachia*'s influence during the maturation of sperm somehow disturbs the carefully choreographed maneuvering that later brings together the sperm's chromosomes with the egg's. "It looks like the paternal and maternal chromosomes are out of sync," says Karr.

While comparing infected and uninfected eggs of a fruit fly species, Karr's group discovered that *Wolbachia* binds to specific egg proteins. One of those proteins normally resides in the nucleus of the fruit fly's egg cell. In an infected egg cell, however, the protein concentrates in sites throughout the cytoplasm.

The same phenomenon appears to occur in the testes, but it is complicated by the eventual eviction of *Wolbachia* from mature sperm, says Karr. "During spermatogenesis, *Wolbachia* binds proteins that should be in that sperm when it finally matures." But because *Wolbachia* gets kicked out of sperm, it takes that protein away, he says.

Though not necessary for early stages of fertilization, this sperm protein is crucial to coordinating the union of the two chromosome sets, proposes Karr. That would explain why infected males, whose sperm are stripped of the protein by *Wolbachia*, have trouble generating progeny in uninfected females, says Karr.

As for the pairings of infected females and infected males, which do produce offspring, Karr contends that the proteins stripped from sperm by *Wolbachia* are the same ones concentrated by the bacteria in the cytoplasm of the egg cell. The *Wolbachia* in the egg cells, along with the host proteins they are bound to, thus "rescues" the defective sperm from infected males, he says.

O'Neill has discovered a *Wolbachia* protein that may be relevant to Karr's theory. This protein varies in size among *Wolbachia* strains, and O'Neill suggests it may explain why some strains induce cytoplasmic incompatibility and others do not. Furthermore, the protein, seemingly from *Wolbachia*'s outer surface, binds to the same host cellular proteins identified by Karr. "It looks like our stories are coming together," says O'Neill.

There is a practical side to all this *Wolbachia* research. Both Werren and Stouthamer, for example, are leading efforts to transfer parthenogenesis-inducing *Wolbachia* into commercially useful parasitic wasps.

"A lot of parasitic wasps are reared for the control of pest insects," notes Werren. "It would be highly desirable for these mass-rearing programs to use [parthenogenesis-inducing *Wolbachia*] because they could generate all females, and it's the females that go out and kill the insects."

O'Neill's ambitions center on eliminating insect-borne diseases, such as malaria and Lyme disease, by making use of *Wolbachia*'s ability to disperse through a population.

In one scenario, researchers would genetically engineer *Wolbachia* to make antibodies or other compounds that kill the malaria-causing parasite carried by mosquitoes in the wild. They would then infect mosquitoes with these *Wolbachia* and seed the countryside with the insects. Since the bacteria induce cytoplasmic incompatibility, the natural mosquito population would quickly give way to a population almost entirely infected with *Wolbachia* and presumably free of the malaria parasite.

The main obstacle to this idea, notes O'Neill, is that *Wolbachia* normally lives in the mosquito's reproductive tissues, whereas the malaria parasite inhabits its gut and salivary glands. Consequently, any compounds made by *Wolbachia* might not reach and kill the parasites.

O'Neill is searching for the *Wolbachia* gene that brings about cytoplasmic incompatibility. He intends to link that gene to a gene engineered to produce an antiparasitic compound in the tissues where the malaria parasite dwells. Once added to the mosquito's complement of genes, the two genes would be inherited by future mosquito generations and, with the aid of cytoplasmic incompatibility, would spread quickly within a mosquito population, says O'Neill.

The most provocative question surrounding *Wolbachia* may be whether the bacteria have played a role in the development of new species.

A central concept in many theories of speciation is reproductive isolation. This idea holds that if two populations of a species cannot breed together, then the genes of each population will evolve independently and diverge (SN: 11/2/96, p. 284). "Reproductive isolation is a key component of speciation because without it, genomes would mix and you can't get divergence," says Werren.

Eventually, he explains, the genes of two populations would diverge so much that they become genetically incompatible for reproduction. At that point, most evolutionary biologists would argue, the single original species has given way to two species.

Wolbachia may serve as an excellent mechanism to engender reproductive isolation, argues Werren. He and other researchers have found that such isolation can arise in an insect species infected by different *Wolbachia* strains. Members of the species infected by one strain cannot reproduce with members infected by the other strain.

While theories about evolution are notoriously difficult to prove, Werren suggests that mapping the diversity of insect species infected and not infected by *Wolbachia* may bolster his theory. Species infected with *Wolbachia* should have many more closely related species than uninfected species do.

Could some *Wolbachia* species infect vertebrates, or even humans, and play a role in their speciation? Decades ago, researchers tried unsuccessfully to infect mice with strains of the bacteria. Moreover, O'Neill notes that all the *Wolbachia* found so far are temperature-sensitive and could not survive inside warm-blooded animals.

Still, Werren says it's too early to dismiss the possibility completely. "We don't have any idea whether these bacteria occur in vertebrates, either cold- or warm-blooded. We haven't really looked," he says. □