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Wolbachia: can we save lives with a great pandemic?

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Abstract

Wolbachia pipientis is the most common bacterial infection in the animal world and wields a vast influence on invertebrate reproduction, sex determination, speciation, and behavior worldwide. These avenues of research have made seminal gains, including the latest use of *Wolbachia* to alter mosquito populations and a strengthened focus on using anti-*Wolbachia* therapies against filarial nematode infections. This work is further bolstered by a more refined knowledge of *Wolbachia* biology spanning mechanisms to relevance. Here we tally the most up-to-date knowledge in the field and review the immense implications that this global infection has for the basic and applied life sciences.

Keywords

Wolbachia pipientis; vector control; filarial disease

The great *Wolbachia* pandemic

Wolbachia pipientis is an obligate, intracellular α-proteobacteria and a member of the Rickettsiales family. These gram-negative bacteria are not culturable outside of host cells and, as a result, knowledge on the symbiosis has only surged in the last two decades owing to readily available molecular techniques. Once considered an obscure bacterium in a few insect species, the most recent meta-analysis estimates that ~40% of all arthropod species are infected with *Wolbachia* [1] as well as 47% of the Onchocercidae family of filarial nematodes [2] (see Box 1 for description of strains). Arthropods are by far the most common group of animals with likely millions of species; thus, from a biodiversity perspective, *Wolbachia* infections are one of the great pandemics in the history of Life.

Box 1

Wolbachia strains

The species *Wolbachia pipientis* is divided into separate but related subclades or supergroups that are denoted by capital letters. For instance, supergroups A, B, E, and H infect arthropods while supergroups C and D reside in nematode species. The F group is unique in that it infects both arthropods and filarial nematodes. These supergroups can be

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further divided into specific *Wolbachia* strains that are named based on the host species they infect, such as the *w*Mel strain found in *Drosophila melanogaster* and the *w*Pip strain in *Culex pipiens*. Some hosts carry multiple supergroups, such as *Aedes albopictus* that harbors both *w*AlbA and *w*AlbB. As with other species of bacteria, the genomes of different *Wolbachia* strains can vary substantially while still maintaining a core set of genes.

While these infections rampantly transfer between different arthropod hosts on an evolutionary time scale, including a recent study that showed ingestion of infected arthropods can lead to infection [3], *Wolbachia* are predominantly transmitted vertically by maternal inheritance via infected stem cells of the ovaries to developing oocytes [4]. On top of the ability to both switch hosts and transmit vertically, *Wolbachia* use several parasitic and mutualistic mechanisms to increase the number of infected females in a new host population. These host manipulations are so successful for spreading the infection that they have led to proposals on how to commandeer *Wolbachia* for human benefit at global scales. For instance, the remarkable discoveries that infected mosquitos show resistance to dengue, Chikungunya virus, yellow fever, and even malaria [5,6] create a potentially cheap and sustainable system in which the great pandemic of *Wolbachia* can be used to control mosquito and other insect vectors. Equally exciting are areas that have focused on the mutualistic role that *Wolbachia* play with filarial nematodes, the causative agents of lymphatic filariasis and river blindness that afflict hundreds of millions of people [7,8]. This research aims to eliminate the *Wolbachia* infection and thereby reduce the fitness of the nematodes that depend on it. Here we will highlight recent advances in understanding the biology and spread of *Wolbachia*, its role in curbing human diseases, and the emerging fields of *Wolbachia* genomics and host-parasite interactions.

How did *Wolbachia* spread worldwide?

W. pipientis have an arsenal of host reproductive manipulations that propagated their worldwide prevalence, including feminization, parthenogenesis (see Glossary), male-killing, and cytoplasmic incompatibility (CI). These phenotypes serve to increase the frequency of infected females in a host population and therefore the scale of maternal transmission of *Wolbachia* to the next generation. While each of these modifications has been characterized at a foundational level, their underlying mechanisms are enigmatic [7].

The most heavily studied adaptation of *Wolbachia* is CI, which occurs as a conditional reproductive failure that manifests as embryonic lethality between eggs from uninfected females and modified sperm from infected males (Figure 1). However, the reciprocal cross as well as crosses between males and females infected with the same *Wolbachia* strain are viable [10]. When compared to uninfected females, this unidirectional incompatibility underscores a dramatic, relative fitness increase for infected females - the transmitting gender of *Wolbachia*. *Wolbachia* have been repeatedly observed to spread via CI in natural and laboratory populations, with recent analyses in tsetse flies [8] and the grasshopper *Chorthippus parallelus* [9].

How is cytoplasmic incompatibility induced?

One of the most pre-eminent questions in the *Wolbachia* field is how does the infection cause CI and other reproductive alterations? Extensive cytological studies of embryonic defects in model insects provide hints as to how CI causes lethality after fertilization. First, the majority of embryonic arrest is associated with shortcomings in the first mitotic division [13]. They include a failure of the paternal nuclear envelope to break down [14], delayed Cdk1 activation [14], an inability to correctly deposit maternal histones in the paternal

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genome, and slowed replication of the sperm DNA [15]. These delays in cell cycle progression are accompanied by severe chromosomal defects, specifically in the paternal DNA, which include incomplete condensation and failure to segregate correctly. In addition, CI embryos contain an excess of centrosomes that are unassociated with the pronuclei [16,17]. This latter defect can be explained by a combination of mitotic delays and incomplete chromosome condensation, which are known to dissociate centrosomes from nuclei [18].

In the absence of data on the exact sperm modification used to cause embryonic lethality, investigators have turned to conceptual models that may explain CI-associated defects. They are based on the positions that (i) *Wolbachia* modify the sperm to cause severe defects in the timing and progression of mitosis, and (ii) this modification can be 'rescued' by a female infected with the same strain [19]. Males and females infected with genetically-distinct strains (Box 1) are bidirectionally or reciprocally incompatible. For many years, the two predominant models for CI have been (i) the lock and key model and (ii) the mistiming model. The lock and key model posits that *Wolbachia* place certain 'locks' on the paternal genome. A female infected with the same strain of *Wolbachia* has the appropriate 'keys' to remove these locks after fertilization and rescue the mitotic defects that may occur [20]. The mistiming model, however, suggests that CI results from mitotic mistiming between the maternal and paternal pronuclei [20]. An infected female is able to rescue this disparity by making compensatory changes in either pronucleus. The mistiming model can be expanded to suggest that the asynchrony actually occurs between the paternal pronuclei and maternal cytoplasm [7,18].

While each of the above models has its merits, neither fully explains current observations for CI. For instance, the lock and key model suggests that each strain has its own encrypted locks and keys. The known strain incompatibilities, however, quickly demand a questionably large number of different locks and keys, especially considering the speed with which new incompatibilities arise [22]. The mistiming model also fails to account for strain specificity. For example, data show that strain *w*San of *Drosophila santomea* is capable of rescuing CI caused by strain *w*Ri of *Drosophila simulans* and that *w*Ri can rescue strain *w*Mel of *D. melanogaster*. When tested, however, *w*San is unable to rescue *w*Mel [23,24], an incongruity not explained by the mistiming model. It is obvious that our understanding is incomplete and new simulations, such as the goalkeeper model proposed by Bossan *et al*. [25], are attempting to fill this gap. Briefly, this model posits that a rescuing strain of *Wolbachia* must act as a 'goalkeeper' to block CI. This action requires the bacteria to utilize two separate 'factors', which can range from mistiming of the parental genomes to various bacterial proteins or even phage components. These two factors are equivalent to a keeper jumping both far enough and high enough to block a soccer goal. They could also be altered by host conditions (equivalent to placing the goalkeeper on a stool or in a trench) and thereby explain the dependence on host genotype. This model is supported by growing evidence that multiple factors are involved in rescue [23].

Finally, it has been known for many years that titers of *Wolbachia* are, in general, positively associated with CI [26,27]. Each of the proposed models, whether it is through failure to place enough locks on the host genome, a struggle to induce mistiming, or a lack of sufficient modifying factors, can easily explain how low *Wolbachia* density fails to induce complete cytoplasmic incompatibility. Similar to mistiming, however, infection load cannot fully clarify the mechanism of CI.

Causal factors for CI

Current efforts to identify the causal factor for CI are varied and have been largely unsuccessful. Initial work looking at host gene expression in infected versus uninfected

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hosts implicated the host histone chaperone Hira [28]. Research has also focused on the unusually large number of *Wolbachia* proteins that contain ankyrin repeat domains. This domain is usually implicated in protein-protein interactions, and was thus a tempting candidate for host modifications. Multiple studies have looked at the link between these proteins and cytoplasmic incompatibility and, while some are regulated in a sex-specific manner, none were shown to be involved in CI [29–31]. Finally, there might be a link between reactive oxygen species (ROS) and the induction of CI. Specifically, *Wolbachia* infection leads to increased levels of ROS in testes and ovaries, and these reactive oxygen species lead to damaged spermatid DNA [32]. This discovery is interesting as DNA damage induced by ROS can account for several hallmarks of CI including defective paternal chromatin, delayed Cdk1 activation, and failed mitosis. Future research should determine how large a role DNA damage induced by reactive species actually plays in the induction of CI.

Future investigations into CI

While the exact mechanism of cytoplasmic incompatibility remains elusive, future studies will be strengthened by several developments within the field. One other bacterium, *Cardinium hertigii*, is known to induce CI, and in hosts infected by both bacteria, there is an additive effect to embryonic lethality [33]. The genome for *Cardinium* is now available, and comparative sequence analyses suggest an independent origin of CI [34]. A renewed focus on the other reproductive alterations induced by *Wolbachia* should also prove informative. Exciting, recent work demonstrates that male-killing, like CI, is associated with damaged paternal chromatin [35]. Further links between CI and male-killing are evident as CI-inducing *Wolbachia* from *Drosophila recens* elicit male-killing when transferred to *Drosophila subquinaria,* with similar effects observed in strains transferred between moths [36,37]. Other work shows that the male-killing strain *w*Inn (from *Drosophila innubila*) fails to induce CI or male-killing in *D. melanogaster* and *D. simulans*. These results, however, can be explained by low titers, especially in sperm cysts, in the recipient hosts [38]. Finally, strains from *Drosophila bifasciata* that exhibit incomplete male-killing can also induce CI [39]. The growing links between the types of *Wolbachia*-induced sexual parasitism suggest a related underlying mechanism that warrants future exploration and modeling.

Commandeering the *Wolbachia* pandemic for vector control

The study of *Wolbachia* is a pre-eminent example of how basic science can translate to biomedical science. Once studied as an obscure reproductive modification, CI is now at the center stage of efforts to control the transmission of human pathogens through mosquito vectors. In particular, species infected with *Wolbachia* have increased resistance against dengue, Chikungunya, yellow fever, and West Nile viruses, as well as malaria and bacteria [6,40–42], though some hosts show increased susceptibility [43]. The twofold advantage of *Wolbachia* to both decrease pathogen replication and deterministically spread via CI in insect vectors has direct implications for quelling the transmission of infections to humans.

Two strategies take advantage of this system to reduce vector numbers and transmission competency. First, a large release of *Wolbachia*-infected, and therefore pathogen-depleted mosquitoes, could replace the local population of *Wolbachia*-uninfected animals through CI. As we discuss below, this Population Replacement Strategy (PRS) (Figure 2A) has made impressive progress in the past few years via the International Eliminate Dengue Project (EDP). A second strategy, known as the Incompatible Insect Technique (IIT) (Figure 2B), is to release only CI-inducing males into uninfected vector populations, which can then sterilize a large fraction of the females and drastically reduce overall vector numbers [44]. This population suppression has been successfully employed to control farm pests [45], and there is an ongoing field study on islands in the Indian Ocean, which aims to reduce *Culex*

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pipiens quinquefasciatus numbers to control filarial parasites and arboviruses [46]. A final technique, which utilizes the lifespan shortening ability of *Wolbachia* infection, has been proposed, but implementation is difficult, as models predict that a shortened lifespan substantially negates any fitness conferred by a genetic drive mechanism such as CI, making it exceedingly difficult to replace native populations [47].

Eliminate Dengue Program

The Eliminate Dengue Program was originally established in Australia with the aim of using *Wolbachia*-based strategies to curb the spread of dengue, a mosquito-borne disease. Early efforts focused on using the *w*MelPop strain of *Wolbachia* [48], but in 2011, the EDP stably infected the mosquito vector of dengue, *Aedes aegypti*, with the *w*Mel *Wolbachia* strain from *D. melanogaster* [49]. The feat was accomplished by passaging the bacteria for several years in an *Aedes albopictus* cell line before microinjection into the mosquitoes. The long term *in vitro* cultivation in mosquito cells led to attenuated virulence in the mosquito species *in vivo* and a normal host lifespan; yet, remarkably the *w*Mel strain retained high rates of maternal transmission, the capacity to spread through experimental populations by CI, and the crucial refractoriness to dengue virus. Controlled release of these mosquitoes into a small number of Australian neighborhoods effectively replaced the native population with a dengue-free vector [50]. While data on whether the population replacement has reduced the incidence of human dengue cases will take many years to assess, the EDP is quickly scaling their approach throughout the world. Recent estimates suggest that dengue infects 390 million people per year with 96 million showing some level of disease severity [51]. The vast majority of these cases are in Southeast Asia and South America where the EDP has research centers in China, Indonesia, Vietnam, and Brazil. These locations will give the EDP a growing influence in the spread of dengue among the most heavily affected areas in the world.

The success of the EDP has inspired a broad push to identify applications for *Wolbachia* in other disease vectors (Box 2). Of particular interest are the anopheline mosquitoes, the main carriers of malaria. Every sampled species of *Anopheles* lacks *Wolbachia*, and while *Anopheles gambiae* can be somatically infected by *Wolbachia* strains from *D. melanogaster* and *Aedes albopictus*, stable germ line infection with high maternal transmission has historically been difficult [52,53]. Recently, however, that hurdle was overcome by stably infecting anopheline mosquitoes with microinjections of *Wolbachia* into eggs. The resultant mosquitoes show few defects, induce CI, and cause refractoriness to *Plasmodium* infection [54]. This exciting new work now places *Wolbachia*-based control of mosquitoes that transmit malaria within sight.

Box 2

Outstanding questions

- How is *Wolbachia pipientis* able to easily manipulate the host reproductive system and, specifically, how does it induce cytoplasmic incompatibility?
- Can the success of the Eliminate Dengue Project be expanded into other mosquito-borne viruses such as Chikungunya and yellow fever?
- Can *Wolbachia*-specific antibacterial drugs with no contraindications be developed to target filarial nematode infections?
- Can bacteriophage WO be developed either into a transgenic vector for *Wolbachia* and/or an anti-*Wolbachia* therapeutic for treating filarial diseases?

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There has also been work to identify the infection status of other mosquito species to test the applicability of population replacement by *Wolbachia* in the vectors of yellow fever and lymphatic filariasis [55]. Additionally, *Wolbachia* have been proposed as a possible means to control bed bugs [56] and tsetse flies [57]. In bed bugs, resistance to pyrethroid insecticides is common, and thus alternative methods using *Wolbachia* are welcome developments. Finally, the use of *Wolbachia* in tsetse flies is especially enticing, as they spread trypanosomes and sleeping sickness, and *Wolbachia* are already known to induce CI in some tsetse species [8]. Possible methods for vector control in tsetse flies, and their comparison to current techniques used in sub-Saharan Arica, are reviewed in Doudoumis *et al*. [57].

Caveats to *Wolbachia*-based vector control

While the discovery of *Wolbachia*-based anti-pathogen resistance [58,59] has attracted widespread attention for its role in vector control, the mechanism behind this super-charged host protection remains an area of active investigation. It was first hypothesized that *Wolbachia*, by virtue of its transgenerational persistence, simply primes the host immune system and thereby encourages the clearance of viral particles. Indeed, *Wolbachia* heat shock and surface proteins stimulate the expression of innate immunity genes including cytokines, defensins, proteases, and peptidoglycan-recognizing proteins [52,60,61]. These results, though, may arise from contamination originating in the *E. coli* expression systems utilized or non-physiologically relevant concentrations of protein. These alternatives are perhaps reinforced by data, which show that infected cell lines gain protection from viruses [62] even though they lack several components of the innate immune system, including fat bodies and phagocytosing blood cells. Interestingly, the *Wolbachia* strain *w*MelPop-CLA confers viral protection to both *A. aegypti* and *D. melanogaster* individuals, but it only upregulates immunity genes in the mosquitoes [63]. While 'priming' of the immune system might be insufficient to confer viral resistance, components of the innate immune system such as ROS and the Toll pathway are known to play large roles in *Wolbachia*-induced protection in *A. aegypti* [64]. Additionally, the innate antiviral siRNA pathway is not required for viral protection in infected *D. melanogaster* [65], and increased immunity is dependent on where *Wolbachia* localizes within *D. simulans* [66]. These conflicting results will hopefully be resolved in coming years by comparative studies of species that do not receive pathogen protection from *Wolbachia* infection [43,67] or are actually weakened by the bacteria [68]. Finally, recent evidence suggests that *Wolbachia*-associated expression of host miRNAs [69,70] assists regulation of dengue virus titers within mosquitoes [71].

Symbiotic transitions: from a pandemic to a mutualist

W. pipientis is well known for its parasitic phenotypes, yet it also has a mutualistic relationship with several invertebrate species. The archetypal example occurs in filarial nematodes, in which 47% of the Onchocercidae family are infected by *Wolbachia* [2], and both host and bacteria are completely dependent upon each other. Interestingly, almost every disease-causing species of filarial nematode are infected with *Wolbachia*. A watershed moment in the science of filarial diseases occurred when studies implicated *Wolbachia* as the chief cause of debilitating ailments such as river blindness and lymphatic filariasis [72], reviewed in [73]. The nature of this mutualism is slowly being elucidated, and there is strong evidence that the bacteria provide essential nutrients to the host, including riboflavin, heme, and flavin adenine dinucleotide (FAD) [74–76]. Recently, based on genome and transcriptome sequencing of *Onchocerca* worms, it was suggested that *Wolbachia* play a defensive, antibacterial role and have possible mitochondria-like actions such as providing energy and metabolites [8]. These observations are supported by work that shows dramatic increases in *Wolbachia* titers when the host is undergoing high levels of growth and division that demand increased metabolism [77–79].

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The symbiosis between *Wolbachia* and filarial nematodes is tightly controlled. Studying specific cell lineages, it has been found that the parasite positions itself in the hypodermal chords of developing zygotes and later is able to specifically invade the gonads before sexual maturity is achieved [80,81]. The nematode maintains strict control over this interaction through host autophagy [82]. This inter-dependence is reinforced by horizontal gene transfer between the bacteria and host, such as in *Brugia malayi* [83]. There is also growing evidence that other filarial nematodes exist independent of *Wolbachia* and may have lost the bacteria after an ancient infection [2].

Although *W. pipientis* is required in many filarial nematodes, its mutualistic relationships with arthropods are more varied. In *Aedes polynesiensis*, infection is associated with decreased larval mortality and increased adult lifespan [84]. In other species, such as *C. pipiens quinquefasciatus* and brown planthoppers, *Wolbachia* increases the number of embryos surviving to adulthood but decreases adult lifespan [85,86]. In *A. aegypti,* however, *Wolbachia* decreases embryo survivability, and in the moth *Ephestia kuehniella*, infection reduces the number of viable sperm [87,88]. In other species, such as rice water weevils and the wasp *Asorbara tabida*, a *Wolbachia* infection is absolutely required for oogenesis [89,90]. Finally in *Drosophila mauritiana*, *Wolbachia* infections in the ovarian stem cells accelerate mitosis, leading to a fourfold increase in egg numbers compared to uninfected counterparts [91]. While these various phenotypes show little correlation with each other, one interesting hypothesis is that they might represent various stages of a parasitic-to-mutualistic continuum between *Wolbachia* and invertebrate hosts. A mutualistic or codependent relationship would be beneficial for both organisms and could be selected for over time. Interestingly, this exact transition has been observed in nature over the course of just a few decades with fruit flies [92].

Removing the mutualist to cure filarial diseases

In contrast to spreading the *Wolbachia* reproductive parasites in arthropods for vector control, the profound health repercussions for *Wolbachia* mutualisms are based on eliminating them. Specifically, in the filarial nematodes, curing *Wolbachia* can halt nematode growth, encourage apoptosis, and eventually lead to death of the worm [93]. These nematodes cause diseases such as lymphatic filariasis and onchocerciasis, which together account for 140 million infections a year. These afflictions threaten 1.4 billion people annually, yet alarmingly over 20 years have passed since the last anti-filarial drug was developed. More importantly, current treatment protocols are losing efficacy, and resistance is of growing concern [94].

Research into *Wolbachia*-nematode interactions was boosted after the genomes for the main causative factor of lymphatic filariasis, *B. malayi,* and its *Wolbachia* symbiont, *w*Bm, were published [74,75]. These studies enabled comparative genomic analyses of the pathways that complement missing functions in both the host and symbiont. As mentioned before, this work showed that *B. malayi* is reliant on factors such as riboflavin, heme, and FAD produced by the bacteria (Figure 3). Interestingly, it also revealed many of the specific metabolites that *Wolbachia* requires from its host. These include coenzyme A, biotin, and nicotinamide adenine dinucleotide (NAD), as well as ubiquinone, lipoic acid, folate, and pyridoxal phosphate. Whether any of these pathways can be successful drug targets is yet to be determined. Further candidates will also be elucidated as comparative genomics of nematodes and their *Wolbachia* continues with the more recent analyses of the uninfected nematode, *Loa loa* [95] and the F group *Wolbachia* [2].

In addition to the factors mentioned above, other work has focused on identifying specific *Wolbachia* pathways and their role in the host-symbiont relationship. Initial results have

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recognized heme biosynthesis, DNA ligases, FtsZ, ClpP peptidase, lipoprotein biosynthesis, and pyruvate phosphate dikinase (PPDK) in the bacteria as promising targets for drug development [96–102]. While these pathways share little in common, the broad range of treatment candidates that they represent could enable *Wolbachia*-specific therapy. Finally, in a directed effort to discover drugs that treat river blindness and filariasis, the Anti-*Wolbachia* Consortium (A-WOL) has recently begun screening compounds that can target the infection in a mosquito cell line. These efforts have looked at over 2600 current drugs as well as 67 000+ other compounds with full results coming soon [73].

The race to find new anti-filarial and anti-*Wolbachia* treatments is urgent. Despite success in eliminating nematode infections with doxycycline in small groups of individuals [103], the lengthy treatment regimes, the potential for evolution of widespread antibiotic resistance in the endogenous microflora, and restrictions against use in children and pregnant women make massive administration of doxycycline problematic. Indeed, the gut flora of treated individuals could act as a reservoir for drug resistance genes [104,105], and intracellular bacteria, while more restricted in horizontal gene transfer than free living species, have still shown a capacity to gain resistance [106]. There is still hope for alternative drugs, such as an anti-filarial vaccine (Box 2), to supplement current treatments. In fact, recent work shows that mice immunized with a single *Wolbachia* protein show strong, although not complete, resistance to nematode infection [107]. More treatment avenues will also open as in-depth research is conducted on the recently sequenced genomes of several filarial nematode species and their accompanying *Wolbachia* infections [108].

Concluding remarks and future perspectives

The field of *Wolbachia* research has matured considerably in recent years into large efforts with basic and applied approaches. From the humble beginnings of simple identification and classification, *Wolbachia* research has evolved into a globally important endeavor. Far from being an interesting side note in arthropod literature, this symbiont has shown great potential as a means for vector and filarial disease control. Success in controlling dengue fever in *A. aegypti* populations will hopefully translate over to other species, including the newly infected anopheline mosquitoes and tsetse flies. This research is paralleled by work in filarial nematodes that targets *Wolbachia* mutualists to eliminate diseases such as lymphatic filariasis and river blindness. Current drug screening efforts place these goals within reach. While there is continuing difficulty in the ability to genetically transform *Wolbachia* or to culture it outside host cells, making many modern techniques unavailable to researchers, much information has been gained thanks to new sequencing efforts. There are currently 19 sequenced strains of *Wolbachia* either completed or in progress [57,109,110] with more certainly to come. There has also been a growing appreciation for the role of bacteriophage WO in *Wolbachia* biology [111], with the lytic phage potentially offering a naturally-evolved anti-*Wolbachia* strategy for the treatment of filarial disease. The research community is compelled by surging amounts of evidence that *Wolbachia* have the potential to mediate diseases ranging from dengue fever and Chikungunya to malaria and lymphatic filariasis. These illnesses account for over 730 million infections every year and can be crushing social burdens for developing countries. The advancement of new techniques, and refinement of current ones, will hopefully allow the most abundant endosymbiont, *W. pipientis*, to make these diseases a thing of the past.

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Glossary

Bacteriophage WO	a temperate virus commonly found in arthropod *Wolbachia*
Cytoplasmic incompatibility (CI)	a post-fertilization defect in chromatin where matings between infected males and *Wolbachia*-free females (or females harboring a different *Wolbachia* strain than that in the male) result in high levels of embryonic death
Eliminate Dengue Project (EDP)	a worldwide effort to eliminate dengue virus through the spread of *Wolbachia*-infected mosquitoes
Incompatible Insect Technique (IIT)	a method to reduce insect populations by sterilizing wild females with large releases of incompatible males
Lock and key model	the concept that CI is caused by certain 'locks' placed on the paternal genome, which can arrest development and are only removed by specific 'keys' found in infected females
Male-killing	the selective killing of male embryos
Mistiming model	the concept that CI is induced by cell cycle timing defects during the first mitosis. Specifically, a delayed paternal pronuclei (when compared to the maternal pronuclei or cytoplasm), could account for cell cycle arrest and embryonic death
Parthenogenesis	asexual reproduction whereby viable embryos develop from unfertilized eggs
Population Replacement Strategy (PRS)	the use of a genetic drive mechanism, such as cytoplasmic incompatibility induced by *Wolbachia* infection, to replace a natural population with a laboratory-reared one
Pronucleus	the nucleus of either the sperm or egg during fertilization
Vertical transmission	the transmission of material from parent to offspring. Maternal transmission is a subtype, involving the specific passage from mother to child

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Highlights

Wolbachia are one of the great pandemics of Life from a biodiversity perspective

The molecular basis of *Wolbachia* parasitism remains enigmatic

Wolbachia parasites can be deployed to curb natural mosquito vectors of dengue virus

Research on anti-*Wolbachia* drugs hold future promise to eradicate filarial disease

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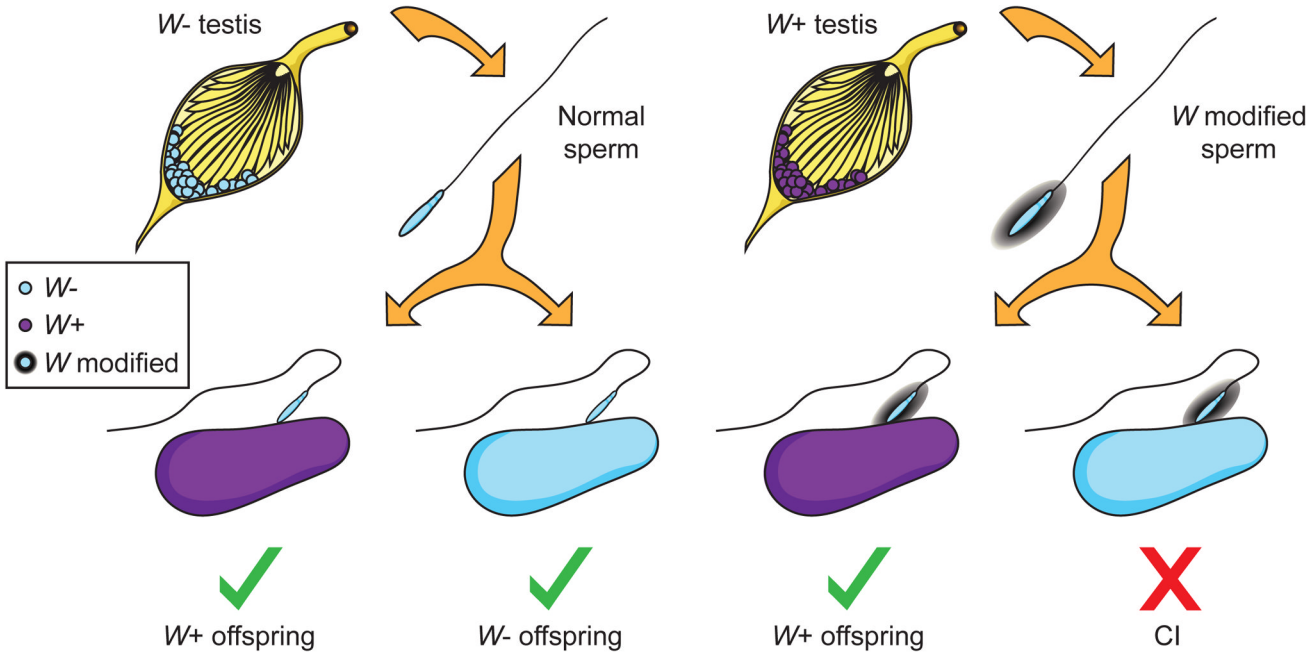

Figure 1. *Wolbachia*-induced cytoplasmic incompatibility

Wolbachia (*W*, purple) infection causes a modification in the sperm that can be rescued by eggs of infected females but leads to embryonic death in uninfected embryos. Abbreviations: *W*−, *Wolbachia*-uninfected; *W*+, *Wolbachia*-infected; *W* modified, *Wolbachia*-modified sperm. Illustration by Robert M. Brucker.

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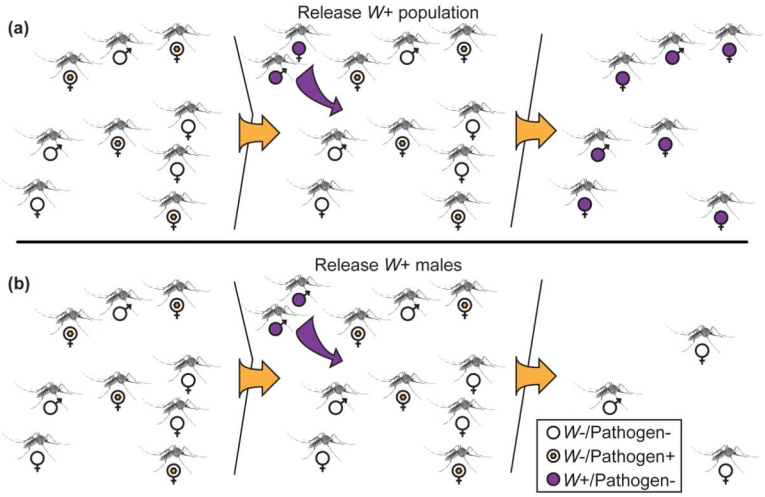

Figure 2. Vector control strategies

(A) Population Replacement Strategy switches a wild population of mosquitoes (pathogen carrying, *Wolbachia* uninfected) with a pathogen-free one through *Wolbachia*-induced CI. **(B)** In the Incompatible Insect Technique, a release of just *Wolbachia* infected males leads to high levels of CI and a reduction in the total vector population. Abbreviations: *W−*, *Wolbachia*-uninfected; *W+*, *Wolbachia*-infected; Pathogen+, pathogen-infected; Pathogen-, pathogen-uninfected. Illustration by Robert M. Brucker.

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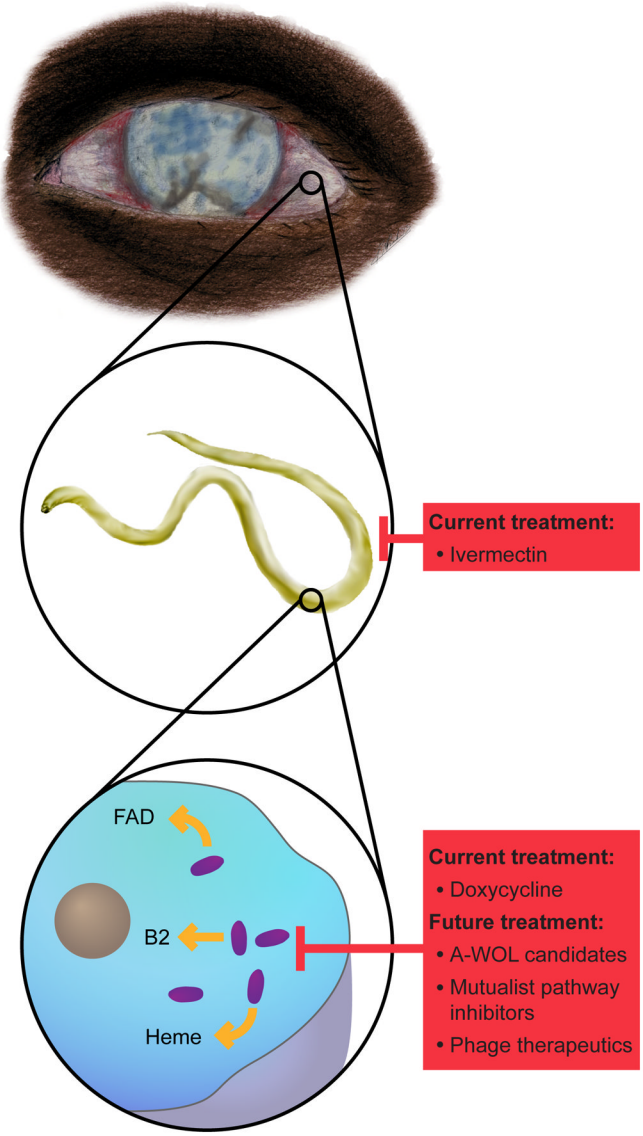

Figure 3. Elimination of filarial nematodes

Diseases such as river blindness and lymphatic filariasis, traditionally treated with anti-filarial medications, could benefit from anti-*Wolbachia* approaches that target the host-bacterial symbiosis. Illustration by Robert M. Brucker.