# The Potentials Of Wolbalchia As A Biocontrol Agent

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## ABSTRACT

Wolbachia are a common and widespread group of bacteria found in reproductive tissues of arthropods. These bacteria are transmitted through the cytoplasm of eggs and have evolved various mechanisms for manipulating reproduction of their hosts, including induction of reproductive incompatibility, pathenogenesis, and feminization. Wolbachia are also transmitted horizontally between arthropod species. Significant recent advances have been made in the study of these interesting microorganisms. In this paper, Wolbachia biology is reviewed, including their biological control implications. Potential directions for future research are also discussed.

## INTRODUCTION

Insect-borne diseases impose an immense burden on global health, and insect crop pests greatly influence economic and agricultural productivity. For example, malaria alone is responsible for over a million deaths every year (Snow *et al*., 2005). With the resurgence of vector borne disease, some have been pessimistic that conventional control measures, such as using insecticides for long-term periods will be effective. Furthermore, continued use of insecticides has led to concerns of negative environmental effects. Thus, the need for novel environmentally friendly control strategies has been suggested to complement current insect control measures. *Wolbachia* are maternally inherited intracellular rickettsiae-like bacteria belonging to the α-Proteobacteria (O'Neill *et al*., 1992; Werren, 1997; Werren *et al*., 2008). The type species for the *Wolbachia* genus is *Wolbachia pipientis*, first described in the mosquito *Culex pipiens* (Hertig and Wolbach, 1924). Since then, *Wolbachia* has been found worldwide in numerous arthropod species, including: insects, mites, spiders, terrestrial isopods, as well as filarial nematodes (Werren, 1997; Zhou *et al*., 1998; Lo *et al*., 2002; Gotoh e*t al*., 2003; Cordaux *et al*., 2004; Goodacre *et al*., 2006; Hilgenboecker *et al.*, 2008; Werren *et al*., 2008). A recent meta-analysis has estimated that >65% of insect species harbor *Wolbachia*, making it one of the most ubiquitous endosymbionts on earth (Hilgenboecker *et al*., 2008). Recently, *Wolbachia* has received attention as a potential bio-control agent that may yield novel insect control strategies. In invertebrates, *Wolbachia* has been shown to manipulate cellular and reproductive processes (Hoffman and Turelli, 1997; Werren, 1997; Sinkins, 2004; Jeong and Suh, 2008; Werren *et al*., 2008). In filarial nematodes, *Wolbachia* appears to behave as a mutualist (Taylor *et al*., 2005). *Wolbachia* may provide metabolic pathways absent in filarial nematodes, which are important for the fecundity of its host (Taylor *et al*., 2005). However, in arthropods, *Wolbachia* behaves more like a reproductive parasite by inducing: feminization of genetic males, parthenogenesis, male-killing, and cytoplasmic incompatibility (CI) (Werren, 1997; Werren *et al*., 2008). These modifications typically give a reproductive advantage to infected individuals and allow for the spread of *Wolbachia* through a population (Turelli and Hoffman, 1991; Dobson *et al.*, 2002a; Dobson *et al.*, 2002b; Dobson, 2003; Xi *et al*.,2005a).

2.0     PERSPECTIVES AND OVERVIEW

Bacteria in the genus Wolbachia are cytoplasmically inherited rickettsiae that are found in reproductive tissues (ovaries and testes) of a wide range of arthropods (76, 86, 103, 126, 127). These bacteria cause a number of reproductive alterations in their hosts, including cytoplasmic incompatibility (CI) between strains (21, 77) and related species (11, 12), parthenogenesis induction (PI) (103), and feminization of genetic males (86). These modifications of host reproduction impart a selective advantage for the bacteria (113, 127).

Wolbachia are extremely widespread. Recent surveys have found these bacteria in over 16% of insect species, including each of the major insect orders (124). Wolbachia have also been found in isopods (86) and mites (54), and a close relative has recently been found in a nematode (97). The limits of Wolbachia distribution in arthropods and other phyla are yet to be determined. Wolbachia have attracted considerable recent interest for several reasons.

Firstly, given their widespread distribution and effects upon hosts, Wolbachia have implications for important evolutionary processes. Of particular interest is their potential role as a mechanism for rapid speciation (12, 23, 62, and 64).

Secondly, these intracellular bacteria are known to alter early development and mitotic processes in their hosts (33, 60, 81 and 104). As a result, Wolbachia may be used to study these basic processes. Thirdly, there is widespread interest in using Wolbachia in biological control as a microbial "natural enemy," to enhance productivity of natural enemies (PI bacteria; 102) or as a vector for spreading desirable genetic modifications in insect populations (3, 26).

A tremendous amount of progress has been made over the past five years in the study of mechanisms of action, population biology, and evolution of Wolbachia. Here I present a brief historical sketch of Wolbachia research; review, recent advances, and discusses potential directions for future research.

3.0     BRIEF HISTORICAL SKETCH

Intracellular bacteria were first reported within the reproductive tissues of the mosquito Culex pipiens by Hertig&Wolbach in 1924 (39), and these rickettsiae were subsequently namedWolbachia pipientis (38). In the 1950s, Ghelelovitch (30) and Laven (61, 62, 64) discovered that certain intraspecific crosses within Culex mosquitos were incompatible, i.e. they produced few or no progeny. Laven (62, 64) established that the incompatibility factor had a cytoplasmic inheritance pattern (i.e. inheritance through females but not through males) and named this phenomenon cytoplasmic incompatibility. A connection between these two discoveries was not formally made until the early 1970s, when Yen & Barr (131) established that CI was associated with the presence of the rickettsial agent by elimination of Wolbachia through antibiotic curing. Males from infected strains were found to be incompatible with antibiotically cured females derived from the same strain, whereas the reciprocal cross was compatible (i.e. a unidirectional incompatibility). This is now known to be the standard pattern in antibiotic curing experiments. Over the next 25 years, new examples of CI were found in a diverse range of insects, including flour beetles (75, 118), alfalfa weevils (49, 68), parasitic wasps (82, 94), plant hoppers (73, 74), flour moths (17), Aedes mosquitos (112), and fruit fly (6, 41, 44, 46, and 69). CI typically was first detected as a reduction in progeny numbers from crosses between certain strains, and cytoplasmic inheritance was shown in subsequent crosses.

In some cases, presence of bacteria in ovaries or testes was established microscopically and/or their involvement implicated by antibiotic or heat-treatment curing. However, the phylogenetic relationships among CI bacteria found in the reproductive tissues of divergent host insects were unknown until the early 1990s. Note that Wolbachia persica, originally assigned to the genus based on ultrastructural similarities, is actually a Gamma division bacterium, and therefore unrelated to true rickettsiae (120). In this paper, Wolbachia therefore refers to W. pipientis and its relatives. The closest bacteria to the Wolbachia are a group of rickettsiae that include Ehrlichia equii, Ehrlichia canis, Cowdria ruminata, and Anaplasma marginale. These are blood parasites of mammals that are vectored by arthropods (91). Ehrlichia sennetsu and Ehrlichia risticii, also disease agents of mammals, represent a more divergent group (91). Bacteria in the genus

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Rickettsia are still more distantly related. This genus includes several arthropod-vectored disease agents, including the causative agents of Rocky Mountain spotted fever, murine typhus, and scrub typhus, as well as a cytoplasmically inherited malekilling bacterium found in ladybird beetles (125). Although most of the species mentioned above are arthropod-vectored disease agents of vertebrates, to date, Wolbachia have only been found associated with arthropod reproductive tissues,and there is no evidence that they cause disease in vertebrates. However, given the abundance of arthropod species infected with Wolbachia (124), this possibility cannot be ruled out.

## 4.0    POPULATION BIOLOGY AND EVOLUTION

Why are Wolbachia selectively favored to cause CI? Both theoretical (19, 27,51, 100, 113) and empirical (114) studies show that cytoplasmically inherited Wolbachia infections can readily spread through uninfected populations due to CI. The basic reason is that infected eggs are compatible with sperm from both infected and uninfected males, but uninfected eggs are incompatible with sperm from infected males. As a result, the uninfected "cytotype" is reduced in the population in proportion to the frequency that uninfected eggs are fertilized by sperm from infected males. Dynamics of CI Wolbachia are interesting and potentially complex (80, 88, 113). Three factors of particular importance are (a) survival and fecundity of infected relative to uninfected females, (b) proportion of infected eggs produced by infected females (transmission), and (c) level of CI expression in incompatible crosses. When infected females suffer a survival/fecundity cost, there exists a threshold frequency for increase of the infection, below which the infection will decrease and above which it will increase, often to near fixation. For example, or a CI strain with 100% expression, the threshold frequency (p) is p D s, where s is the fecundity cost to infected females (19, 113). Thus, fecundity cost of an infection is crucial to its initial spread in a population. Fecundity costs range from nearly negligible to over 10%, depending on the host species (45, 115). Wade & Chang (117) report that sperm from Wolbachia-infected males has a competitive advantage relative to sperm from uninfected males. This effect could accelerate spread of the infection. Turelli & Hoffmann (115) have shown that in spatially structured populations, infections with relatively low cost can readily drift above the threshold frequency in a local population and then quickly spread throughout the larger population in a process analogous to spread of under-dominant chromosome arrangements (113). They have documented such a spread of CI Wolbachia in North American populations of D. simulans (128). One consequence of the spread of an initial Wolbachia infection within a population will be "hitchhiking" of the associated mitochondrial haplotype (89, 90, 116). This should result in a significant reduction in mitochondrial variation and association of the infection with particular mitochondrial haplotypes, as observed in D. simulans (89, 98, 116).

## 5.0    SPECIATION AND WOLBACHIA

Wolbachia may promote rapid speciation by causing reproductive incompatibility between populations (12, 22,111), especially when bidirectional incompatibility occurs. Partial to complete bidirectional incompatibility has been found between strains of D. simulans (21) and C. pipiens (64), and between sibling species of Nasonia (12). Nasonia wasps are a complex of three sibling species (N. vitripennis, N.giraulti, and N. longicornis). N. vitripennis is cosmopolitan, whereas the other two occur allopatrically in North America and are microsympatric with N. vitripennis over much of their ranges. The three species show complete to nearly complete reproductive incompatibility with each other, owing toWolbachia.

Each Nasonia species harbors double infections with distinguishable strains of A- and B-Wolbachia (11, 127). Hybrids do not normally occur in crosses between the species unless they are antibiotically cured of their associated Wolbachia strains (12, 15). Introgression crosses show that the interspecies bidirectional incompatibility is due to bacterial strain differences, not to interactions with host genotype (14). Subsequent studies of hybrids between N. vitripennis and N. giraulti reveal recessive hybrid inviability genes, indicating significant divergence between these species (15). The Nasonia complex suggests that Wolbachia could be involved in speciation. However, in this system it is not yet known whether bidirectional incompatibility

preceded the speciation event (and potentially promoted it) or followed divergence of the incipient species. A number of bidirectional incompatibility types are found in D. simulans (21); however, incompatibility is relatively weak and is apparently insufficient to prevent nuclear gene flow between 600 WERREN different compatibility types. Bidirectional reproductive isolation is found between geographic populations of the parasitic wasp Trichopria drosophilae and is associated with different strains of Wolbachia (61, 62, 64) uncovered incompatibility relationships between different geographic isolates of the mosquito C. pipiens, and subsequent workers have further studied this system (2,132). A complex pattern of unidirectional and bidirectional compatibilities occurs. However, caution must be exercised in attributing Wolbachia involvement in all cases of cross incompatibility found in C. pipiens (25, 70). In only a fewspecific cases has the inheritance pattern been shown to be cytoplasmic (109, 131), and the distribution of bacterial strains and possible host genetic effects on compatibility have yet to be determined. Unidirectional and bidirectional incompatibilities and associated microorganisms are found within and between species of Aedes mosquitos (70, 112). Reproductive incompatibility between populations of the two-spotted spider mite (7) and citrus red mite (110) may also involve Wolbachia strains. Resolving the relative roles of Wolbachia versus other factors in reproductive isolation in such complexes is a particularly promising research area. Wolbachia-induced CI need not be the only isolating mechanism between species for the bacteria to be important as a speciation mechanism. For example, unidirectional CI combined with other reproductive isolating mechanisms in the reciprocal direction, such as hybrid sterility and inviability or premating isolation, could result in bidirectional reproductive isolation. A possible example occurs between North American Gryllus species. Wolbachia-induced bidirectional incompatibility is a possible mechanism for

rapid speciation in arthropods, as suggested by the examples above. The recent finding that over 16% of insects harbor these bacteria further supports this intriguing possibility (124). However, it remains to be demonstrated how often Wolbachia are associated with reproductive incompatibility between populations within a species or between recently diverged species, a prerequisite for determining their potential importance as a speciation mechanism.

5.1     Biological Control and Public health Importance

There is considerable interest in using CI Wolbachia in biological control (1, 3, 24,). Early studies considered use of Wolbachia to eradicate host populations in a method analogous to sterile-male release (63); however, this approach is logistically difficult except in small isolated populations. An alternative is to establish CI infections that will reduce the reproductive potential of insect populations. An obvious difficulty is that, as the infection approaches fixation in a population, the frequency of incompatibility declines dramatically.

Therefore, CI strains with very high transmission rates would be less useful for this form of biological control. However, strains with lower transmission rates that achieve polymorphic equilibria within host populations might be employed with effect. For example, population models indicate that a CI strain with no fertility cost to infected females, complete (100%) expression, and a transmission rate of 80% will achieve a polymorphic equilibrium of 0.72, causing a 20% reduction in fertility of the population (113). There is also the potential of "stacking" additional incompatibility types within a population and studies indicate that double infections can be used in populations already fixed for Wolbachia (3, 95). However, the effectiveness of such an approach would be quite sensitive to specifics of the population parameters (fertility costs, expression, and transmission).

A more ambitious use of Wolbachia involves genetically engineered organisms.

Several projects are underway to genetically engineer vector arthropods for refractoriness to disease agents (3). One approach is to transform mutualistic symbionts found in these vectors. Success has been achieved in developing transformation systems for the symbionts of tsetse flies (vector of African trypanosomiasis) and kissing bugs (vector of Chagas' disease) using plasmid-based constructs (3). If effective expression of anti-parasitic or anti-viral genes is achieved, there is yet another major hurdle: replacement of natural populations with the refractory genotypes. The ability of a CI Wolbachia strain to sweep through a population, bringing along with it other maternally inherited factors (such as genetically altered endosymbionts) could be an

effective mechanism for genetic replacement (3, 24, 26). However, as with direct use of CI Wolbachia, a fuller understanding of the population dynamics of this process will be crucial to its implementation.


6.0     CONCLUSIONS AND FUTURE PERSPECTIVE

As reviewed above, the prospect of using Wolbachia to control insects shows considerable promise. To date, a major obstacle for Wolbachia-based strategies has been the ability to transfer Wolbachia infections to novel hosts. However, this obstacle has been overcome by recently demonstrated successful transfers into novel hosts, using microinjection of cytoplasm into embryos (Zabalou et al., 2004; Xi et al., 2005a; Xi et al., 2005b; McMeniman et al., 2009). The mosquito A. aegypti, an important vector of dengue virus and yellow fever virus, was recently infected with a Wolbachia type from Aedes albopictus (Xi et al., 2005a). The demonstrated successful transfections are encouraging for the likelihood of transfer to major economic pests and disease vectors lacking naturally occurring Wolbachia infections.

Successful transfers promote the development of novel control programs using the Wolbachia-based strategies described in this review. Further work is needed to define the underlying molecular mechanisms of Wolbachia induced reproductive modifications, particularly CI. Genome wide analyses using available Wolbachia genome sequences should help in the development of a transgenic system that will help define the mechanism(s) of CI. Understanding the mechanism of CI is important from both a basic scientific standpoint and to facilitate Wolbachia based control strategies, specifically gene drive strategies. The release of non-transgenic incompatible males may be a logical segue before the release of fertile transgenic or paratransgenic insects, which may yield improved efficacy and/or cost. Furthermore, public acceptance of transgenic insect releases may be increased via an approach that is integrated with Wolbachia-induce CI. Specifically, if released transgenic males are cytoplasmically incompatible with the targeted population, the released transgene has a reduced probability of establishing in the field. This approach would allow for the examination of the dynamics of a transgene in a population with less risk.

Research into Wolbachia is likely to undergo an explosive growth in the near future. There is widespread interest in these bacteria, and tools are now available for detailed studies. Key questions to be investigated include the following:

What are the biochemical mechanisms of CI, PI, and feminization? How widely distributed are Wolbachia (e.g. do they occur in vertebrates)? How do

Wolbachia move between species? What are the evolutionary trajectories of Wolbachia infections within and between species? Do Wolbachia promote speciation? Can Wolbachia be effectively used in biological control? Significant progress is now likely to be made in answering these questions, and the next decade of Wolbachia research therefore promises to be an exciting one.


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