

Commentary

Wolbachia run amok

John H. Werren

Department of Biology, University of Rochester, Rochester, NY 14627

Symbiotic associations between microorganisms and higher eukaryotes are extremely common, and they range from mutualistic (beneficial) to commensal (neutral) and parasitic (harmful). Vertical vs. horizontal transmission mode is believed to play an important role in determining whether parasitic or mutualistic interactions evolve (1–5). According to this view, frequent horizontal (infectious) transmission favors virulent microbes (especially if virulence increases propagule number), whereas vertical (inherited) transmission favors evolution of benign and mutualistic associations. The level of virulence, therefore, is expected to depend on the relative importance of vertical and horizontal transmission for maintenance of the microbe. The process may apply, for example, to bacteriophage and other viruses that have both lytic (horizontal) and lysogenic (vertical) transmission modes (5).

A variety of symbiotic microorganisms are known that are predominantly or exclusively transmitted through host reproduction. Examples include *Buchnera aphidicola*, a nutritional bacterial symbiont of aphids (6), and *Wolbachia*, a group of intracellular rickettsia found in invertebrates (7). The expectation that vertical transmission will lead to avirulence makes intuitive sense. If the primary mode of transmission of a microbe is through the reproduction of the host, then harming the host will reduce transmission of the microbe, whereas a benign or beneficial effect will enhance transmission.

Contrary to this expectation, Min and Benzer (8) report discovering a highly virulent strain of *Wolbachia* that is vertically transmitted but causes tissue degeneration and adult death in *Drosophila melanogaster*. Their discovery has several practical and theoretical implications, which are discussed below. But first, some background information is necessary.

Wolbachia are intracellular bacteria (rickettsia) that are transmitted within the egg cytoplasm and found in reproductive and other tissues of invertebrates. These remarkable bacteria are extremely common and widespread. They are estimated to occur in 15–20% of insect species (9) and have also been found in arachnids, crustaceans, and nematodes (10–12). The limits of their distribution are currently unknown. Although routinely transmitted vertically, *Wolbachia* have also undergone extensive intertaxon transmission, even between different orders of insects and between insects and crustaceans. Within the intracellular environment, they seem to have a wide host tolerance. Equally remarkable are the phenotypic effects of these bacteria. *Wolbachia* are known to cause a number of reproductive alterations in hosts, including cytoplasmic incompatibility in a broad range of insects, parthenogenesis in hymenopterans, and feminization of genetic males in isopods (12–14). In some cases, these effects are accomplished by manipulation of mitosis and early development of the host. *Wolbachia* are of wide interest as a potential mechanism for rapid speciation (7) and as a mechanism for genetic modification and biological control of pest arthropods (15). The number of research groups working on these bacteria has grown exponentially in the past few years, spurred in part by a recently acquired ability to detect and characterize these

fastidious intracellular bacteria by using molecular genetic techniques.

Cytoplasmic incompatibility (CI) is the most common phenotype known for *Wolbachia*, and it has been demonstrated in a wide range of insects. CI is a *Wolbachia*-induced incompatibility between the sperm of infected males and eggs of uninfected females or females infected with a different *Wolbachia* strain. CI typically causes disruption of early mitotic events and zygotic death. Genetic crosses indicate that CI involves a modification–rescue system. *Wolbachia* present in testes induce modifications of the sperm, and the same or similar strain of *Wolbachia* must be present in the egg to rescue this modification. Although the biochemical mechanisms of CI are unknown, modifications of heat shock protein profiles in sperm have recently been implicated (T. Karr, personal communication). CI-inducing *Wolbachia* are found in *D. melanogaster*, both in natural populations and in laboratory strains (16–19). In *D. melanogaster*, CI induction is rather weak, causing only approximately 0–30% mortality among embryos in incompatible crosses (19). Besides their CI effect, these *Wolbachia* are quite avirulent. Infected and uninfected females have nearly identical survival and fecundity, and the bacteria are relatively low in abundance in somatic tissues.

Min and Benzer (8) have found, in contrast, a strain of *Wolbachia* in *D. melanogaster* that massively proliferates in adult tissues, causing early death. They accidentally detected this bacterial variant in an X-chromosome deficiency stock being used to map mutations involved in brain degeneration. To their surprise, they found that a severely reduced adult lifespan in this stock did not segregate with the deficiency, but was rather maternally inherited to both male and female offspring. Light microscopy revealed progressive degeneration of brain and retina in adults. Electron micrographic examination of the tissues detected massive infections with rickettsia-like organisms (RLOs) similar in structure to *Wolbachia*. PCR and sequencing using *Wolbachia*-specific primers and staining with monoclonal antibodies that preferentially bind to *Wolbachia* have established that *Wolbachia* are present in abundance in these infected tissues and in ovaries. On the basis of sequence information, the bacterium belongs to the A division of *Wolbachia*, the same division as *D. melanogaster* CI *Wolbachia* (7). Antibiotic treatment with tetracycline both restored normal adult lifespan and eliminated the *Wolbachia* infections. Although the case is sound, a few potential problems exist. First, the specificity of the *Wolbachia* monoclonal antibodies is not yet known relative to binding with other RLOs. Second, it is possible (although unlikely) that another maternally inherited bacterium is also present in the tissues that actually causes the syndrome. However, the case is strong that a virulent *Wolbachia* does proliferate within adult tissues and is the causative agent of early adult death. Min and Benzer have named the strain “popcorn,” due to the popcorn-like appearance of infected cells in adult tissues.

Strictly speaking, most *Wolbachia* are not mutualistic symbionts, but are more accurately regarded as “reproductive parasites” (20). The reproductive alterations they cause enhance vertical transmission of the parasite, often to the detriment of the host. Nevertheless, due to their cytoplasmic

transmission, *Wolbachia* are expected to be “prudent” parasites, replicating sufficiently within host tissues to ensure transmission through the eggs but otherwise restraining replication to reduce negative effects on hosts through which they are transmitted. The popcorn strain clearly has either lost or overcome these replicative restraints.

How do we account for a vertically inherited *Wolbachia* that is highly virulent in adults, since this trait should be selected against? There are two basic explanations. Either the popcorn strain represents a mutant *Wolbachia* that expresses a non-adaptive pathology of overreplication in adult tissues, or it is a natural variant of *Wolbachia* that has been selected for higher virulence. The latter scenario would imply that the bacterium has an increased horizontal transmission component, to compensate for the reduced survival and fecundity of infected adult females. Min and Benzer did not test for infectious transmission of the bacterium. According to theoretical models, even a relatively small rate of horizontal transmission could be sufficient to favor increased virulence (3). As mentioned, popcorn may simply represent a nonadaptive virulence mutant. As such, it would be interesting to determine what genetic changes are associated with this loss of replicative control. It has been previously observed that a major problem of maintaining symbionts is the possibility of “symbiont cancers”—symbiont mutations that cause overreplication of the microbe and harm to the host (20). The popcorn strain could represent just such a case.

In either scenario, the popcorn bacterium will be a useful system for studying replication of intracellular bacteria and the mechanisms and evolution of virulence. One question concerns why overreplication occurs in adult and not larval tissues. This may be due to a tissue-specific tropism or could simply be a consequence of relative replication rates of host cells vs. bacterial cells. Many host cells cease dividing in adults—continued unregulated bacterial division could then result in the adult pathologies observed. A second important question concerns the role of bacterial and host genetic factors in regulating intracellular symbiont numbers. Clearly, the wide array of genetic tools available in *D. melanogaster* make it highly suitable for such investigations.

The popcorn strain has other potential applications. *Wolbachia*, like other rickettsia, cannot be cultured outside of host cells. As a result, molecular genetic and biochemical studies of *Wolbachia* have been hampered by the difficulty of obtaining large and relatively pure amounts of these bacteria, which typically have low infection levels in hosts. However, the massive infections of popcorn *Wolbachia* in adult tissues (and potentially in tissue culture) may provide enough material to overcome this problem.

Bacterial relatives of *Wolbachia* include a number of agents that have arthropods as vectors and cause serious human diseases such as typhus, scrub typhus, ehrlichiosis, and Rocky Mountain spotted fever (21). Studies of these bacteria require special containment facilities. In contrast, *Wolbachia* have so far been found only in invertebrates and are not known to cause mammalian disease. The relative ease of rearing large numbers of the popcorn strain could therefore make it a useful general system for genomic studies of rickettsia. In recent years, efforts have been made to obtain complete DNA sequences for representatives of the major bacterial groups.

However, so far bacteria in the Rickettsiaceae have not been targeted for such studies, perhaps for some of the reasons described above. At some future point, the popcorn strain of *Wolbachia* may provide a suitable representative for complete rickettsial sequencing.

As pointed out by Min and Benzer (8) and previously by others (16), *Drosophila* researchers should beware. *Wolbachia* are common in laboratory cultures of *D. melanogaster*, and their potential effects on phenotypes under study cannot be ignored. The warning should be generalized to other organisms as well. *Wolbachia* are conservatively estimated to occur in one to five million species (9). Given this incredible abundance, we can expect to find a wide range of *Wolbachia* types, including strains with phenotypic effects on hosts ranging from mutualism to pathogenicity. Global disturbance of ecological communities is believed to be important in the emergence of new diseases (22). By bringing together organisms not previously in contact, such disturbances could also increase the movement of *Wolbachia* between host species.

1. Fine, P. E. M. (1975) *Ann. N.Y. Acad. Sci.* **503**, 295–306.
2. Ewald, P. (1995) *Evolution of Infectious Diseases* (Oxford Univ. Press, New York).
3. Lipsitch, M., Nowak, M. A., Ebert, D. & May, R. M. (1995) *Proc. R. Soc. London Ser. B* **260**, 321–327.
4. Frank, S. A. (1996) *Q. Rev. Biol.* **71**, 37–78.
5. Stuart, F. M. & Levin, B. R. (1984) *Theor. Popul. Biol.* **26**, 93–117.
6. Moran, N. & Baumann, P. (1994) *Trends Ecol. Evol.* **9**, 15–20.
7. Werren, J. H. (1997) *Annu. Rev. Entomol.* **42**, 587–609.
8. Min, K.-T. & Benzer, S. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 10792–10796.
9. Werren, J. H., Guo, L. & Windsor, D. W. (1995) *Proc. R. Soc. London Ser. B* **262**, 147–204.
10. Breeuwer, J. A. J. & Jacobs, G. (1996) *Exper. Appl. Acarol.* **20**, 421–434.
11. Sirioni, M., Bandi, C., Sacchi, L., Di Sacco, B., Damiani, G. & Genchi, C. (1995) *Mol. Biochem. Parasitol.* **74**, 223–227.
12. Rousset, F., Bouchon, D., Juchault, P. & Solignac, M. (1992) *Proc. R. Soc. London Ser. B* **250**, 91–98.
13. Southamer, R., Breeuwer, J. A. J., Luck, R. F. & Werren, J. H. (1993) *Nature (London)* **361**, 66–68.
14. O’Neill, S. L., Giordana, R., Colbert, A. M. E., Karr, T. L. & Robertson, H. M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 2699–2702.
15. Sinkins, S. P., Curtis, C. F. & O’Neill, S. L. (1997) in *Influential Passengers: Inherited Microorganisms and Arthropod Reproduction*, eds. O’Neill, S. L., Hoffmann, A. A. & Werren, J. H. (Oxford Univ. Press, New York), pp. 155–175.
16. Glover, D. M., Raff, J., Karr, T. L., O’Neill, S. L., Lin, H. & Wolfner, M. F. (1990) *Nature (London)* **348**, 117.
17. Solignac, M., Vautrin, D. & Rousset, F. (1994) *C. R. Acad. Sci.* **317**, 461–470.
18. Hoffmann, A. A., Clancy, D. & Duncan, J. (1996) *Heredity* **76**, 1–8.
19. Hoffmann, A. A. & Turelli, M. (1997) in *Influential Passengers: Inherited Microorganisms and Arthropod Reproduction*, eds. O’Neill, S. L., Hoffmann, A. A. & Werren, J. H. (Oxford Univ. Press, New York), pp. 42–80.
20. Werren, J. H. & O’Neill, S. L. (1997) in *Influential Passengers: Inherited Microorganisms and Arthropod Reproduction*, eds. O’Neill, S. L., Hoffmann, A. A. & Werren, J. H. (Oxford Univ. Press, New York), pp. 1–41.
21. Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991) *J. Bacteriol.* **173**, 697–703.
22. Garret, L. (1994) *The Coming Plague* (Penguin, New York).