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Review Article

Wolbachia-mediated reproductive alterations in invertebrate hosts and biocontrol implications of the bacteria: an update

M. Saiful Islam*
Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, United Kingdom
*Present address: Dept. of Zoology, University of Rajshahi, Rajshahi 6205, Bangladesh; e-mail : saifulzoo@yahoo.co.uk

Abstract: *Wolbachia* are obligatory intracellular bacteria that have evolved to manipulate reproduction and/or metabolism of their arthropod and nematode hosts in a number of ways, all designed to the benefit of their own survival and transmission through hosts' populations. An updated account of the occurrence, identification, phylogeny and genetics, phenotypic effects, distribution, mechanisms of action, horizontal transmission, infection dynamics, evolutionary consequences and biocontrol implications of the bacteria are presented. Associations between these maternally heritable bacteria and their hosts not only cover the entire range of interactions from parasitism to mutualism but also a complex interplay of both. *Wolbachia* are transmitted vertically from mothers to offspring, and also horizontally within or between arthropod taxa. They are known to induce cytoplasmic incompatibility (CI) *via* unviable brood, parthenogenesis induction (PI) through asexual reproduction, feminization (F) by converting males into functional females, and male killing (MK) by causing death to sons of the infected mothers. How these bacteria influence host fitness and population dynamics, and could play an important role in speciation have been reviewed. Possible uses of the bacteria and their predominant phenotypes in control programmes for agricultural pests and human disease vectors have been discussed.

Key words: *Wolbachia*, reproductive manipulation, cytoplasmic incompatibility, parthenogenesis induction, feminization, male killing, biocontrol implications

Introduction

Wolbachia (**Fig. 1**) are a group of obligate, intracellular and maternally inherited Gram negative, purple bacteria that belong to the Kingdom Eubacteria, Phylum Proteobacteria, Class Alpha Proteobacteria, Order Rickettsiales and Family Rickettsiaceae (Wu *et al*., 2004; Lo *et al*., 2007). The closest known relatives of *Wolbachia* are *Cowdria* and *Anaplasma* species that cause arthropod-borne diseases of mammals. Important arthropod and nematode pests and disease vectors harbour these highly adaptive bacteria. *Wolbachia*-arthropod relationships have variously been described as mutualistic (Girin & Bouletreau, 1995), parasitic (Werren *et al*., 1995a), pathogenic (Min & Benzer, 1997) and symbiotic (James & Ballard, 2000) whereas *Wolbachia*-nematode relationships have been shown to be mutualistic and reciprocal co-adaptive (Bandi *et al*., 1999; Hoerauf *et al*., 2000). However, it is not always simple to characterize them because the bacteria are capable of inducing both positive and negative range of effects on different host species (Wade, 2001; Zimmer, 2001; Weeks *et al*., 2002).

Hertig & Wolbach (1924) first described *Wolbachia* from the gonadal tissues of the mosquito *Culex pipiens* and subsequently the bacteria were given the name *Wolbachia pipientis* which are irregular rods of 0.5-1.3 µm long, coccoids of 0.25-0.5 µm diameter or oval of 1-1.8 µm diameter (Hertig, 1936). Morphology of the bacteria was further described by Wright & Barr (1980) in *Aedes scutellaris*, by Hsiao & Hsiao (1985) in *Hypera postica* and by O'Neill (1989) in *Tribolium confusum.* They could be artificially cultured outside the host in insect and mammalian cell lines (O'Neill *et al.,* 1997; Dobson *et al*., 2002a). As it would be evident from the foregoing pages of this review, the unique biology of *Wolbachia* has attracted a growing number of researchers interested in questions ranging from the evolutionary implications of infection through to the use of this agent for pest and disease control.

Occurrence

Wolbachia infect all major groups of insects, isopod crustaceans, mites, spiders, springtails and thrips (Weeks *et al*., 2002; Iturbe-Ormaetxe & O'Neill, 2007). Outside Arthropoda, the bacteria infect filarial nematodes including those causing river blindness and elephantiasis in humans as well as heart worms in dogs (Hoerauf *et al*., 2003; Tsillassie & Legesse, 2007). PCR-based screenings revealed 16-20% *Wolbachia* infections in studies of Neotropical (Werren *et al*., 1995b), Palaearctic (West *et al*., 1998) and Nearctic

(Werren & Windsor, 2000) insects. Six of 16 species of spider mites, four of seven species of predatory mites, 35% of terrestrial isopods and nine of 10 filarial nematode species are infected (Stouthamer *et al.,* 1999). Seventy six percent of Nearctic arthropods (Jeyaprakash & Hoy, 2000), 50% of Indo-Australian and Indonesian ants (Wenseleers *et al*., 1998, 2002) and 100% of Panamanian leafcutter ants (Van Borm *et al*., 2001) are found positive for the bacteria. Surveys across a taxonomically diverse range of samples demonstrate *Wolbachia* in 17% of Panamanian insects, 19% of North American insects and 22% of British hymenopteran and lepidopteran insects (Jiggins *et al.,* 2001b). To sum up, *Wolbachia* are believed to infect between 20% and 76% of all insect species (Stevens *et al*., 2001; Weeks *et al.,* 2002), thus making *Wolbachia* among the most abundant intracellular bacteria. Recent systematic surveys in Japan revealed that 16.7% spider mite (Gotoh *et al*., 2003) and 44.9% Lepidoptera (Tagami & Miura, 2004) are infected with the bacteria. A brief account of the occurrence of *Wolbachia* in major invertebrate taxa is given in Table 1.

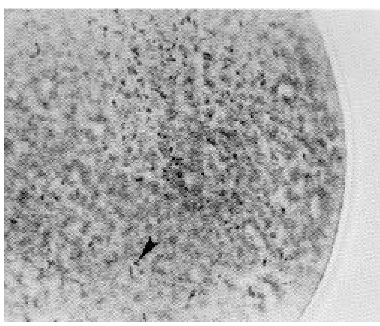

Fig. 1. *Wolbachia* (darkly stained dots) in a *Nasonia* egg (Bordenstein *et al*., 2001).

Identification and nomenclature

Traditional microbiological procedures are not suitable for studying *Wolbachia*. Polymerase chain reaction (PCR) and DNA sequencing techniques have provided major breakthroughs in the study of these bacteria. PCR primers specific to 12S, 16S or 23S rDNAs, and *wsp* (*Wolbachia* surface protein), *ftsZ* (bacterial cell division) and *groELI* (bacterial heat shock protein) genes are used to detect the presence of the bacteria in host tissues. A system of naming for various *Wolbachia* strains of *Drosophila* uses *w* followed by the name of the host from which the bacteria were first collected (*Stouthamer et al*., 1999). For instance, *w*Ri stands for *Wolbachia* of *D. simulans* collected in Riverside, California; whereas *w*Ha, *w*Ma, *w*Au and *w*Ki represent the bacteria from Hawaii, Madagascar, Australia and Mount Kilimanjaro in Tanzania, respectively. However, complications in naming may arise due to recombination between *Wolbachia* strains (Jiggins *et al*., 2001a). The need for a more generalized system of nomenclature therefore is felt for naming a large number of *Wolbachia* strains that are either described already or to be discovered in the future.

Phylogeny and genetics

Wolbachia strains described so far fall under six major supergroups or clades from A to F. The diversity of the bacteria is mostly analyzed using fast-evolving genes like *ftsZ* and *wsp*. Based on *ftsZ* sequences, most of the *Wolbachia* from insects, crustaceans and mites are classified into A and B (Werren *et al*., 1995a). The A and B supergroups are divided further into a number of groups based on *wsp* sequences (Zhou *et al*., 1998). The bacteria from filarial nematodes belong to C and D (Bandi *et al*., 1998), springtails to E (Vandekerckhove *et al*., 1999), and termites and scorpions to F (Lo *et al*., 2002; Baldo *et al*., 2007). According to Weisburg (1989) *Wolbachia* might have acquired an intracellular symbiotic life-style more than 100 million years ago (mya). Supergroups A and B are estimated to have diverged some 60 mya (Werren *et al*., 1995a) and they have been separated from C and D some 100 mya (Bandi *et al*., 1998).

The complete sequencing of *w*Mel strain of *Wolbachia* by Wu *et al*. (2004) from naturally infected *Drosophila simulans* reveals that the bacteria have a small genome consisting of a single circular molecule of about 1.3 million base pairs, very similar to the closely related strain *w*MelPop described by Sun *et al*. (2003). This is about a third of the size of the genome of *Escherichia coli*. Masui *et al*. (2000) reported a bacteriophage *WO* from *Wolbachia*-infected insects, suggesting that *WO* exchanges genetic material between different *Wolbachia* lineages (Gavotte *et al*., 2004). The *WO* locus *orf7* varies between *Cx. pipiens* species complex in copy number and sequence (Sanogo & Dobson, 2004). Recently the genome of *Wolbachia* from *Brugia malayi* have been sequenced (Foster *et al*., 2005), and a complete copy of the *Wolbachia* genome are found within the genome of *D. ananassae* (Hotopp *et al*., 2007). Information on the genetic makeup of the phage has exciting potential for discovering the mechanisms of bacterial action and understanding the diversity of these bacteria-host interactions. Moreover, use of the phage to manipulate *Wolbachia* could be one of the keys in future for using the bacteria to control medically and agriculturally important pests.

***Wolbachia* phenotypes**

Wolbachia manipulate host reproduction to promote their own spread and maintenance in hosts' populations by a number of phenotypes. Much of the success of these bacteria can be attributed to the diverse phenotypes that result from infection. These range from incompatibility in early embryos to override chromosomal sexdetermination such as induction of parthenogenesis and feminization, and to selectively kill males. The nature of manipulation varies with host taxa, their genetic systems, and with bacterial strains. Reviews by Werren (1997a), Hoffmann & Turelli (1997), Stouthamer *et al.* (1999), Bandi *et al.* (2001), Stevens *et al.* (2001), Weeks *et al*., (2002) and Iturbe-Ormaetxe & O'Neill (2007) provide an extensive account of the phenomena associated with the bacteria. Given below is a brief description of *Wolbachia*-induced predominant phenotypes (Table 1). Other effects of the bacteria on their hosts are summarized in Table 2.

Cytoplasmic incompatibility (CI): The most common phenotype that *Wolbachia* induce on arthropod reproduction is cytoplasmic incompatibility (CI) which typically results in zygotic death in diploid species and some haplodiploid mite species, or haploid male production in haplodiploid parasitic wasps (Werren, 1997a). One-way or unidirectional CI is manifested in crosses between *Wolbachia* single- or superinfected males and uninfected females, whereas two-way or bidirectional CI is shown in crosses between individuals infected with two different infection types of the bacteria. CI therefore offers single and superinfected females, a reproductive advantage relative to uninfected and single-infected females, respectively (**Fig. 2**). Yen & Barr (1973) offered experimental evidence in support of their hypothesis that *W. pipientis* cause CI in *Cx. pipiens*. The exact mechanism by which the bacteria induce CI in their hosts is yet not known. Early meiotic defects and loss of paternal chromosomes (Jost, 1971; Wright & Barr, 1981; Reed & Werren, 1995; Callaini *et al*., 1996), and delayed breakdown of nuclear envelope (Tram & Sullivan, 2002) are shown to be related to CI.

Parthenogenesis induction (PI): Another phenotypic effect of *Wolbachia* is thelytokous parthenogenesis induction (PI) where unfertilized eggs of the host develop into diploid females instead of the usual haploid males. In other words, PI bacteria prevent arrhenotokous parthenogenesis (*i.e.* production of males from unfertilized haploid eggs) and allow the infected females to produce daughters without mating (**Fig. 3**). This favours the bacteria because they are only transmitted through females. Cytological analyses revealed that the chromosomes of infected unfertilized embryos fail to segregate in the first meiotic anaphase, resulting in completely homozygous diploid individuals that develop as females (Stouthamer & Kazmer, 1994). The bacteria infect at least 40 species of the parasitic wasps including *Trichogramma* (Stouthamer, 1997). In some of these hymenopterans the ability to reproduce sexually has been lost over time; in others the infection remains at a polymorphic equilibrium where both infected and uninfected individuals co-exist (Bandi *et al.,* 2001). Outside Hymenoptera, PI is described in springtails (Vandekerckhove *et al*., 1999), predatory thrips (Arakaki *et al*., 2001) and phytophagous mites (Weeks & Breeuwer, 2001).

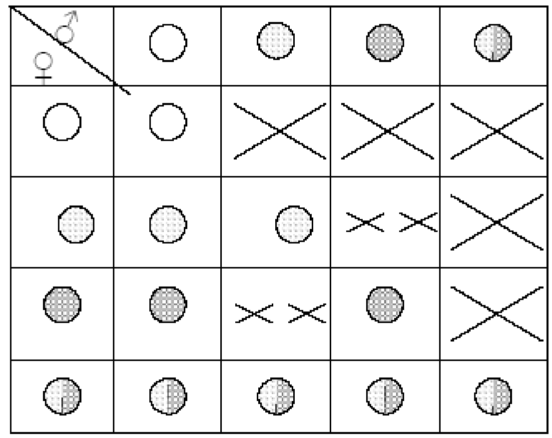

Fig. 2. A generalized crossing pattern showing unidirectional (marked ×) and bidirectional (marked xx) cytoplasmic incompatibility (CI) that results from crosses of *Wolbachia* uninfected (unshaded), single-infected (shaded) and superinfected (shaded plus striped) hosts. Incompatibility is observed when the male harbours an infection type that is absent in his female partner. Due to maternal transmission of the bacteria, the infection type in offspring is similar to that of the mother. Single and superinfected females have the reproductive advantage relative to uninfected and single-infected females, respectively. See text for further detail.

Feminization (F): Wolbachia infections in some terrestrial isopod crustaceans (Juchault *et al*., 1994) and lepidopteran insects (Fujii *et al*., 2001; Hiroki *et al*., 2002; Kageyama *et al*., 2002) convert genetic males into phenotypic, functional females (**Fig. 4**). The feminized insects still require fertilization by phenotypic males to produce progeny. Similar to PI, this conversion of males into females is an advantage to *Wolbachia* because infections are transmitted only through mothers (Rigaud & Juchault, 1995; Rigaud, 1999). The bacteria induce feminization of males either through action on the androgenic glands and androgen reception or through disrupting gland development or blocking the formation of the glands that would produce the hormone responsible for male differentiation (Martin *et al*., 1999). Apart from F, *Wolbachia* induce femalebiased sexratio in *Eurema hecabe* butterflies (Narita *et al*., 2007a).

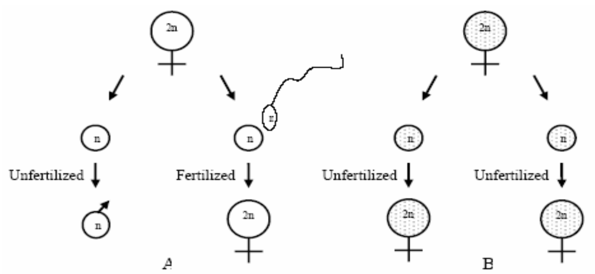

Fig. 3. (A) Arrhenotokous parthenogenesis in *Wolbachia* uninfected wasp species where an unfertilized egg develops into a haploid (n) son and a fertilized egg develops into a diploid (2n) daughter. (B) Thelytokous parthenogenesis induction (PI) in *Wolbachia* infected wasp species where all eggs develop into diploid daughters without fertilization. PI is advantageous to *Wolbachia* because the bacteria are transmitted only through females.

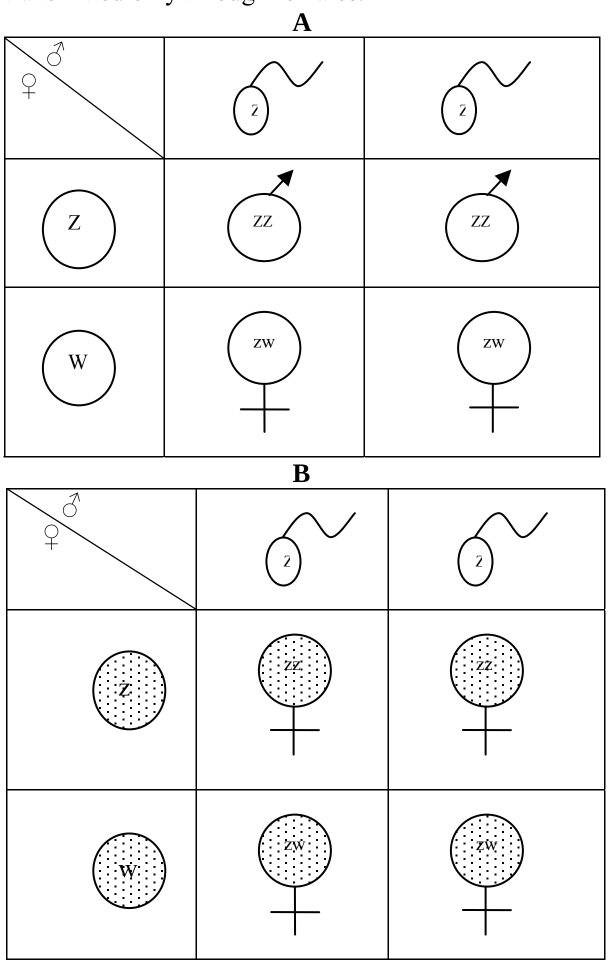

Fig. 4. Feminization in isopod crustaceans. In the checker board, male (♂) and female (♀) gametes, and the gender of the progeny are shown. (A) In the absence of *Wolbachia* both males (ZZ) and females (ZW) are produced. (B) *Wolbachia* infection suppresses the androgenic glands of the genetic males and converts them into phenotypic females, resulting in all-female progeny. Similar to PI as shown in Fig. 3, this conversion of males into females is an advantage to the bacteria since infections are transmitted only through females. In some infected populations, however, female determining W chromosome has been lost over time, resulting in all-male individuals.

Male killing (MK): Wolbachia increase the production of daughters at the expense of sons by killing embryonic males in some insects (**Fig. 5**). They are found in hosts differing in their system of sexdetermination (*i.e*. in both male and female heterogamy), suggesting that these bacteria are relatively unconstrained with respect to the range of hosts in which they can induce the MK phenotype. Apparently, these bacteria can detect host sex and act to kill males, or interfere directly with sexdetermination to produce malespecific lethality. They have been reported in ladybird beetles (Hurst *et al.,* 1999a,b), *Drosophila* (Hurst *et al.,* 2000, 2001), flour beetle (Fialho & Stevens, 2000), butterflies (Jiggins *et al.,* 2000a,b, 2001b,c) and leafcutter ants (Van Borm *et al*., 2001, 2003).

Factors affecting expression of *Wolbachia* phenotypes

Both host and bacterial factors and the interactions between these two appear to determine the type and efficiency of the reproductive manipulation caused by *Wolbachia* (Charlat *et al*., 2003a). Host diapause (Perrot-Minnot *et al*., 1996), density, food quality and antibiotics, and rearing temperature (Hoffmann *et al*., 1990; Clancy & Hoffmann, 1998), mating frequency (Karr *et al.,* 1998), age (Hoffmann *et al*., 1990), genotype, mating history and larval environment, and bacterial strains and load (Poinsot *et al.,* 2000; Clark *et al.,* 2002) all affect the strength of CI. Moreover, maternal transmission rates (Turelli & Hoffmann, 1995), heat shock (Feder *et al*., 1999), and host nuclear background (Olsen *et al*., 2001) influence *Wolbachia* expression and their dynamics. However, it is to be borne in mind that some unicellular eukaryotes and bacteria other than *Wolbachia* are reported to cause PI (Weeks *et al*., 2001; Zchori-Fein *et al*., 2001), F (Rigaud, 1999; Bandi *et al*., 2001; Weeks *et al*., 2002) and MK (Lawson *et al*., 2001; Von der Schulenburg *et al*., 2001), even though CI so far appears to be the most widespread and only *Wolbachia*-specific phenomenon.

Distribution of *Wolbachia* in host tissues

Wolbachia are present in mature eggs, but not in mature sperm. Though predominantly limited to the reproductive tissues in most hosts, somatic infections by the bacteria is a common event for many insects, and distribution of the bacteria depends on the particular *Wolbachia*-host association (Dobson *et al.,* 1999; Cheng *et al.,* 2000). Cytoplasm of cells in the reproductive organs, Malpighian tubules, muscle tissues next to the body cavity, nervous tissue, haemocytes, nurse cells of the ovaries and microtubules in the eggs are the common sites for the bacteria (Clark *et al*., 2002). The density of the bacteria per host varies substantially (reviewed in Stouthamer *et al.,* 1999): an infected female *Armadillidium vulgare* for example, may harbour between 66,000 and 164,000 bacteria, there are *ca*. 250-670 bacteria per *Trichogramma* egg, a single egg of *D. simulans* contains as many as 500,000 bacteria, while a male *D. simulans* harbours up to 36.5×10^6 bacteria.

Mechanisms of *Walbachia* action

Owing to an amazing diversity in the virulence of the *Walbachia*-host interactions, the exact mechanisms of the bacterial action are still unclear. The bacteria are abundant in the testes of infected males, but they are not physically associated with mature sperm (Binnington & Hoffmann, 1989; Bressac & Rousset, 1993). The bacteria are shed with the cytoplasm into 'waste bags' during individualization in spermatogenesis, indicating that *Wolbachia* do not cause CI directly, but modify developing sperm, which then transmit the CI-inducing effects to eggs. Attempting to account for *Wolbachia*-induced CI, the 'bacterial dosage' model suggests that unidirectional incompatibility results from the relative dose of bacteria in males *versus* females (Breeuwer & Werren, 1993; Solignac *et al.,* 1994). The dosage alone appears to be insufficient to explain all aspects of CI and superinfections (Hoffmann & Turelli, 1997). Werren (1997a) proposed a two-component system consisting of *Wolbachia*-induced modification (*mod*) of sperm and bacterial rescue (*resc*) in the fertilized egg, analogous to ‘poison-antidote’ or the restriction-modification defense system in bacteria. *Wolbachia* can only rescue sperm chromosomes that have been modified by the same bacterial strain (*i.e.* $mod^{+}resc^{+}$) which can induce CI by modifying sperm chromosome but can rescue these when in the egg; whereas mod^{-} $resc^{-}$ strain cannot induce CI because it can neither modify sperm nor rescue egg. Incompatibility occurs when a modified sperm cannot be rescued in the fertilized egg so that crosses involving *Wolbachia*-infected males with modified sperm and uninfected females are incompatible (**Fig. 6**). Crosses between different strains of *Wolbachia* are also incompatible because these strains have different ‘*mod-resc*’ systems. Almost parallel to these mechanisms, Curtis & Sinkins (1998) proposed a sperm ‘imprint’ and egg ‘rescue’ model to explain *Wolbachia*-induced CI. Charlat *et al*. (2001) further elaborated the *mod-resc* model and evolution of *Wolbachia* compatibility types. Three models describing molecular mechanisms involved in CI are: (1) lock-and-key, (2) titration-restitution, and (3) slow-motion, of which the first one appears to be the most parsimonious and fits the available observations best (Poinsot *et al*., 2003). Host chromatin-binding proteins and microtubules associated with the early divisions in eggs are implicated to explain some of the phenotypic effects of

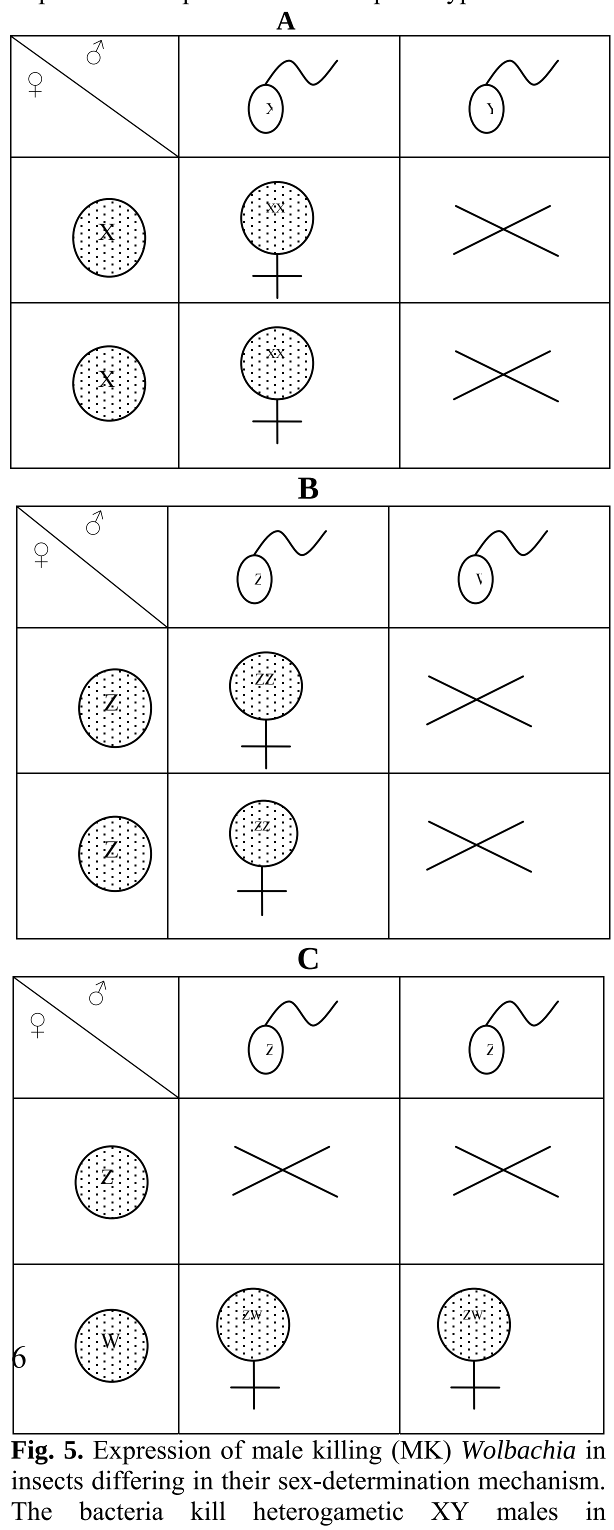

Fig. 5. Expression of male killing (MK) *Wolbachia* in insects differing in their sex-determination mechanism. The bacteria kill heterogametic XY males in *Drosophila* (A), and ZW males in coccinelids (B), or homogametic ZZ males in butterflies (C). MK is not

deleterious to the bacteria because they are transmitted only by females.

Wolbachia on their hosts. In addition, maternally derived chromatin packaging proteins *ms(3)K81* (Yasuda *et al*., 1995), and *Wolbachia*-induced sperm modification due to impairment of male pronuclei and/or interference with post-fertilization chromosome remodeling steps (Presgraves, 2000), have been suggested as probable mechanisms involved in CI. PI *Wolbachia* in parasitic wasps act through doubling the chromosome constitution of unfertilized eggs (Stouthamer & Kazmer, 1994) whereas F *Wolbachia* in isopods prevent formation of the androgenic glands that induce male differentiation (LeGrand *et al*., 1987; Martin *et al*., 1999). But very little is known about the mechanisms of MK *Wolbachia* which can detect the sex of the embryo and specifically kill only males. Because the models currently available do not explain exact mechanisms of the bacterial action, a more realistic model(s) needs to be described to elucidate *Wolbachia* phenotypes in a wide range of hosts. The distribution, behaviour and fate of *Wolbachia* in host tissues appear to be crucial to a full understanding of the mechanisms of the bacterial action.

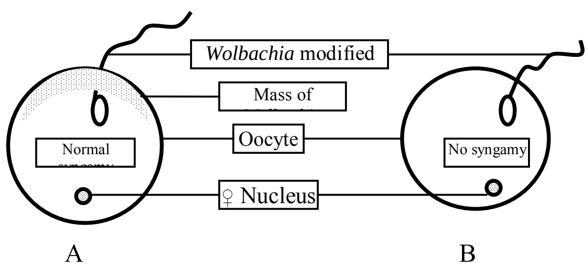

Fig. 6. A simplified '*mod-resc*' model explaining the mechanism of *Wolbachia*-induced CI. (A) A compatible cross between an infected male and an infected female. Under the micropyle of an infected oocyte, a mass of *Wolbachia* is present. Normal syngamy proceeds when *Wolbachia*-modified spermatozoon enters the oocyte and fusion of gametic nuclei takes place, indicating that maternal *Wolbachia* rescues fertility. (B) When the mass of *Wolbachia* is absent and *Wolbachia*-modified spermatozoon enters an uninfected oocyte, syngamy is not achieved, demonstrating that paternal *Wolbachia* induces CI.

Horizontal or intertaxon transmission of *Wolbachia*

Studies suggest that *Wolbachia* are likely to undergo frequent horizontal transmission (Breeuwer *et al*., 1992; O'Neill *et al*., 1992). Phylogenetic and laboratory data further imply that the bacteria are capable of moving horizontally between species (Braig *et al.,* 1994; Werren *et al.,* 1995a) and between different orders of insects and between insects and crustaceans (Werren, 1997b). Though the precise means by which horizontal transmission of the bacteria is achieved in nature are not known, the biology of *Wolbachia*-host association suggests that parasitic insects and their hosts are probably one of the routes. Uninfected *A. vulgare* might acquire an infection of *Wolbachia* through blood-to-blood contact with their host (Rigaud & Juchault, 1995). Parasitic wasps *Nasonia* and their fly host *Protocalliphora avium*, for example, all are infected with similar *Wolbachia* strain, whereas a drosophilid larval parasitoid *Asobara tabida* shares identical strain of *Wolbachia* with its host *D. melanogaster* (Werren *et al*., 1995a). Moreover, *Wolbachia* strains from a parasitic mite and its host, *Trichogramma* and its moth host *Ephestia*, and *Nasonia* and its flesh fly host *Sarcophaga bullata* are similar, although evidence of a recent horizontal transmission of the bacteria among the parasitic wasps and their hosts is not established (Schilthuizen & Stouthamer, 1997). A possible horizontal transmission of the bacteria between frugivorous *Drosophila* and their hymenopteran parasitoids (Vavre *et al*., 1999a), between species of the ten-spot ladybird beetle (Von der Schulenburg *et al.*, 2001), between species of the leafcutter ants (Van Borm *et al.*, 2003) and between terrestrial heteropteran bugs (Kikuchi & Fukatsu, 2003) has been suggested. Recent data suggest that *Wolbachia* have transmitted large segments of its genome into at least seven multicellular eukaryotic species (Hotopp *et al*., 2007).

Artificial transfer of the bacteria by microinjection from an infected host to an uninfected novel host has been achieved. Examples include intraspecific transfer of CI *Wolbachia* between *T. confusum* (Chang & Wade, 1994), and interspecific transfer of the bacteria from *Ae. albopictus* to *D. simulans* (Braig *et al*., 1994), from *D. simulans* to *D. serrata* (Clancy & Hoffmann, 1997), and from *D. melanogaster* to *D. simulans* (Poinsot *et al*., 1998). Transfer of F *Wolbachia* between species of isopods (Juchault *et al*., 1994) and inter-class transfer of PI *Wolbachia* from *Muscidifurax* to *D. simulans* (Van Meer & Stouthamer, 1999) have been fruitful. Moreover, transfer of naturally infecting *Wolbachia* from *D. simulans* into *Laodelphax striatellus* and maintenance of the infections for 12 generations is perhaps the first report to establish a horizontal transfer of the bacteria between phylogenetically distant insects (Kang *et al*., 2003).

Recently, Weiss *et al*. (2006) have assessed infection establishment and host fitness after interspecific transfer of *Wolbachia* between tsetse fly species.

***Wolbachia* infection dynamics**

The overall frequencies of *Wolbachia* strains and their transmission in host populations either in a laboratory or in natural habitat are referred to as infection dynamics of the bacteria. The consequence of an introduction of *Wolbachia* infected individuals into uninfected populations would be a rapid increase in frequency and spread of infected individuals in the mixed population because infected mothers (who gain a reproductive advantage relative to uninfected females) would produce infected progeny but the bacteria do not appear in progeny from uninfected mothers (**Fig. 7**). This spreading of the bacterial infections is referred to as 'cytoplasmic drive' and has been documented in both laboratory and field populations of *D. simulans* (Turelli & Hoffmann, 1991, 1995; Turelli, 1994) and in *L. striatellus* (Hoshizaki, 1997). In the presence of two or more different *Wolbachia* infections, the most common infection will spread to fixation. This is because females infected with the common infection type are more likely to mate with males infected with the same *Wolbachia* strain, producing fertile offspring from compatible crosses, while rest of the infections types will be maintained at equilibrium frequencies (Weeks *et al*., 2002).

All reproductive alterations mediated by *Wolbachia* have the same goal in common. They favour the spread of the infection in host populations either by providing more infected hosts of the female sex that would transmit the infection vertically as found in PI and F *Wolbachia* phenotypes, or by eliminating/decreasing the fitness of the non-transmitting, uninfected individuals as evident in CI and MK phenotypes. While PI and F *Wolbachia* have selective advantages in host populations due to increased production of the vertically transmitted sex *i.e.* females, the infection dynamics of MK *Wolbachia* is clearly parasitic where malekilling increases the number of daughters produced by infected females who, in turn, produce all-female progeny. Factors that affect infection dynamics of the bacteria in host populations include environmental curing (Stevens, 1989; Stevens & Wicklow, 1992), maternal transmission rates (Turelli & Hoffmann, 1995) and mutations (Hurst & McVean, 1996). Differential extinction and speciation rates of infected and uninfected host species are other factors influencing the dynamics of the bacteria in host populations (Werren & Windsor, 2000). Geographical distribution and diversity of *Wolbachia* in *D. melanogaster* and *E. hecabe* populations have been studied recently (Corby-Harris *et al*., 2007; Narita *et al*., 2007b).

Evolutionary consequences of *Wolbachia* infections

Wolbachia is capable of sterilizing uninfected females, turning infected individuals into females, killing males, and behaving as a perfect mutualistic symbiont. These apparent interactions between the bacteria and their hosts would give rise to selection pressure favouring host gene mutations that would prevent the actions of the bacteria in a number of ways (Charlat *et al*., 2003a). *Wolbachia* strategies influence sexdetermination in ladybirds (Hurst *et al*., 1999b), sex differentiation and gametogenesis in the parasitic wasps (Dedeine *et al*., 2001) or *Drosophila* (Starr & Cline, 2002), cell-cycle mechanisms through gamete duplication in parasitic wasps (Stouthamer, 1997), loss or improper segregation of paternal chromosomes in *Drosophila* (Callaini *et al*., 1997) and delay in nuclear envelope breakdown in *Nasonia* (Tram & Sullivan, 2002). The bacterial infection may be deleterious for males because it reduces fertility in crosses with uninfected females and lowers male fitness and spermatogenesis efficiency (Snook *et al*., 2000), or beneficial for males by increasing their mating rates (Crespigny *et al*., 2006).

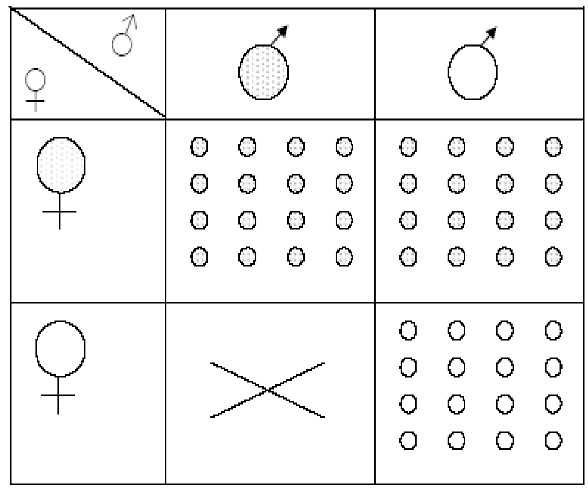

Fig. 7. The consequence of an introduction of *Wolbachia* infected individuals into uninfected populations, illustrating how a rapid increase in the frequency of *Wolbachia* infected individuals could take place. Infected females (shaded) can mate and produce progeny successfully with both infected (shaded) and uninfected (unshaded) males, and all offspring of infected females are themselves infected (shaded). Uninfected females (unshaded), on the other hand, fail to produce any offspring (marked ×) when mated with infected males, and can produce uninfected progeny only when mated with uninfected males (unshaded).

CI *Wolbachia* are likely to increase extinction risks directly by decreasing population productivity during the process of invasion of an uninfected population in which numerous incompatible crosses lead to inviable progeny (Turelli & Hoffmann, 1995). Because PI *Wolbachia* induce females to reproduce without males, males are very rare or, indeed, absent from some parasitic wasp populations. These males fail either to fertilize females or to mate successfully, a tendency to degenerate or lose male sex due to bacterial infection (Charlat *et al*., 2003a). The spread of the F *Wolbachia* in certain populations of isopod, for example, has caused the loss of the female-determining W chromosome from infected populations in which all individuals are males (ZZ). Here selection on the host to promote the production of male progeny apparently favoured host genes that prevent either action or transmission of such feminizing bacteria (Rigaud, 1999). MK *Wolbachia* in ladybirds, as Stevens *et al*. (2001) assert, has enhanced survivorship or fecundity effects on the surviving infected females due to (i) sibling cannibalism (as surviving females feed on dead eggs), (ii) absence of competition between sibs for food and space (because the death of males reduces competition), and (iii) lack of disadvantageous inbreeding (as consanguineous mating is avoided by the death of brothers). Moreover, it seems that MK *Wolbachia* could potentially perturb host reproductive behaviour from the rule of male-male competition and female choosiness to female-female competition and male choice as seen in some female lekking swarms of the butterfly *A. encedon* (Jiggins *et al*., 2000b; Randerson *et al*., 2000). In the long run, the F and MK *Wolbachia* also could increase extinction risk by reducing genetic diversity in all-female or female-biased populations (Charlat *et al*., 2003a). Finally, turning to filarial nematode case in which *Wolbachia* are necessary for host embryogenesis and other developmental stages, the bacteria tend help to evade oxidative damage caused by the mammalian host's immune system in response to nematode infection. In this regard, Henkle-Duhrsen *et al*. (1998) found evidence that *Wolbachia* produce a catalytic enzyme that is functional in the detoxification of hydrogen peroxide.

Biocontrol implications of *Wolbachia*

Since *Wolbachia* infect a significant number of insect pests of human diseases, crops, and livestock, there are growing interests in using these bacteria in biocontrol programmes in which the bacteria could be used as vectors for spreading desirable genetic modifications in pest populations or as microbial agents to enhance productivity of natural predators and parasites. Control strategies involving CI *Wolbachia* have the potential to be a powerful addition to the traditional sterile insect techniques (SIT) of pest suppression by repeated sweeps with infected insects (Laven, 1967a), or pest replacement through cytoplasmic drive for a number of pest insects (Sinkins *et al.,* 1997; Dobson *et al*., 2002b). Rapid advances in DNA based technologies have expanded the range of possibilities for the utilization of *Wolbachia* for such long-term goals as creation and release of paratransgenic and/or transgenic insects (Turelli & Hoffmann, 1999; Durvasula *et al*., 1999; Sinkins & O'Neill, 2000). In addition, the presence of *Wolbachia* infections in the somatic tissues of insects opens up the possibility of expressing anti-parasitic gene products directly into these bacteria, which could then invade vector populations in the field by virtue of the CI phenomenon they confer (Sinkins *et al.,* 1997; Dobson *et al.,* 1999; Cheng *et al.,* 2000). This also gave hope for transferring many of the desirable traits that could interfere with arthropod-borne diseases that require *Wolbachia* expression in tissues like gut or haemolymph (Riehle *et al*., 2003), resulting in an increased use of *Wolbachia* in biocontrol research (Floate *et al*., 2006; Tagami *et al*., 2006; Tsillassie & Legesse, 2007).

Traditional SIT used to suppress pest insects like mosquitoes, screwworm flies, med flies and tsetse flies is logistically difficult except in small isolated populations (Knipling, 1998; Benedict & Robinson, 2003). An alternative strategy is aimed at establishing *Wolbachia* infections that will suppress the target insect populations by reducing their reproductive potential as has been reported in a hymenopteran parasitoid *C. sesamiae* (Mochiah *et al*., 2002). Unidirectional CI can be utilized, perhaps integrated with existing SIT programme, for the suppression of certain agricultural insect pests in developed countries where they have infrastructures for supporting such strategies (Werren, 1997a). One problem associated with population suppression, however, is the risk of accidentally releasing *Wolbachia*-infected females, which may result in the replacement of the uninfected target population due to an inherent reproductive advantage of *Wolbachia*-infected females over uninfected females. The intentional release of infected females, however, is the foundation of *Wolbachia*-mediated population replacement strategy. Classic example involves an anopheline mosquito carrying a trait making it refractory to a malaria infection. Natural population replacement events have been demonstrated in California populations of *D. simulans* where southern cytotype was shown to be migrating northward replacing northern cytotype often times in excess of 100 km per year (Turelli & Hoffmann, 1991). This ability of *Wolbachia* infections to spread through a population could be harnessed as a mechanism to help drive a genetically altered trait through a population given that the trait 'hitchhikes' with the *Wolbachia*-infected cytoplasm, and replacement of target insect population might be particularly useful for African trypanosomiasis, tick-borne diseases of humans and livestock, and leishmanial or viral infections (Beard *et al.,* 1993). Such a trait, however, must be linked to the *Wolbachia* infection for effective disease control and preventive strategies, otherwise, the trait will become separated from the *Wolbachia* infection, leading to eventual loss of the trait (Curtis, 1994).

Wolbachia-mediated reproductive alterations

Model simulations show that release of *Wolbachia* infected hosts would not only allow the host population size to be reduced and maintained at low levels or eliminated, but it would also permit multiple generations of control resulting from a small release samples, indicating a cost effective means of such a programme (Sinkins & O'Neill, 2000; Dobson *et al*., 2002c). Applicable to both natural *Wolbachia* infections and artificial insect transgenesis (*i.e*. the genetic alteration of insects by inserting novel genes into them), bacterial infections could be used in accelerating cytoplasmic drive rates, as apparent from increased host fitness (Dobson *et al*., 2002b, 2004), promoting population replacement strategies *via* desired transgenes through natural populations (Dobson *et al.,* 2002c; Dobson, 2003). Although this promising strategy has not yet been applied to field populations, a recent study on horizontal transfer route for *Wolbachia* between phylogenetically distant insects, from *D. simulans* to *L. striatellus*, demonstrates a novel way to generate insect lines capable of driving genes into *Wolbachia* infected populations to start population replacement (Kang *et al*., 2003).

Apart from insect transgenesis, paratransgenesis in insects is the genetic alteration of microbes living in association with insect disease vectors. This approach attempts at decreasing pathogen transmission without adverse effects on the vectors themselves and employs the interactions between vectors (*e.g*. host insects), bacterial symbionts of the vectors (*e.g. Wolbachia*), and the pathogen (*e.g*. malaria parasite or dengue virus). The bacteria are isolated and genetically transformed *in vitro* to export molecules that interfere with pathogen transmission, genetically altered bacteria are then introduced into the host vector, where expression of engineered molecules affects ability of the host to transmit the pathogen (Turelli & Hoffmann, 1999; Beard *et al*., 2002). Engineering a gene refractory to a human pathogen (*Trypanosoma cruzi,* the agent of Chagas' disease) into a bacterial symbiont *Rhodococcus rhodnii* of the insect vector *Rhodnius prolixus* Stål (Hemiptera: Reduviidae) is an encouraging precedence (Durvasula *et al.,* 1997, 1999). *Wolbachia*-mediated CI could also be used to drive genetically engineered symbionts into pests like tsetse fly population for sweeping (Wilkinson, 1998). However, the absence of any proven technique for driving a refractory construct into a field population is still a major obstacle (Benedict & Robinson, 2003). So far, insect paratransgenesis is the most promising avenue of research in tsetse flies and kissing bugs (Kramer, 2004). But as Curtis (2007) very wisely pointed out, whether these transgenics might become vectors of lethal pathogens such as HIV or whether transgenes could be picked up and become active in host predators (such as spiders for mosquitoes), must be considered during desiging such control programmes.

Possible uses of PI *Wolbachia* in control programmes by enhancing productivity of parasitic wasps have been suggested (Stouthamer, 1993; Stouthamer *et al*., 1993; Stouthamer *et al*., 1999). Similar to the modification of their disease-transmitting abilities by CI *Wolbachia*, isolates from PI *Wolbachia* are also of interest in the improvement of natural predators and parasitoids. Artificial transfer of PI *Wolbachia* between *Trichogramma* species (Grenier *et al*., 1998) and from *Trichogramma* or *Muscidifurax* into other hymenopteran species aimed at producing all-female progenies in the latter could be utilized against a number of lepidopteran pests (Takagi, 2000; Knight, 2001).

Wolbachia represent a very useful target for the control of filarial diseases. Use of simple antibiotics that kill *Wolbachia* is found effective for eliminating microfilaria production and killing the adult worms (Hoerauf *et al.,* 2000; Taylor *et al*., 2000), and the bacteria appear to be an excellent target for chemotherapy against elephantiasis and onchocerciasis (Taylor & Hoerauf, 2001; Blanke, 2002). Studies demonstrate that *Wolbachia* provoke the immune response of *Onchocerca*, resulting in an intense skin disease, visual impairment and eventual blindness, and treating the patients with antibiotics like ivermectin and doxycycline help control the dreadful river blindness (Andre *et al.,* 2002; Frankish, 2002; Viney, 2002). Elimination of the bacteria from filarial nematodes generally results in either death or sterility (Hoerauf *et al*., 2003). Current strategies for control of filarial nematode diseases include: elimination of *Wolbachia via* the simple doxycycline antibiotic rather than far more toxic antinematode medication (Foster *et al*., 2005; Taylor *et al*., 2005).

Concluding remarks

Wolbachia are fascinating and amazing bacteria not only because they induce an impressing range of effects on their hosts, but also because they appear to have 'framed' the biology of their hosts in a number of unique ways. The bacteria are perhaps one of the world's most common parasitic microbes and are potentially the most successful reproductive manipulator in the biosphere (Werren, 1998). The complete genome sequencing of *Wolbachia* strains provides a new impetus to understand the mechanistic basis of the bacteria/host interactions, and the current flurry of activities generated by research groups around the world on the impacts of *Wolbachia* on pest species will yield further insights into these bacteria. The outcome of the *Wolbachia* Genome Project is expected to alleviate human sufferings (Slatko *et al*., 1999; Ware *et al*., 2002; Tsillassie & Legesse, 2007), help understand the mechanisms that *Wolbachia* use to influence host reproduction and the diversity of ways the bacteria affect natural populations (Iturbe-

Ormaetxe & O'Neill, 2006; Narita *et al*., 2007b). Being over half a century-old riddle, unravelling *Wolbachia* mechanisms of action need further efforts because it

Table 1 Predominant *Wolbachia* phenotypes in different arthropod taxa.

Wolbachia phenotypes*	Arthropod taxa (relevant references)
Cytoplasmic incompatibility (CI)	Diptera (Laven, 1956, 1967a; Wright & Barr, 1981; Hoffman *et al*., 1986; Hoffman, 1988; Cheng *et al*., 2000; Islam & Dobson, 2006; Tagami *et al*., 2006; Kassem & Osman, 2007)
	Lepidoptera (Kellen *et al.*, 1981; Sasaki & Ishikawa, 1999)
	Homoptera (Noda, 1984; Hoshizaki & Shimada, 1995; Noda *et al*., 2001)
	Coleoptera (Hsiao & Hsiao, 1985; Wade & Stevens, 1985; O'Neill, 1989; Fialho & Stevens, 1996; Giordano *et al*., 1997; Islam et al., 1997; Heddi *et al*., 1999; Clark *et al*., 2001; Perez & Hoy, 2002; Sokolova *et al*., 2002)
	Hymenoptera (Ryan *et al*., 1985; Breeuwer & Werren, 1993; Reed & Werren, 1995; Bordenstein *et al*., 2001; Van Borm *et al*. 2001; Perlman *et al*., 2006)
	Isopoda (LeGrand *et al*., 1987; Rousset *et al*., 1992)
	Acari (Breeuwer & Jacobs, 1996; Breeuwer, 1997; Johanowicz & Hoy, 1998; Vala *et al*., 2000; Gotoh *et al*., 2007)
	Heteroptera (Giordano *et al*., 1997; Kamoda *et al*., 2000; Kikuchi & Fukatsu, 2003)
	Arachnida (Oh *et al*., 2000)
Parthenogenesis induction (PI)	Hymenoptera (Stouthamer, 1993; Stouthamer *et al*., 1993, 1999; Werren *et al*., 1995a; Stouthamer, 1997; Dedeine *et al*., 2001; Stahlhut *et al*., 2006)
	Collembola (Vandekerckhove *et al*., 1999)
	Acari (Weeks & Breeuwer, 2001; Enigl & Schausberger, 2007; Xie *et al*., 2007)
	Thysanoptera (Arakaki *et al*., 2001)
Feminization (F)	Isopoda (Juchault *et al*, 1994; Rigaud & Juchault, 1995; Rigaud, 1999; Bouchon *et al*., 1998; Rigaud *et al*., 2001; Verne *et al*., 2007)
	Lepidoptera (Fujii *et al*., 2001; Hiroki *et al*., 2002; Kageyama *et al*., 2002; McGraw & O'Neill, 2007; Narita *et al*., 2007a)
	Hemiptera (Negri *et al*., 2006; Curley *et al*., 2007)
Male killing (MK)	Coleoptera (Hurst *et al*., 1999a,b; Fialho & Stevens, 2000; Von der Schulenburg *et al*., 2001; Nardon, 2006)
	Diptera (Hurst *et al*., 2000, 2001)
	Lepidoptera (Jiggins *et al*., 2000a,b; 2001b,c; Li *et al*., 2007)
	Hymenoptera (Van Borm *et al*., 2001, 2003)

*See text for description

Table 2 *Wolbachia*-induced effects on different invertebrate hosts.

Phenomenal effects	Examples	References
No effect	Non-CI inducing effect in *D. simulans*	Charlat *et al.* , 2003b
	No reproductive or fitness benefit in *Drosophila* spp.	Giordano *et al*., 1995; Hoffmann *et al*., 1996
Host fitness		
(a) Positive effects	Increase in progeny production in *Trichogramma* sp.	Girin & Bouletreau, 1995
	Increase in male fertility in *T. confusum*	Wade & Chang, 1995
	Protection of *Hypera* sp. from its parasitoid	Hsiao, 1996
	Fecundity enhancement in *Trichogramma* sp.	Vavre *et al*., 1999b
	Ogenesis and fecundity enhancement in *Asobara* sp.	Dedeine *et al*., 2001
	Restoration of fertility in *D. melanogaster*	Star & Cline, 2002
	Increase in longevity, fecundity and hatch rate in *Aedes* sp.	Dobson *et al*., 2004
	Insecticide resistance in *C. pipiens*	Berticat *et al*., 2002
	Increase in fitness in sand flies	Kassem *et al*., 2003
	Beneficial for metabolism and fertility in nematodes	Bandi *et al*., 1998; Hoerauf *et al*., 2000; Langworthy *et al*., 2000
	Increased male mating rate	Crespigny *et al*., 2006
(b) Negative effects	Reduction in longevity in *D. melanogaster*	Min & Benzer, 1997
	Reduction in egg-laying and hatch rate in *Nasonia* and *Trichogramma* spp.	Bordenstein & Werren, 2000; Huigens *et al*., 2000
	Reduction in reproductive fitness in transfected *Drosophila*	McGraw *et al*., 2002
Hybrid sterility/ breakdown	Production of sterile or no hybrids in *T. urticae*	Vala *et al*., 2000
Speciation	Acceleration of speciation events in the following:	
	C. pipiens	Rozeboom & Kitzmiller, 1958; Laven, 1967b
	Gryllus spp.	Giordano *et al*., 1997
	D. simulans	Shoemaker *et al*., 1999; Rokas, 2000
	Nasonia spp.	Breeuwer & Werren, 1995 Werren, 1998; Hurst & Schilthuizen, 1998 Bordenstein *et al*., 2001 Wade, 2001
	Aedes spp. complex	Dean & Dobson, 2004
Host mtDNA	Evolutionary divergence in *D. simulans*	Hale & Hoffmann, 1990; Ballard, 2000; James & Ballard, 2000
	Variability in woodlice *Porcellionoides* spp.	Marcade *et al*., 1999

would help better understand the bacteria-mediated control of public health and agricultural pests. The other much-needed tasks for the coming days would be to bring together advances made in transgenic and paratransgenic pest and vector technologies from laboratory bench to field practice.

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