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# *Wolbachia*: more than just a bug in insects genitals

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Research on the intracellular bacterial symbiont *Wolbachia* has grown on many levels, providing interesting insights on various aspects of the microbe's biology. Although data from fully sequenced genomes of different *Wolbachia* strains and from experimental studies of host–microbe interactions continue to arise, most of the molecular mechanisms employed by *Wolbachia* to manipulate the host cytoplasmic machinery and to ensure vertical transmission are yet to be discovered. Apart from the well-established role of *Wolbachia* in triggering reproductive alterations, a new fascinating aspect is emerging, related to the ecological benefits that the symbiont provides to the host. The mutualistic relationship of *Wolbachia* strains with disease vectors remains among the top research priorities with new insights having an impact on putative anti-filarial strategies.

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## Introduction

*Wolbachia* is a maternally transmitted intracellular symbiont belonging to the α-proteobacteria. *Wolbachia* is mainly localized in the reproductive tissues of arthropods and it is responsible for the induction of a number of reproductive alterations including feminization, parthenogenesis, male-killing and cytoplasmic incompatibility (CI). Apart from reproductive parasitism, *Wolbachia* also participates in mutualistic relationships with nematode hosts [1•]. 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 [2]. The aim of this review is to present the most recent advances in this field.

## *Wolbachia* distribution, diversity and evolution

*Wolbachia* was first discovered in the gonads of the mosquito *Culex pipientis* in 1924. Since then, *Wolbachia* has been detected in many host tissues (Figure 1). *Wolbachia* has evolved several strategies to ensure vertical transmission through the manipulation of host reproductive system. These strategies include feminization, parthenogenesis, male killing and cytoplasmic incompatibility. All the above phenotypes, commonly referred to as 'reproductive parasitism', increase the frequency of infected females in the host population.

PCR-based screening methods revealed the widespread distribution of the symbiont in diverse hosts, covering numerous taxa of arthropods and filarial nematodes. Several studies estimate the levels of infected insect species to be up to 70% worldwide, emphasizing the 'pandemic' nature of *Wolbachia* [1•,3]. Recently, the association of *Wolbachia* with *Radopholus similis*, a plant parasitic nematode, was also reported, broading the host range even more [4]. The genus *Wolbachia* is taxonomically subdivided into different supergroups on the basis of phylogenetic inference of several genes including 16S rDNA, *wsp*, *ftsZ*, and others. Recent studies using multiple gene sequencing extended the number of *Wolbachia* supergroups up to eleven (namely A–K) [5–7].

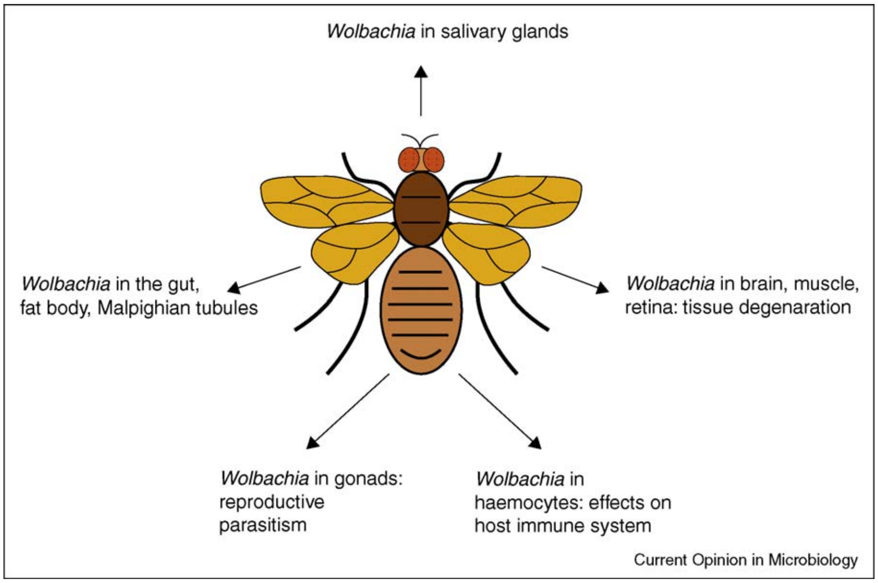
Despite the significant number of phylogenetic and taxonomic studies, the evolutionary root of *Wolbachia* is still subject of scientific debate. The examination of *Wolbachia* strains found in nematodes and arthropods led to the preliminary conclusion that the root of the *Wolbachia* clade lies between the nematode and arthropod symbionts [8]; however, current phylogenetic analysis methods are prone to long-branch attraction artifacts and therefore, the definite evolutionary relationships of the *Wolbachia* parasites and mutualists remain unresolved [5].

## *Wolbachia*–host interactions

Interesting aspects of the host–symbiont genomic interplay have been recently revealed. Lateral gene transfer is well established between *Wolbachia* and hosts in both insect and nematode species, although the direction of the transfer is in some cases unclear [9–13]. It is also worth noting that an aphid gene acquired via lateral gene transfer from a bacterial symbiont closely related to *Wolbachia* has been found to functionally compensate the absence of its homolog from the primary symbiont *Buchnera* [14]. Recently, genetic exchange between *Wolbachia* and other symbionts has also been reported; Darby *et al.* describe the presence of a *Wolbachia*-like surface protein in *Arsenophonus nasoniae* genome [15].

Maternally inherited *Wolbachia* is also an excellent candidate for inducing epigenetic alterations to the host

**Figure 1**




*Wolbachia* distribution in insect tissues and effects on host biology.

genome. In the leafhopper *Zyginidia pullula*, the feminizing strain of *Wolbachia* was found to alter the host's genomic imprinting by methylation, influencing the expression of genes involved in sex differentiation and development [16]. Similar mechanisms may operate in other *Wolbachia*-induced phenotypes as well, although this remains to be proved.

Evidence exists that *Wolbachia* manipulates host antioxidant systems in a manner that is beneficial to its survival. The stable symbiotic interaction with *Wolbachia* in an *Aedes albopictus* cell line involves reactive oxygen species (ROS) generation and induction of antioxidant enzymes [17]. In isopods, the localization of *Wolbachia* within hemocytes clearly affects the hemocyte density and the degree of septicaemia in the haemolymph. The effect of the symbiont on the host's immunocompetence and survival significantly varied within the same population, depending on the *Wolbachia* strain infecting the host [18].

Microarray analysis of *Wolbachia*-infected and uninfected cells revealed a number of differentially expressed genes involved in reproduction, immunity, and heat stress response. Among these, the angiotensin converting enzyme (*Ance*) emerged as a candidate CI mediator [19]. Moreover, the role of an insulin/IGF-like signalling enhancer has also been recently proposed for *Wolbachia*, although mechanism and biological significance of such an effect remain unclear [20].

Undoubtedly, the most extensively studied but still evasive *Wolbachia*-associated phenotype is cytoplasmic incompatibility. The study of CI led to the assumption that there are at least two distinct functions involved in CI, the 'modification' and the 'rescue' function. When the female lacks the 'rescue' function, the 'modification' of the male results in embryonic lethality. Later studies revealed more of the complexity of the phenomenon, unraveling the multiple rescue determinants that a given *Wolbachia* strain can carry [21].

Although the exact mechanism of CI remains unclear, the incompatibility phenotype is associated with an asynchrony in the development of the male and female pronuclei (reviewed in [22•]). Subsequent studies extended this knowledge to prove that CI produces a delay in recruitment of the histone H3.3/H4 complex to the male pronucleus, which normally occurs immediately after protamine removal; this may be the cause of the later observed mitotic condensation and segregation defects [23•]. The way *Wolbachia* promotes these defects is obscure, given the fact that the presence of the symbiont is not required in individual spermatocytes or spermatids for sperm modification in some systems [24].

A possible functional link between different *Wolbachia*-induced phenotypes emerged with the observation that suppression of the male-killing phenotype can lead to the instantaneous induction of CI in the butterfly *Hypolimnas bolina* [25]. The ability of a given *Wolbachia* strain to cause multiple reproductive phenotypes in different hosts has also been reported [26]. The picture of host population dynamics and ecology has been additionally complicated by the report that *Wolbachia*-associated sex ratio distortion was found altered over short time scales [27].

One of the most intriguing aspects of *Wolbachia* biology is the relationship of the symbiont with other symbiotic bacterial communities and the resulting effect on the host. *Wolbachia* and *Cardinium*, both of which have been documented to cause CI in insects, have also been reported to be hosted by a common host, causing diverse reproductive phenotype. In the spider mite *Bryobia sarothamni*, *Cardinium* induces strong CI, but *Wolbachia* does not, nor do doubly infected individuals [28]. In this case, *Cardinium* can neither rescue nor modify in the presence of *Wolbachia*, which is also true for doubly infected individuals of the parasitoid wasp *Encarsia inaron* [29].

## Wolbachia genomics

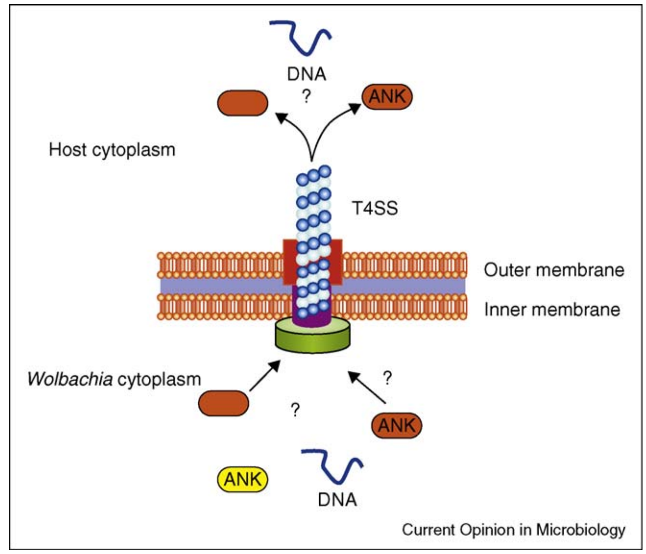
Since 2004, when the first genome of *Drosophila melanogaster w*Mel was released [30], three other *Wolbachia* genomes have been completed [31–33] and a number of other sequencing projects are in progress (reviewed in [1•]). The comparison of the genetic profile of strains related to various reproductive phenotypes provided important hints on the symbiont biology and evolution.

Similar to other obligate intracellular bacteria, *Wolbachia* have relatively small genomes, but they contain a high number of mobile and repetitive elements. A striking difference between *Wolbachia* and its close relatives is the presence of a large number genes encoding ankyrin repeat carrying proteins. Ankyrin repeats are common in higher eukaryotes, where they mediate protein-protein interactions, but are rather rare and of unknown origin and importance in bacteria. Genome sequencing has also revealed the presence of WO prophages in the arthropod-infecting *Wolbachia*. The total number of ankyrin repeat genes and prophage sequences varies in the complete *Wolbachia* genomes available. A few studies have attempted to correlate this variation with the induction of cytoplasmic incompatibility and/or other reproductive phenotypes.

The Type IV Secretion System (T4SS) is a transport apparatus employed by several bacteria for transfering DNA and/or proteins to eukaryotic cells. In *Wolbachia*-related bacterial pathogens like *Anaplasma phagocytophilum*, *Legionella pneumophila* and *Coxiella burnetii*, ankyrin repeat containing proteins are delivered into the host cytoplasm via T4SS, where they modulate diverse functions [34]. The *Wolbachia* genome also possesses transcriptionally active genes encoding a complete set of T4SS components. The sequence and organization of these genes are conserved among *Wolbachia* strains inducing various phenotypes, suggesting that whether a DNA release system or protein effector translocator, T4SS is of major importance for *Wolbachia* biology (Figure 2) [35–37].

Despite its highly reduced genome, insertion sequences constitute more than 2% of *w*Bm genome and although transpositionally silent, they persist because of frequent recombinational gene conversion [38]. The high frequency of recombination events in the *Drosophila simulans w*Ri genome, together with the incidence of co-infections, suggests that the global *Wolbachia* population is likely to consist of many subpopulations with freely recombining mosaic genomes [31]. The mosaic nature of *Wolbachia* genome was further supported by a microarray-based comparative genome hybridization analysis that showed that prophages, ankyrin-repeat encoding genes, and predicted genes of unknown function make up the majority of divergent or absent genes across several closely related *Wolbachia* genomes [39]. This increased genomic exchange probably contributes to *Wolbachia*'s ecological and evolutionary success.

**Figure 2**




Schematical representation of the *Wolbachia* type IV secretion system. The depiction of DNA and ankyrin repeat containing proteins as substrates is purely hypothetical.

## Host dependence on *Wolbachia*

Although in arthropods *Wolbachia* is primarily described as a reproductive manipulator, evidence of mutualistic-like functions has been reported recently. For example, the originally parasitic *w*Ri *Wolbachia* from *D. simulans* evolved to a beneficial symbiont in natural insect populations within the inconceivable short period of 20 years [40]. In addition, bacterial regulation of host programmed cell death has been described in the parasitoid wasp *Asobara tabida*. The presence of *Wolbachia* prevents apoptosis of nurse cells and therefore enables oocyte maturation [41].

Theory predicts that in the absence of strong reproductive parasitism, *Wolbachia* should confer a positive selection advantage to the host. A clear benefit to *Drosophila* hosts is offered by *Wolbachia* in respect to RNA antiviral protection [42•,43•]. *Wolbachia* infection was found to significantly delay host mortality induced by inoculation of diverse RNA viruses in the lab, possibly by interfering with virus proliferation. The induced resistance to viral pathogens may explain *Wolbachia*'s high prevalence in natural populations and support the idea that the response of a host to a particular pathogen also depends on its interactions with other microorganisms. *Wolbachia* may also play a role as a nutritional mutualist of female *Drosophila*, positively affecting the fecundity when reared in low/high iron nutritional environments [44]. In addition, it was recently shown that: (i) *Wolbachia* may have a key role in ferritin expression and iron metabolism in insects and (ii) iron homeostasis may play a role in the regulation of oxidative stress that, in turn, plays a key role in apoptosis [45].

The classical mutualistic strains of *Wolbachia* in filarial nematodes have been implicated in therapeutic strategies against lymphatic filariasis and onchocerciasis. The causative agents of these diseases (*Wuchereria bancrofti*, *Brugia malayi*, and *Onchocerca volvulus*) depend on *Wolbachia* for development and survival, as genes of the biosynthetic pathways of cofactors like riboflavin and heme seem to exist only in the symbiont's genome [33,46]. The significant evolutionary divergence between *Wolbachia* and human heme biosynthesis genes suggests the usage of these proteins as candidate drug targets [47].

Filariasis provokes inflammatory responses due to the death of nematodes within infected tissues. Recently, the *Wolbachia*-derived link between the stimulation of inflammatory activity and disease manifestation was proposed to be a lipoprotein synthesized by the symbiont [48]. Interestingly, the lipid II biosynthesis pathway was found to be functional and essential for *Wolbachia* cell division. It could be exploited as an antibacterial target for filarial infections [49].

## Pest-disease control

*Wolbachia*'s potential as a novel, environmentally friendly, bio-control agent was recognized early due to the manipulation of the host reproductive system and the polyphyletic distribution of the symbiont. Several strategies for disease/pest control management have now been proposed, most of which take advantage of *Wolbachia* virulent strains or the induction of cytoplasmic incompatibility [2]. *Wolbachia* may serve as an important tool for the 'Incompatible Insect Technique', the use of a symbiont-associated reproductive incompatibility for the control of insect pests and disease vectors. A major bottleneck of the *Wolbachia*-based Incompatible Insect Technique is the necessity of employing an efficient sexing strain of the insect pest, so that only infected males are released. Zabalou *et al.* [50] describe a *Wolbachia*-infected line of the medfly carrying 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 also provide a useful tool to complement current control strategies. 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 *Wolbachia*-infected females. On the negative side, the high recombination rate *of Wolbachia* may prove to be a negative factor in this kind of approach.

A wide range of insect-transmitted human pathogens require a period of extrinsic incubation in the host before pathogen transmission. This developmental period of the pathogen often comprises a significant proportion of the expected lifespan of the vector. Since age is a critical determinant of the ability of most insect vectors to transmit pathogens, only a small proportion of the population, the oldest one, contributes to pathogen transmission [51]. The identification of insect symbionts that shorten the host lifespan could therefore offer new tools for the control of vector-borne diseases. McMeniman *et al.* [52•] reported the successful transfer of *w*MelPop, a life-shortening strain of *Wolbachia* from *D. melanogaster*, into the major mosquito vector of dengue, *Aedes aegypti*. The association halved host life span under laboratory conditions, and the symbiont induced complete cytoplasmic incompatibility, which could facilitate its invasion into natural field populations.

In addition to life-shortening, *w*MelPop-infected mosquitoes obtain fewer and smaller blood meals as they age, possibly due to abnormal bending of the proboscis [53••]. The decrease in feeding success in older mosquitoes may further improve the ability of *w*MelPop to limit transmission of human pathogens in association with the life-shortening trait. Interestingly, comparative analysis has shown that *w*MelPop upregulates a variety of immune response-related genes of *A. aegypti* and its presence inhibits the development of filarial nematodes in the mosquito. The constitutive host immune upregulation by *Wolbachia* may contribute to the life-shortening phenotype [54••]. *w*MelPop-infected mosquitoes have also been reported to exhibit increased daytime locomotor activity and metabolic rates [55], which underlines the necessity of further investigation of the *Wolbachia*-induced effects for future advancement of bio-control strategies.

In the mosquito vector *Aedes albopictus*, coexistence of two *Wolbachia* strains together with other bacterial symbionts in tissues where transmissible pathogens reside is of special interest and raises the issue of the impact of such multiple infections on the vectorial competence of the mosquitoes [56]. Recently, infection of this host with the *w*MelPop strain proved to be pathogenic, demonstrating the unpredictability of phenotypes resulting from artificial *Wolbachia*/host associations [57].

## Conclusions

Intensive research in this field keeps underlining the biological, ecological, and evolutionary significance of *Wolbachia*. Unravelling the molecular mechanisms underlying the establishment of the symbiosis and the induction of the reproductive phenotypes will promote the development of novel and environment friendly biotechnological strategies using *Wolbachia* for the control of insect pests and disease vectors.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