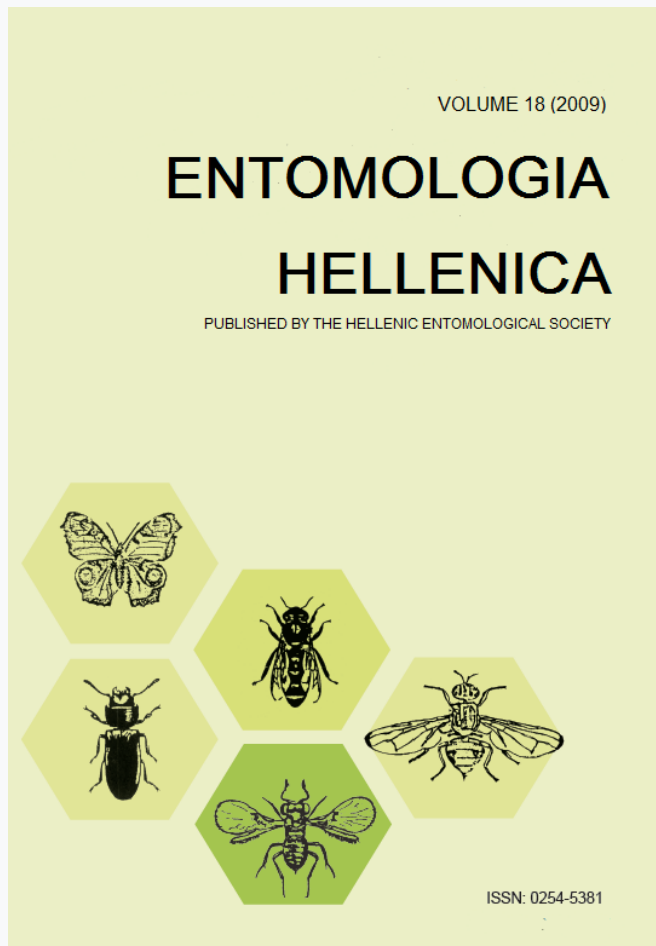


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## ***Wolbachia*-induced reproductive parasitism and applications**

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### **ABSTRACT**

Insects have been reported to be associated with a broad variety of microorganisms, affecting the host biology in many different ways. Among them, *Wolbachia*, an obligatory intracellular and maternally-inherited symbiont, has recently attracted a lot of attention. Beside insects, *Wolbachia* are found in association with a wide variety of other invertebrate species, including mites, scorpions, spiders, crustaceans, filarial nematodes. Several surveys have indicated that *Wolbachia* may be symbiont of up to 70% of all insect species, rendering *Wolbachia* the most ubiquitous intracellular symbiotic organism on Earth. *Wolbachia*-host interactions range from many forms of reproductive parasitism to mutualistic symbioses. Different *Wolbachia* strains have been found to induce a number of reproductive alterations such as feminization, parthenogenesis, male-killing or cytoplasmic incompatibility. Despite their common occurrence and major effects on host biology, speciation and ecological diversity, little is known on the molecular mechanisms that mediate *Wolbachia*-host interactions. Recent studies focus on the potential of *Wolbachia*-based methods for the biological control of insect pests and disease vectors of agricultural, environmental and medical importance.

**KEYWORDS:** insect symbiosis, *Wolbachia*, parthenogenesis, feminization, male-killing, cytoplasmic incompatibility, incompatible insect technique.

### **Introduction on Insect Symbiosis**

Several types of insect-microbe associations are present in nature, many of which are accountable to a more or lesser extent for the evolutionary success of insects. Microbes are ubiquitous both inside and outside the insect bodies, inside or outside the insect cells and interact with their host in a broad variety of relationships that range from mutualism, which is beneficial to the host, to parasitism, where the symbiont has a negative impact on host's biology (Ishikawa 2003). The most intimate association is intracellular symbiosis, with

the symbiont being vertically transmitted among generations. Intracellular symbionts of insects are divided into two groups. The first one, the primary symbionts, covers symbiotic microbes that usually supply hosts with nutrients and are harboured by the host bacteriocyte, a special cell differentiated for this purpose (Buchner 1965, Ishikawa 1989). Secondary symbionts, also known as "guest microbes", are not restricted to a particular cell type, but are present in many cell types of the host insect. Unlike primary symbionts, guest microbes can colonize naive hosts through horizontal transmission among host individuals and species. These associations

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are typically facultative from the perspective of the host and can be deleterious to the host (parasitism), beneficial only to the symbiont (commensalism) or beneficial to both parties (mutualism). During the last three decades, a novel type of symbiosis has been described for bacteria like *Wolbachia*, *Cardinium*, *Rickettsia*, *Spiroplasma* and *Arsenophonus*, that manipulate the host reproduction system to their advantage (reproductive parasitism). *Wolbachia*, the best-studied symbiont of this group, will be the focus of this review.

### Introduction on *Wolbachia*

Collaborative studies between Marshall Hertig, an entomologist, and Samuel Wolbach, a pathologist, on the presence and identification of microorganisms in arthropods, resulted in the discovery of *Wolbachia* in the gonads of the *Culex pipientis* mosquito in 1924 (Hertig and Wolbach 1924); however, the complete description of this symbiotic association was published in 1936 (Hertig 1936). For decades, *Wolbachia* was known only from mosquitoes; the development of PCR-based screening methods clearly indicated that *Wolbachia* is widespread in nature (O'Neill et al. 1992). It has been demonstrated that *Wolbachia* infects up to 70% of insect species, a large number of other arthropods (including spiders, scorpions, mites, springtails, terrestrial isopods) as well as filarial nematodes (Werren et al. 1995, Bandi et al. 1998, Jayaprakash and Hoy 2000, Werren and Windsor 2000, Hilgenboecker et al. 2008, Werren et al. 2008). These studies place *Wolbachia* among the most common intracellular bacteria known, with estimates of several million infected species (Werren et al. 1995, Jayaprakash and Hoy 2000, Werren and

Windsor 2000, Hilgenboecker et al. 2008, Werren et al. 2008).

Molecular phylogenetic analysis based on the 16S rRNA gene indicated that *Wolbachia* belongs to  $\alpha$ -Proteobacteria, being evolutionary related to other intracellular bacterial species of the genera *Anaplasma*, *Ehrlichia* and *Rickettsia* (Breeuwer et al. 1992, O'Neill et al. 1992, Rousset et al. 1992). A significant amount of *Wolbachia* genomic information is available since the genome of four *Wolbachia* strains (*wMel*, *wRi*, *wPip* and *wBm*) has been completed (Wu et al. 2004, Foster et al. 2005, Klasson et al. 2008, Klasson et al. 2009). The available genomic information allowed the development of two multi locus sequencing typing (MLST) systems which can be used for the genotyping of any given *Wolbachia* strain (Baldo et al. 2006, Paraskevopoulos et al. 2006); they have also facilitated the classification of the *Wolbachia* strains into 10 major phylogenetic clades which have been named 'supergroups' (Werren et al. 1995, Bandi et al. 1998, Zhou et al. 1998, Lo et al. 2002, 2007, Rowley et al. 2004, Bordenstein and Rosengaus 2005, Ros et al. 2008, Bordenstein et al. 2009).

Several studies have shown that *Wolbachia* is mainly localized in the reproductive tissues of arthropods and is responsible for the induction of a number of reproductive alterations including feminization, thelytokous parthenogenesis, male-killing and cytoplasmic incompatibility (CI) (Werren 1997, Bourtzis and O'Neill 1998, Bourtzis and Braig 1999, Stouthamer et al. 1999, Werren et al. 2008). The widespread distribution of *Wolbachia* as well as the manipulation of host's reproductive system places this symbiont among the most promising targets for disease/ pest control. The aim of this review is to present the *Wolbachia*-induced reproductive manipulations with an

emphasis on how this symbiont could be used for the control of insect pests and disease vectors.

### ***Wolbachia*-induced Phenotypes**

*Wolbachia* is maternally inherited and has evolved a number of strategies to ensure transmission by manipulating the host reproductive system (Werren et al. 2008). These strategies include: a) feminization, the conversion of genetic males into females, b) parthenogenesis, the production of diploid offspring in the absence of sexual reproduction, c) male killing, the killing of infected males to the benefit of infected female siblings and d) cytoplasmic incompatibility (CI), the inability of infected males to successfully fertilize eggs from either uninfected females or from females infected with different *Wolbachia* types. Each of these phenotypes increases the frequency of infected females in the host population and therefore they represent bacterial adaptations to increase transmission of the microorganisms. Such parasite effects on hosts are commonly referred to as “reproductive parasitism” (Bandi et al. 2001).

Feminization is the most obviously beneficial strategy for a maternally inherited bacterium such as *Wolbachia*. The conversion of genetic male offspring into females doubles the potential *Wolbachia* transmission to the following generation. However, the *Wolbachia*-induced feminization is the most infrequently described of the *Wolbachia*-induced phenotypes, reported most commonly in several species of terrestrial isopods (Bouchon et al. 1998, Rigaud et al. 1999a, Michel-Salzat et al. 2001). In these isopod hosts, *Wolbachia* within genetic males inhibits the development of the androgenic gland and the production of the androgenic hormone (Azzouna et al. 2004). These

“feminized” males may however suffer a fitness disadvantage compared to genetic females, with males preferring to mate with genetic females (Moreau et al. 2001). A feminizing *Wolbachia* infection with complete penetrance would eliminate phenotypic males and lead to the extinction of both the host population as well as the symbiont. Such events, although difficult to be observed, may occur in nature. On the other hand, populations that do persist take advantage of imperfect transmission of feminizing *Wolbachia* strains (Rigaud et al. 1999b) or constrain the ability of *Wolbachia* to spread by exploiting the local scarcity of males (Moreau and Rigaud, 2003). Recent studies suggest that *Wolbachia* can also induce feminization in insect species as reported for the leafhopper *Zyginidia pullula* (Hemiptera: Cicadellidae) and the butterfly *Eurema hecabe* (Lepidoptera: Pieridae) (Negri et al. 2006, Narita et al. 2007).

Parthenogenesis, the production of female offspring in the absence of sperm fertilization offers an obvious advantage to a maternally inherited microorganism. If a 100% occurrence is assumed, parthenogenesis as well as feminization doubles the potential transmission of *Wolbachia* to the offspring. Interestingly, all currently documented cases of *Wolbachia*-induced parthenogenesis are found only within haplodiploid species belonging to Thysanoptera (Arakaki et al. 2001), Acari (Weeks and Breeuwer 2001) and Hymenoptera (Stouthamer et al. 1993, Zchori-Fein et al. 1995). Haplodiploidy describes the development of (diploid) females from fertilized eggs, while (haploid) males develop from unfertilized eggs. In this particular sex determination system, parthenogenesis may occur either by complete suppression of meiosis (apomixis) or by restoration of diploidy upon meiosis (automixis). *Wolbachia*-

induced parthenogenesis has been found to be apomictic in mites (Weeks and Breeuwer 2001) and automictic in wasps (Zchori-Fein et al. 1995).

The killing of genetic males by *Wolbachia* has been described in four different Arthropod orders namely Diptera (Hurst et al. 2000, Dyer and Jaenike 2004), Coleoptera (Fialho and Stevens 2000; Majerus et al. 2000), Lepidoptera (Jiggins et al. 2000) and Arachnida (Zeh et al. 2005). Male killing may be advantageous under limited conditions, where resource reallocation from dead males to female siblings increases the fitness of infected females (Hurst 1991, Hurst et al. 2003). In all cases detected, the *Wolbachia*-induced male killing meets the above criterion. Another predicted benefit would be the resulting avoidance of the inbreeding (Werren 1987).

Despite the fact that cytoplasmic incompatibility (CI) is the most commonly described reproductive abnormality induced by *Wolbachia*, the underlying mechanism still remains under investigation. CI has been described in many different arthropod orders: Diptera (Yen and Barr 1973), Coleoptera (Wade and Stevens 1985), Acari (Breeuwer and Jacobs 1996), Isopoda (Moret et al. 2001), Lepidoptera (Brower 1976), Hymenoptera (Reed and Werren 1995), Homoptera (Hoshizaki and Shimada 1995) and Orthoptera (Kamoda et al. 2000). As shown in Figure 1, CI can be unidirectional or bidirectional, depending on the number of *Wolbachia* strains involved in the phenotype (Breeuwer and Werren 1990, O'Neill and Karr 1990, Bourtzis et al. 1998, Bourtzis et al. 2003). Unidirectional CI describes the embryonic lethality observed when a *Wolbachia*-infected male mates with an uninfected female. All the other possible crosses are fully compatible, favoring the relative fitness of infected females and the spread of *Wolbachia*. Bidirectional CI

occurs between populations infected with different *Wolbachia* strains, when an infected male mates with a female lacking the same *Wolbachia* strain. The second type of incompatibility reproductively isolates two populations and may contribute to speciation (Werren 1998, Bordenstein 2003, Telschow et al. 2005).

The types of the incompatible crosses lead to the assumption that there are at least two distinct functions involved in CI, the "modification" and the "rescue" function (Werren 1997, Bourtzis et al. 1998, Merçot and Poinso 1998). When the female lacks the "rescue" function, the "modification" function of the male results in embryonic lethality. Although the exact mechanism remains unclear, the incompatible phenotype is associated with an asynchrony in the development of the male and female pronuclei probably due to impaired histone deposition in the male pronucleus (Lassy and Karr 1996, Tram and Sullivan 2002, Landmann et al. 2009).

In addition to the above mentioned reproductive abnormalities, *Wolbachia* can positively or negatively influence other aspects of host fitness. In *Aedes albopictus* (Diptera: Culicidae), fitness benefits resulting from *Wolbachia* infection affect both fecundity and longevity (Dobson et al. 2002). Both single and doubly infected females produce more eggs and live longer than uninfected females; no effect on males has been observed. It should be noted that similar observations were recently reported for *Drosophila simulans* (Diptera: Drosophilidae) (Weeks et al. 2007). Negative effects of *Wolbachia* in host longevity have been well documented due to *wMelpop* strain (Min and Benzer 1997). Flies bearing *wMelpop* suffer significant reduction in longevity, most likely due to overproliferation of the symbiont in the neuronal tissue (Min and Benzer 1997,

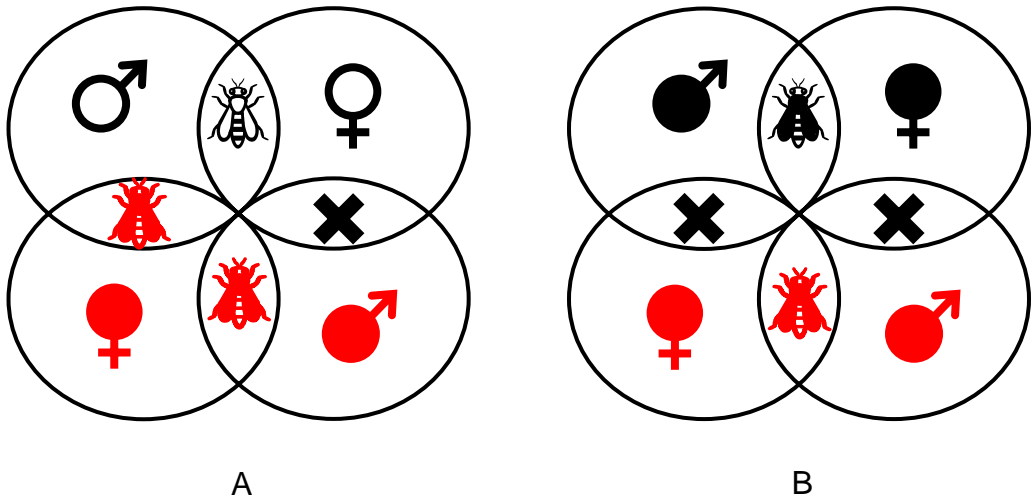


FIG. 1. Schematic representation of unidirectional (A) and bidirectional (B) cytoplasmic incompatibility. Insects bearing incompatible *Wolbachia* strains are marked with red or black.

McGraw et al. 2002, McMeniman et al. 2009).

Although *Wolbachia* successfully evades the host immune system and does not induce the normal antibacterial response (Bourtzis et al. 2000), *Wolbachia* infection has been shown to be a key player in host immunity. In at least one host-parasitoid system, the presence of *Wolbachia* decreases fitness in both the host and the parasitoid (Fytrou et al. 2006). *D. simulans* infected with *Wolbachia* is less effective in killing the eggs laid by the parasitoid *Leptopilina heterotoma* (Hymenoptera: Eucoilidae). Similarly, *Wolbachia* infection of *L. heterotoma* makes the parasitoid more vulnerable to the host defenses. The exact nature of these interactions is currently unknown. On the other hand, recent reports suggest that *Wolbachia* infections provide virus protection in insect hosts (Hedges et al. 2008, Teixeira et al. 2008).

### Practical Applications

The widespread distributions as well as the manipulation of host's reproductive system

render *Wolbachia* as key-player in pest control management (Table 1). *Wolbachia*'s potential as novel environmentally friendly bio-control agent has already attracted a lot of attention (Beard et al. 1993, Sinkins et al. 1997, Bourtzis and Braig 1999, Sinkins and O'Neill 2000, Aksoy et al. 2001). Several strategies have been proposed, most of which take advantage of the induction of cytoplasmic incompatibility (Bourtzis, 2008).

Despite the global distribution of *Wolbachia*, many important agricultural pests (e.g. *Bactrocera oleae*) and disease vectors (*Aedes aegypti*, *Anopheles gambiae*) are not naturally *Wolbachia*-infected. However, many studies have shown that *Wolbachia* can be transferred and established into a naive host resulting in the expression of the expected reproductive phenotype (Boyle et al. 1993, Braig et al. 1994, Poinsoot et al. 1998, Sasaki and Ishikawa 2000, McGraw et al. 2001, Zabalou et al. 2004a, b, Xi et al. 2005). Based on these observations, *Wolbachia* may serve as an important tool for the "Incompatible Insect Technique", the use of a symbiont-associated

TABLE 1. Synopsis of *Wolbachia*-based applications.

Application	<i>Wolbachia</i> -induced phenotype	<i>Wolbachia</i> strain / host
Incompatible Insect Technique (pest population control)	Cytoplasmic Incompatibility	<i>wCer2</i> / <i>Ceratitis capitata</i> (Zabalou et al. 2004a, 2009)
Drive strategies	Cytoplasmic Incompatibility	<i>wAlbB</i> / <i>Aedes aegypti</i> (Xi et al. 2005)
Control of vector-borne diseases	Shortening of host lifespan	<i>wMelPop</i> / <i>Aedes aegypti</i> (McMeniman et al. 2009)

reproductive incompatibility as for the control of insect pests and disease vectors (Bourtzis and Robinson 2006).

A successful example of stable transinfection of a *Wolbachia*-free agricultural pest has been reported for *Ceratitis capitata* (Diptera: Tephritidae) (Zabalou et al. 2004b). *Wolbachia* strains from the host *Rhagoletis cerasi* (Diptera: Tephritidae) have been used to stably infect the Mediterranean fruit fly through embryonic injection. Crosses between uninfected females and *Wolbachia*-infected males resulted in 100% egg mortality, while crosses between fly lines bearing different *Wolbachia* strains were 100% bidirectionally incompatible. The major advantage of the *Wolbachia*-based Incompatible Insect Technique over the Sterile Insect Technique lies on the fact that the insects do not have to be irradiated before release. However, the necessity of employing an effective sexing strain of the insect pest, so that only infected males are released, still remains (Bourtzis and Robinson 2006). Zabalou et al. (2009) described a *Wolbachia*-infected line of the VIENNA 8 genetic sexing strain of the medfly that carried the selectable marker *temperature sensitive lethal (tsl)*. Transferred *Wolbachia* induced high levels of CI even after the temperature treatment required for the male-only production.

Genetic manipulation that reduces the fitness of a pest population would provide a

useful tool to complement current control strategies. Drive systems are an important component of population replacement strategies that provide mechanisms for the autonomous spread of desired genotypes/transgenes into the targeted population (Dobson 2003). Besides autonomous transposons, primary candidates for drive strategies are bacterial symbionts used as expression vehicles (Curtis and Sinkins 1998, Turelli and Hoffmann 1999). The reproductive advantage afforded by CI to *Wolbachia*-infected females promotes the spread of the maternally inherited *Wolbachia* infection. Thus, desired genotypes / transgenes linked to a *Wolbachia* infection would be expected to spread into a targeted population following the seeding of the targeted population with proper *Wolbachia*-infected females. Xi et al. (2005) demonstrated the ability of *wAlbB* to spread into an *A. aegypti* population after seeding of an uninfected population with infected females, reaching infection fixation within seven generations in laboratory cage tests.

Age is a critical determinant of the ability of most insect vectors to transmit a range of human pathogens. This is due to the fact that most pathogens require a period of extrinsic incubation in the insect host before pathogen transmission can occur. This developmental period for the pathogen often comprises a significant proportion of the expected lifespan of the

vector. As such, only a small proportion of the population that is oldest contributes to pathogen transmission (Cook et al. 2008). Given this, strategies that target vector age would be expected to obtain the most significant reductions in the capacity of a vector population to transmit disease. The identification of insect symbionts that shorten the host lifespan would offer new tools for the control of vector-borne diseases (Sinkins and O'Neill 2000). McMeniman et al. (2009) reported the successful transfer of *w*Melpop, a life-shortening strain of *Wolbachia*, into the major mosquito vector of dengue, *Aedes aegypti* (Diptera: Culicidae). The association halved host life span under laboratory conditions and the symbiont induced complete cytoplasmic incompatibility, which should facilitate its invasion into natural field populations.

### Concluding Remarks

During the last decades, insect symbiosis gained a lot of attention as a widespread phenomenon affecting the host biology in many ways. Among the bacteria related to insects in a positive or negative aspect, *Wolbachia* is doubtless the most ubiquitous. Causing a broad range of reproductive phenotypes to the hosts, *Wolbachia* is a key player with biological, ecological and evolutionary significance. Due to its unique properties, *Wolbachia* offers the potential for development of novel and environment friendly biotechnological strategies for the control of insect pests and disease vectors.

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## ***Wolbachia* – Επαγόμενος αναπαραγωγικός παρασιτισμός και εφαρμογές**

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### **ΠΕΡΙΛΗΨΗ**

Οι συμβιωτικές σχέσεις εντόμων-μικροοργανισμών (και ιδιαίτερα βακτηρίων) είναι ένα ευρέως διαδεδομένο φαινόμενο με ποικίλες συνέπειες στη βιολογία του ξενιστή, θετικές ή αρνητικές. Ένα από τα πιο διαδεδομένα και καλά μελετημένα βακτήρια είναι το βακτήριο *Wolbachia*. Το βακτήριο *Wolbachia* είναι ένα υποχρεωτικά ενδοκυττάριο και μητρικά κληρονομούμενο βακτήριο που συμβιώνει με ένα μεγάλο εύρος ειδών ασπόνδυλων όπως ακάρεα, αράχνες, ισόποδα, νηματώδεις της φιλαρίας και κυρίως έντομα. Μελέτες έχουν δείξει ότι το βακτήριο *Wolbachia* πιθανώς συμβιώνει με το 70% όλων των ειδών των εντόμων, καθιστώντας το ως τον πιο διαδεδομένο συμβιωτικό οργανισμό του πλανήτη. Οι αλληλεπιδράσεις του βακτηρίου με τον ξενιστή κυμαίνονται μεταξύ της αμοιβαίας συμβίωσης και της επαγωγής αναπαραγωγικών ανωμαλιών όπως θηλυκοποίηση, παρθενογένεση, θανάτωση των αρσενικών ατόμων ή κυτταροπλασματική ασυμβατότητα. Παρά την ευρεία εξάπλωση και τις επιδράσεις του βακτηρίου στη βιολογία του ξενιστή, στην ειδογένεση και στην οικολογική ποικιλότητα, λίγα είναι γνωστά για τους μοριακούς μηχανισμούς της αλληλεπίδρασης του ξενιστή με το συμβιωτικό αυτό βακτήριο. Πρόσφατες μελέτες εστιάζουν στη δυνατότητα ανάπτυξης μεθόδων βιολογικού ελέγχου εντόμων γεωργικής, περιβαλλοντικής ή ιατρικής σημασίας, οι οποίες βασίζονται στις ιδιότητες του βακτηρίου *Wolbachia*.