

THE MANIPULATION OF ARTHROPOD REPRODUCTION BY
WOLBACHIA ENDOSYMBIONTS

DENISE L. JOHANOWICZ AND MARJORIE A. HOY

Department of Entomology and Nematology, University of Florida
Gainesville, FL 32611-0620

ABSTRACT

Wolbachia are intracellular bacteria that manipulate the reproduction of their arthropod hosts. The nature of the manipulation varies with *Wolbachia* strain, arthropod taxa, and arthropod genetic system. Nonreciprocal and reciprocal reproductive incompatibilities, sex ratio biases, and induction of thelytoky are some of the results of *Wolbachia* symbiosis. The *Wolbachia* present in the predatory mite *Metaseiulus occidentalis* are genetically similar to those found in insects, and are correlated with nonreciprocal incompatibility in crosses between infected males and uninfected females. The incompatibility phenotype includes reduced numbers of eggs, shriveled eggs, and a male-biased sex ratio of the few resulting progeny, which may be related to the parahaploid genetic system of this phytoseiid mite.

Key Words: symbiont, incompatibility, sex ratio, Phytoseiidae

RESUMEN

Wolbachia son bacterias intracelulares que manipulan la reproducción de sus hospederos artrópodos. La forma de esta manipulación varía con la sepa de *Wolbachia*, el taxón del artrópodo, y el sistema genético del artrópodo. Incompatibilidades reproductivas recíprocas y no-recíprocas, variaciones del coeficiente sexual, e inducción de telitokia son algunos de los resultados de la simbiosis con *Wolbachia*. La sepa de *Wolbachia* que se encuentra en el ácaro depredador *Metaseiulus occidentalis* es genéticamente similar a la que se encuentra en insectos, y está correlacionada con incompatibilidad no-recíproca en cruza entre machos infectados y hembras no infectadas. El fenotipo de incompatibilidad incluye la producción de un número reducido de huevos, huevos arrugados, y un coeficiente sexual de la poca progenie que resulta inclinado hacia el macho, el cual puede estar relacionado al sistema genético parahaploide de este ácaro fitoseido.

HISTORICAL OVERVIEW

Wolbachia are intracellular, transovarially-transmitted, rickettsial-like endosymbionts in the alpha-subdivision of the proteobacteria (purple bacteria). *Wolbachia* bacteria were first described from the gonadal tissues of the mosquito *Culex pipiens* L. in 1924 by Hertig and Wolbach. Unusual reproductive incompatibilities were described later in *Culex pipiens* mosquitoes by Ghelelovitch (1952) and Laven (1951). One type of these incompatibilities was nonreciprocal, meaning that crosses of males from population A with females of population B resulted in normal hybrid progeny, but crosses of males from population B with females from population A (the reciprocal cross) resulted in few viable hybrid progeny. Because the nuclear genetic makeup of the two hybrid crosses is essentially the same and the main difference is which mother's cytoplasm is interacting with the nuclear genes, the incompatibilities have a cytoplas-

mic inheritance pattern, also called "cytoplasmic incompatibility" (Laven 1959). In the 1970s Yen & Barr (1971) first correlated these nonreciprocal, cytoplasmic incompatibilities with the presence of *Wolbachia* endosymbionts. They found that when *Wolbachia*-infected males were treated with tetracycline (which is toxic to rickettsia-like microorganisms), they could reproduce successfully with uninfected females.

Because *Wolbachia*'s morphological characters are of limited value and *Wolbachia* are difficult to culture outside the host (Weiss & Moulder 1984, O'Neill et al. 1992), their presence in other arthropods was merely speculative. However, in 1992, *Wolbachia*-specific polymerase chain reaction (PCR) primers were developed (O'Neill et al. 1992). These primers, designed to be specific to *Wolbachia*, are also general enough to amplify *Wolbachia* 16S ribosomal DNA from various insects. Reproductive anomalies associated with the presence of an unknown rickettsia could now be correlated with the presence of *Wolbachia*. For example, *Wolbachia* infection was confirmed in some California populations of *Drosophila simulans* Sturtevant (O'Neill et al. 1992). This symbiont was previously suspected as the causative agent of nonreciprocal reproductive incompatibilities between geographical populations of *D. simulans* (Hoffmann et al. 1986).

WOLBACHIA BIOLOGY

Wolbachia are transmitted through the egg cytoplasm, and therefore solely by females, except for one reported case of male transmission in laboratory populations of *D. simulans* (Hoffmann & Turelli 1988). *Wolbachia* are sensitive to high temperatures (Stevens 1989, Stouthamer et al. 1990, Girin & Bouletreau 1995, Louis et al. 1993), and the antibiotics rifampin and tetracycline (Stouthamer et al. 1990). The only success to date in culturing them outside the host has been in an *Aedes albopictus* (Skuse) cell line (O'Neill et al. 1995).

Because *Wolbachia* cannot be studied with traditional microbiological techniques (Weiss & Moulder 1984), techniques such as the polymerase chain reaction (PCR) and DNA sequencing have provided major breakthroughs in the study of these endosymbionts (O'Neill et al. 1992, Breeuwer et al. 1992, Rousset et al. 1992b, Stouthamer et al. 1993). The PCR allows the amplification of a specific region of *Wolbachia* DNA more than a million-fold. The presence or absence of the symbiont then can be determined by visual detection of the expected size fragment of DNA in an ethidium bromide-stained agarose gel under UV light. This amplification also yields ample DNA for sequencing and further description and characterization. DNA sequence analyses indicate a lack of concordance between the phylogenies of the symbiont and of the hosts, suggesting that this symbiont might sometimes be transmitted horizontally from species to species (Rousset et al. 1992b, O'Neill et al. 1992). Recent studies with the PCR determined that 16% of all insect species examined are infected with *Wolbachia* (Werren et al. 1995), and include representatives from a wide variety of orders and families (Giordano et al. 1997). For an extensive review of the current knowledge about *Wolbachia*, see Werren (1997).

WOLBACHIA'S EFFECTS

The effects of *Wolbachia* can be influenced by several factors. The strain of *Wolbachia* is important; some strains have been demonstrated to cause no reproductive alterations (Giordano et al. 1995). The phenotype of *Wolbachia*-mediated reproductive alterations also depends on the taxonomic status of the affected arthropod (Insecta, Arachnida, Isopoda) (see Werren 1997), as well as the genetic system of the arthropod.

It is important to consider arthropod genetic systems in order to better appreciate the diversity of *Wolbachia's* effects on reproduction.

Diplo-diploid arthropods produce both sexes from fertilized eggs, each sex carrying both the maternal and paternal sets of chromosomes throughout their lives. In haplo-diploid arthropods, female progeny arise from fertilized eggs and are diploid, but the male progeny arise from unfertilized eggs and are haploid, carrying only the maternal set of chromosomes. Thelytoky is a genetic system in which virgin females produce diploid daughters parthenogenetically, rarely producing males. In a parahaploid genetic system, both sexes initially arise from fertilized (diploid) eggs, with both sets of chromosomes. However, one chromosome set is subsequently lost in males, and the adult male is haploid, producing sperm by a mitotic process.

When males of infected diplo-diploids mate with females lacking *Wolbachia*, the paternal chromosome set becomes abnormal in the fertilized egg (Kose & Karr 1995, O'Neill & Karr 1990), resulting in the death of both male and female progeny (Hoffmann et al. 1986, Hsiao & Hsiao 1985). The reciprocal cross is normal. Although the molecular mechanism of this incompatibility is not yet fully understood, it is speculated that *Wolbachia* somehow "imprints" or "modifies" the paternal set of chromosomes during spermatogenesis (Werren 1997), even though the bacteria themselves are not present in the mature male gametes. If *Wolbachia* is present in the egg cytoplasm, it can "rescue" the paternal chromosomes so that they remain normal and produce the normal diploid sons and daughters. If no *Wolbachia* is present, there is no "rescue" and those paternal chromosome set becomes abnormal, leading to embryonic death.

This same mechanism may occur in haplo-diploid insects, but with different consequences. When infected haploid males mate with uninfected diploid females, the male (haploid) progeny remain normal, but the normally diploid female embryos become haploid due to abnormalities in the paternal set of chromosomes (Ryan & Saul 1968, Reed & Werren 1995). The resulting phenotype of *Wolbachia*-mediated incompatibilities in haplo-diploid species is a strongly male-biased sex ratio because of the loss of female progeny. The haploid female embryos may die, as in some strains of the mite *Tetranychus urticae* Koch (Chelicerata: Arachnida) (Vala & Breeuwer 1996), or the haploid female embryos can become males thereby increasing the total number of expected males, as in the wasp *Nasonia vitripennis* Walker (Mandibulata: Insecta) (Breeuwer & Werren 1990, Ryan & Saul 1968).

Wolbachia can also cause bidirectional incompatibility in diplo-diploid species (O'Neill & Karr 1990) and haplo-diploid species (Perrot-Minot et al. 1996). In this situation, two populations apparently host two different *Wolbachia* strains. The result is reciprocal incompatibility, where both interpopulation crosses are incompatible.

Wolbachia induces thelytoky in some hymenopteran species, such as *Trichogramma* (Stouthamer et al. 1990) and *Aphytis* (Zchori-Fein et al. 1995). *Wolbachia* allows these females to produce diploid daughters parthenogenetically by causing gamete or chromosomal duplication early in the first mitotic division (Stouthamer & Kazmer 1994).

Wolbachia causes a typical diplo-diploid incompatibility phenotype in some isopods (Rousset et al. 1992a), as well as an unusual phenotype in the species *Armadillium vulgare* Latr. In this species, *Wolbachia* suppresses the androgenic gland in genetically male individuals, causing these male isopods to become functional females (Rigaud et al. 1991). It is likely that, with the diversity of *Wolbachia's* effects on the arthropod taxa and genetic systems described to date, there may be more *Wolbachia*-mediated reproductive anomalies remaining to be described.

Because uninfected females are reproductively incompatible with infected males, and infected females can reproduce successfully with infected and uninfected males, infected females tend to have a reproductive advantage in polymorphic populations

(Caspari & Watson 1959, Turelli & Hoffmann 1991). The *D. simulans* *Wolbachia* infection has spread within and among California populations (Turelli & Hoffmann 1991, Turelli et al. 1992) since it was first documented in 1986 (Hoffmann et al. 1986). However, the ability of *Wolbachia* to spread through a population is modulated by various factors, including the stability of the infection as a function of maternal transmission frequency, fitness costs associated with infection, and the strength of incompatibility (Hoffmann et al. 1990, Turelli et al. 1992, Clancy & Hoffmann 1997).

The potential for *Wolbachia*-infected individuals to sweep through a population may be a useful phenomenon in the control of arthropod-borne pathogens. Efforts are under way to genetically engineer insects to be refractory to disease agents like those causing malaria or Chagas' disease (Beard et al. 1993). A mechanism is needed to enable these transformed arthropods to replace the wild-type insects already present in the field population. The ability of *Wolbachia* infection to spread through a population, as documented in *D. simulans*, could be harnessed as a mechanism to help drive a genetically altered trait through a population if the trait "hitchhikes" with the *Wolbachia*-infected cytoplasm (Caspari & Watson 1959, Beard et al. 1993). However, a fuller understanding of *Wolbachia* biology will be necessary before it can be used successfully as a drive mechanism (Werren 1997).

Wolbachia symbiosis may have other important effects. *Wolbachia*-mediated reproductive isolation may be one mechanism that could allow sympatric speciation to occur (Laven 1959, Werren 1997, Giordano et al. 1997). *Wolbachia* alters sex ratios and progeny survival and, as a consequence, may affect laboratory experiments and insect management in field programs. *Wolbachia* infection may have implications for mass rearing projects, especially if the bacteria have an influence on the quality of the natural enemies (Steiner 1993) or affect the rate of population increase of the individuals being reared.

WOLBACHIA IN THE PREDATORY MITE *METASEIULUS OCCIDENTALIS*: A CASE STUDY

The predatory mite *Metaseiulus* (= *Typhlodromus* or *Galendromus*) *occidentalis* (Nesbitt) is an important natural enemy of *Tetranychus* species, including *Tetranychus urticae* Koch. This predatory mite is used as a biological control agent in various crops in the western United States (Hoyt 1969, Flaherty & Huffaker 1970, Hoyt & Caltagirone 1971, Hoy 1985). Biological characteristics that affect these predators' rate of population increase are of particular importance (Sabelis 1985), including the presence of nonreciprocal reproductive incompatibilities between different strains or populations (Hoy 1985). Such nonreciprocal reproductive incompatibilities have been reported in *M. occidentalis*, and are associated with shriveled eggs, low numbers of eggs, low survival of immature stages, and reduced fecundity in surviving F_1 individuals (Croft 1970, Hoy & Knop 1981, Hoy & Standow 1982, Hoy & Cave 1988). Studies on the mode of inheritance of pesticide resistance have been affected by nonreciprocal cross incompatibilities (Hoy & Knop 1981, Hoy & Standow 1982). Nonreciprocal incompatibilities have interfered with hybridization studies between different phytoseiid mite populations (one method of determining species designations), including studies with *M. occidentalis* (Croft 1970), *Typhlodromus annectens* DeLeon (McMurtry & Badii 1989), and *Amblyseius addoensis* van der Merwe and Ryke (McMurtry 1980).

The cause of the nonreciprocal reproductive incompatibilities was unknown in these phytoseiids. An intracellular rickettsia-like microorganism was found by Hess & Hoy (1982) in *M. occidentalis* eggs and ovaries through light and electron microscopy. This observation, along with the nonreciprocal nature of the incompatibilities, led Hoy & Cave (1988) to speculate that a cytoplasmic factor may be responsible for

the observed reproductive aberrations seen in *M. occidentalis*, perhaps due to the presence of *Wolbachia*.

By using *Wolbachia* specific PCR primers which amplify the 16S ribosomal RNA and *ftsZ* genes, we determined that *Wolbachia* was present in both the predatory mite and its prey, *Tetranychus urticae* (Johanowicz & Hoy 1996). Unexpectedly, the *Wolbachia* DNA sequences from the two mite species were nearly identical to each other and to those from insects, including the type species *Wolbachia pipientis* from the mosquito *Culex pipiens*. Whether the *Wolbachia* from the mites are truly this similar to each other and to the symbionts from insects remains to be answered, because these genes are too conserved to resolve this question.

In order to study the biological effects of *Wolbachia* infection, it is crucial to obtain a population without the symbionts with which infected individuals can be crossed or compared. Rearing these mites at high temperatures (33°C) eliminated *Wolbachia* in the treated mites, as indicated by a PCR assay for infection status, and allowed crossing studies to be conducted. Interestingly, the incompatibility phenotype in *M. occidentalis* was a unique combination of reduced progeny production (as in diplo-diploids) and a skewed sex ratio (as in haplo-diploids) of the few resulting progeny, when infected males were crossed with cured females (Johanowicz & Hoy 1998). This phenotype may be due to the parahaploid genetic system (Hoy 1979) of this mite. Nelson-Rees et al. (1980) demonstrated cytologically that both male and female *M. occidentalis* are diploid at the beginning of embryonic development, but at the onset of the reductional division 24-48 hours after egg deposition, one of the sets (most likely the paternal set) becomes heterochromatinized and excluded from the nucleus, resulting in haploid males.

CONCLUSION

Wolbachia manipulate arthropod reproduction by causing nonreciprocal incompatibility, bidirectional incompatibility, skewed sex ratios, and thelytoky, depending on the *Wolbachia* strain, arthropod taxa, and genetic system. For example, *Wolbachia* is associated with both reduced egg production and a male-biased sex ratio of the few remaining progeny in *M. occidentalis*, a predatory mite with a parahaploid genetic system.

Interesting questions remain to be answered about *Wolbachia* symbiosis in *M. occidentalis* and in other arthropods. The exact mechanism of the reproductive manipulations remains unknown. A more detailed phylogenetic analysis of the *Wolbachia* in its various hosts using less conserved genes should provide a better estimate of the evolutionary relationships between the symbionts. Further study of *Wolbachia* infection dynamics may determine its potential use as a drive mechanism. Other consequences of *Wolbachia* infection remain to be described. For example, Hsiao (1996) indicated that *Wolbachia* infection may be responsible for protecting the Western biotype of the alfalfa weevil from a parasitoid. Because of the complex interactions between this microorganisms and its arthropod hosts, a multidisciplinary approach will be helpful in answering many of these remaining questions.

ACKNOWLEDGMENTS

Thanks to J. Sivinski and T. Walker for including this paper in the Behavioral Ecology Symposium, to J. Sivinski and J. Nation for helpful comments on this manuscript, and to L. Keal and A. Jeyaprakosla for technical assistance during this project. This work was supported with funds from the Davies, Fischer, and Eckes Endowment in Biological Control. This is University of Florida journal publication R-06194.

REFERENCES CITED

- BEARD, C. B., S. L. O'NEILL, R. B. TESH, F. F. RICHARDS, AND S. AKSOY. 1993. Modification of arthropod vector competence via symbiotic bacteria. *Parasitol. Today* 9: 179-183.
- BREEUWER, J. A. J., R. STOUTHAMER, S. M. BARNES, D. A. PELLETIER, W. G. WEISBURG, AND J. H. WERREN. 1992. Phylogeny of cytoplasmic incompatibility microorganisms in the parasitoid wasp genus *Nasonia* (Hymenoptera: Pteromalidae) based on 16S ribosomal DNA sequences. *Insect Mol. Biol.* 1: 25-36.
- BREEUWER, J. A. J., AND J. H. WERREN. 1990. Microorganisms associated with chromosome destruction and reproductive isolation between two insect species. *Nature* 346: 558-560.
- CASPARI, E., AND G. S. WATSON. 1959. On the evolutionary importance of cytoplasmic sterility in mosquitoes. *Evolution* 13: 568-570.
- CLANCY, D. J., AND A. A. HOFFMANN. 1997. Behavior of *Wolbachia* endosymbionts from *Drosophila simulans* in *Drosophila serrata*, a novel host. *Am. Nat.* 149: 975-988.
- CROFT, B. A. 1970. Comparative studies on 4 strains of *Typhlodromus occidentalis* (Acarina: Phytoseiidae) I. Hybridization and reproductive isolation studies. *Ann. Entomol. Soc. Amer.* 63: 1558-63.
- FLAHERTY, D. L., AND C. B. HUFFAKER. 1970. Biological control of Pacific mites and Willamette mites in San Joaquin Valley vineyards: Part I. Role of *Metaseiulus occidentalis*. Part II. Influence of dispersion patterns of *Metaseiulus occidentalis*. *Hilgardia* 40: 267-330.
- GHELELOVITCH, S. 1952. Sur le déterminisme génétique de la stérilité dans le croisement entre différentes souches de *Culex autogenicus* Roubaud. *C. R. Acad. Sci. Paris* 24: 2386-2388.
- GIORDANO, R., S. L. O'NEILL, AND H. M. ROBERTSON. 1995. *Wolbachia* infections and the expression of cytoplasmic incompatibility in *Drosophila sechellia* and *D. mauritiana*. *Genetics* 140: 1307-1317.
- GIORDANO, R., J. J. JACKSON, AND H. M. ROBERTSON. 1997. The role of *Wolbachia* bacteria in reproductive incompatibilities and hybrid zones of *Diabrotica* beetles and *Gryllus* crickets. *Proc. Natl. Acad. Sci. USA* 94: 11439-11444.
- GIRIN, C., AND M. BOULÉTREAU. 1995. Microorganism-associated variation in host infestation efficiency in a parasitoid wasp, *Trichogramma bourarachae* (Hymenoptera: Trichogrammatidae). *Experientia* 51: 398-401.
- HESS, R. T., AND M. A. HOY. 1982. Microorganisms associated with the spider mite predator *Metaseiulus* (= *Typhlodromus*) *occidentalis*: Electron microscope observations. *J. Invertebr. Pathol.* 40: 98-106.
- HOFFMANN, A. A., AND M. TURELLI. 1988. Unidirectional incompatibility in *Drosophila simulans*: inheritance, geographic variation and fitness effects. *Genetics* 136: 993-999.
- HOFFMANN, A. A., M. TURELLI, AND L. G. HARSHMAN. 1990. Factors affecting the distribution of cytoplasmic incompatibility in *Drosophila simulans*. *Genetics* 126: 933-948.
- HOFFMANN, A. A., M. TURELLI, AND G. M. SIMMONS. 1986. Unidirectional incompatibility between populations of *Drosophila simulans*. *Evolution* 40: 692-701.
- HOY, M. A. 1979. Parahaploidy of the "arrhenotokous" predator, *Metaseiulus occidentalis* (Acarina: Phytoseiidae) demonstrated by X-irradiation of males. *Ent. Exp. Appl.* 26: 97-104.
- HOY, M. A. 1985. Recent advances in genetics and genetic improvement of the Phytoseiidae. *Annu. Rev. Entomol.* 30: 345-370.
- HOY, M. A., AND F. E. CAVE. 1988. Premating and postmating isolation among populations of *Metaseiulus occidentalis* (Nesbitt) (Acarina: Phytoseiidae). *Hilgardia* 56: 1-20.
- HOY, M. A., AND N. F. KNOP. 1981. Selection for and genetic analysis of permethrin resistance in *Metaseiulus occidentalis*: genetic improvement of a biological control agent. *Ent. Exp. Appl.* 30: 10-18.

- HOY, M. A., AND K. A. STANDOW. 1982. Inheritance of resistance to sulfur in the spider mite predator *Metaseiulus occidentalis*. Ent. Exp. Appl. 31:316-323.
- HOYT, S. C. 1969. Population studies of five mite species on apple in Washington, pp. 117-133 in Proceedings, 2nd International Congress Acarology. Sutton-Bonnington, England. Acad Kiado, Budapest.
- HOYT, S. C., AND L. E. CALTAGIRONE. 1971. The developing programs of integrated control of pests in Washington and peaches in California, pp. 395-421 in C. B. Huffaker [ed.] *Biological Control*. Plenum New York.
- HSIAO, T. 1996. Studies of interactions between alfalfa weevil strains, *Wolbachia* endosymbionts, and parasitoids, pp. 51-71 in W. O. C. Symondson and J. E. Liddell [eds.] *The Ecology of Agricultural Pests*. Chapman and Hall, London.
- HSIAO, C., AND T. H. HSIAO. 1985. Rickettsia as the cause of cytoplasmic incompatibility in the alfalfa weevil, *Hypera postica*. J. Invertebr. Path. 45: 244-246.
- JOHANOWICZ, D. L., AND M. A. HOY. 1996. *Wolbachia* in a predator-prey system: 16S ribosomal analysis of two phytoseiids (Acari: Phytoseiidae) and their prey (Acari: Tetranychidae). Ann. Entomol. Soc. Amer. 89: 435-441.
- JOHANOWICZ, D. L., AND M. A. HOY. 1998. Experimental induction and termination of non reciprocal reproductive incompatibility in a parahaploid mite. Ent. Exp. Appl. (In press)
- KOSE, H., AND T. L. KARR. 1995. Organization of *Wolbachia pipientis* in the *Drosophila* fertilized egg and embryo revealed by anti-*Wolbachia* monoclonal antibody. Mech. Dev. 51: 275-288.
- LAVEN, H. 1951. Crossing experiments with *Culex* strains. Evolution 5: 370-375.
- LAVEN, H. 1959. Speciation by cytoplasmic isolation in the *Culex pipiens* complex. Cold Spring Harb. Symp. Quant. Biol. 24: 166-173.
- LOUIS, C., B. PINTEREAU, AND L. CHAPELLE. 1993. Recherches sur l'origine de l'unisexualité: la thérapie élimine à la fois rickettsies et parthénogénèse thélytoque chez un Trichogramme (Hym., Trichogrammatidae). C. R. Acad. Sci. Paris 336: 27-33.
- MCMURTRY, J. A. 1980. Biosystematics of three taxa in the *Amblyseius finlandicus* group from South Africa with comparative life history studies (Acarina: Phytoseiidae). Int. J. Acar. 6: 147-156.
- MCMURTRY, J. A., AND M. H. BADI. 1989. Reproductive compatibility in widely separated populations of three species of phytoseiid mites (Acari: Phytoseiidae). Pan-Pac. Entomol. 65: 397-402.
- NELSON-REES, W. A., M. A. HOY, AND R. T. ROUSH. 1980. Heterochromatinization, chromatin elimination and haploidization in the parahaploid mite *Metaseiulus occidentalis* (Nesbitt) (Acarina: Phytoseiidae). Chromosoma 77: 263-276.
- O'NEILL, S. L., R. GIORDANO, A. M. E. COLBERT, T. L. KARR, AND H. M. ROBERTSON. 1992. 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. Proc. Natl. Acad. Sci. U.S.A. 89: 2699-2702.
- O'NEILL, S. L., AND T. L. KARR. 1990. Bidirectional incompatibility between conspecific populations of *Drosophila simulans*. Nature 348: 178-180.
- O'NEILL, S. L., M. M. PETTIGREW, T. G. ANDREADIS, AND R. B. TESH. 1995. An in-vitro system for culturing *Wolbachia* symbionts of arthropods. J. Cell. Biochem. 21A: 210.
- PERROT-MINNOT, M.-J., L. R. GUO, AND J. H. WERREN. 1996. Single and double infections with *Wolbachia* in the parasitic wasp *Nasonia vitripennis*: effects on compatibility. Genetics 143: 961-972.
- REED, K. M., AND J. H. WERREN. 1995. Induction of paternal genome loss by the paternal sex ratio chromosome and cytoplasmic incompatibility bacteria (*Wolbachia*): a comparative study of early embryonic events. Mol. Reprod. Dev. 40: 408-418.
- RIGAUD, T., C. SOUTY-GROSSET, R. RAIMOND, J. P. MOCQUARD, AND P. JUCHAULT. 1991. Feminizing endocytobiosis in the terrestrial crustacean *Armadillium vulgare* Latr. (Isopoda): recent acquisitions. Endocytobiosis Cell. Res. 7: 259-273.

- ROUSSET, F., D. BOUCHON, B. PINTUREAU, P. JUCHAULT, AND M. SOLIGNAC. 1992a. *Wolbachia* endosymbionts responsible for various alterations of sexuality in arthropods. Proc. R. Soc. Lond. B. 250: 91-98.
- ROUSSET, F., D. VAUTRIN, AND M. SOLIGNAC. 1992b. Molecular identification of *Wolbachia*, the agent of cytoplasmic incompatibility in *Drosophila simulans*, and variability in relation to host mitochondrial types. Proc. R. Soc. Lond. B 247: 163-168.
- RYAN, S. L., AND G. B. SAUL II, 1968. Post fertilization effect of incompatibility factors in *Mormoniella*. Mol. Gen. Genet. 103: 29-36.
- SABELIS, M. W. 1985. Reproduction and sex allocation, pp. 73-94 in W. Helle and M. W. Sabelis [eds.] Spider Mites Their Biology, Natural Enemies and Control. Elsevier, Amsterdam.
- STEINER, M. Y. 1993. Quality control requirements for pest biological control agents. AECV93-R6. Alberta Environmental Centre, Vegreville, AB. 112 pages.
- STEVENS, L. 1989. Environmental factors affecting reproductive incompatibility in flour beetles, genus *Tribolium*. J. Invertebr. Path. 53: 78-84.
- STOUTHAMER, R., J. A. J. BREEUWER, R. F. LUCK, AND J. H. WERREN. 1993. Molecular identification of microorganisms associated with parthenogenesis. Nature 361: 66-68.
- STOUTHAMER, R., AND D. J. KAZMER. 1994. Cytogenetics of microbe-associated parthenogenesis and its consequences for gene flow in *Trichogramma* wasps. Heredity 73: 317-327.
- STOUTHAMER, R., R. F. LUCK, AND W. D. HAMILTON. 1990a. Antibiotics cause parthenogenetic *Trichogramma* (Hymenoptera/Trichogrammatidae) to revert to sex. Proc. Natl. Acad. Sci. U.S.A. 87: 2424-2427.
- TURELLI, M., AND A. A. HOFFMANN. 1991. Rapid spread of an inherited incompatibility factor in California *Drosophila*. Nature 353: 440-442.
- TURELLI, M., A. A. HOFFMANN, AND S. W. MCKECHNIE. 1992. Dynamics of cytoplasmic incompatibility and mtDNA variation in natural *Drosophila simulans* populations. Genetics 132: 713-723.
- WEISS, E., AND J. W. MOULDER. 1984. Order I. Rickettsiales Gieszczykiewicz, pp. 687-729 in N. R. Kreig, J. G. Holt [eds.] Bergey's Manual of Systematic Bacteriology, Vol. 1. Willians and Wilkins, Baltimore.
- WERREN, J. H. 1997. Biology of *Wolbachia*. Annu. Rev. Entomol. 42: 587-609.
- WERREN, J. H., L. GUO, AND D. W. WINDSOR. 1995. Distribution of *Wolbachia* in neotropical arthropods. Proc. R. Soc. Lond. B 262: 147-204.
- VALA, F., AND J. A. J. BREEUWER. 1996. Hybrid breakdown associated with *Wolbachia* induced cytoplasmic incompatibility in spider mites. Symbiosis 96! Meeting Abstract pp. 58.
- YEN, J. H., AND A. R. BARR. 1971. New hypothesis on the cause of cytoplasmic incompatibility in *Culex pipiens*. Nature 232: 657-658.
- ZCHORI-FEIN, E., O. FAKTOR, M. ZEIDAN, Y. GOTTLIEB, H. CZOSNEK, AND D. ROSEN. 1995. Parthenogenesis-inducing microorganisms in *Aphytis* (Hymenoptera: Aphelinidae). Ins. Mol. Biol. 4: 173-178.