



Review

The impact of environmental and host factors on *wolbachia* density and efficacy as a biological tool

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ABSTRACT

Wolbachia, a bacterium found naturally in some species of *Aedes* and *Culex* mosquitoes, has gained significant attention for its potential in controlling mosquito-borne diseases and suppressing mosquito populations. However, *Wolbachia*-mediated pathogen blockage, *Wolbachia* dynamics in field populations and vertical transmission have been reported to be density-dependent. Several factors, including host genetics, diet, temperature, and co-infections can influence *Wolbachia* titers within its host. The interplay between these factors can have significant influence on the effectiveness of *Wolbachia*-mediated pathogen blockage and cytoplasmic incompatibility. However, there is a knowledge gap regarding the regulation of *Wolbachia* density within its host, which could affect its effectiveness as a biocontrol tool. Therefore, this review aims to understand the complex tripartite association between the environment, host, and endosymbiont, and how these relationships are crucial in harnessing the full potential of *Wolbachia* as a biological tool. Further, we highlight how host, pathogen, and environmental factors influence *Wolbachia* density and how their interplay can impact CI and WMPB. We further review the strategies adopted to maintain/control *Wolbachia* densities in field populations.

1. Introduction

Mosquitoes are notorious for transmitting various diseases that cause significant morbidity and mortality worldwide.¹ Despite efforts to develop effective vaccines and chemotherapies, controlling mosquito-borne diseases remains a daunting task.^{2,3} Chemicals have been used for mosquito control, but their continuous and large-scale applications have resulted in the emergence of resistance in mosquitoes.^{4,5} Therefore, there is a pressing need for environmentally friendly approaches to reduce or block pathogen transmission and reduce mosquito populations.^{6,7}

Biological control tools such as fungi, bacteria, viruses, natural enemies, and genetically modified mosquitoes have been proposed as promising strategies. Among these, *Wolbachia*, an endosymbiont

bacterium of some mosquito species, has gained increasing attention as a modern biological control tool.^{17,18} *Wolbachia* confers protection to mosquitoes against pathogens and has the ability to reduce mosquito populations.

Wolbachia colonizes the germ cells where it manipulates the host's reproductive mechanisms through cytoplasmic incompatibility (CI), parthenogenesis, and feminization¹⁹ in certain insects. This property maintains *Wolbachia* populations in different generations through vertical transfer to off-springs by increasing the rates of infected females or by inducing a female-biased population. The most important of all is CI, where *Wolbachia* induces modification of the male nucleus leading to failure of progeny to develop unless the same strain is present in the eggs to exert the rescue function. Moreover, *Wolbachia*'s ability to reside in the somatic tissues of mosquitoes has led scientists to explore its

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potential in controlling mosquito-borne diseases. *Wolbachia* can block the release of pathogens from mosquito salivary glands, enhance mosquito immunity to pathogens,²⁰ regulate egg laying,^{21,22} and regulate food searching and metabolism^{23,24} in female mosquitoes.

However, the effectiveness of *Wolbachia* in controlling mosquito transmitted diseases is dependent on the level of titers present within the host. These titers affect the frequency of maternal transmission, *Wolbachia* field dynamics and pathogen blockage. Consequently, there are mechanisms within the host that regulate *Wolbachia* titers to ensure they are not eliminated by the mosquito's immune system and that high titers do not harm the host. *Wolbachia* titers in mosquitoes are influenced by host, pathogen, symbiont, and environmental factors. However, there is insufficient information on how the interplay between these factors influences *Wolbachia* titers and how their relationships impact CI and WMPB.

This review aims to explore the complex relationship between the environment, host, and endosymbiont and how they affect *Wolbachia*'s potential as a biological tool. This knowledge can provide insights into how *Wolbachia* density in mosquito populations can vary over time and space, ultimately impacting *Wolbachia*-mediated phenotypes.

2. *Wolbachia* in mosquitoes

The prevalence of *Wolbachia* infection across different mosquito species is not uniform Table 1. Some mosquito species are naturally infected with *Wolbachia*, while others lack the infection.²⁵ This variation in *Wolbachia* distribution can be attributed to several factors, including the evolutionary history of the mosquito species and the ecological conditions in which they live.^{26,27} Titers may vary when considering different *Wolbachia* strains, host species or host populations.²⁸

Culex (*C.*) *pipiens*, *Aedes* (*A.*) *albopictus*, *C. quinquefasciatus*, *A. bromeliae* and *A. fluviatilis*, *Coquilletidia perturbans* and *Mansonia titillans* naturally carry *Wolbachia*,^{29–33} however, most mosquitoes including the *Anopheles* complex, *A. Aegypti*, *C. Tritaeniorhynchus* among others do not.^{18,34} Nevertheless, attempts have been made in the laboratory to infect these mosquitoes with *Wolbachia*. For instance, the highly proliferating wMelPop strain was directly transferred from *Drosophila melanogaster* into *A. aegypti*, resulting in stable *Wolbachia* densities.¹¹ Similarly, embryonic microinjections with the wAlbB strain from *A. albopictus* were used to establish stable *Wolbachia* densities in *A. stephensi*, marking the first successful transfer in *Anopheles* species.¹⁶ However, when *Wolbachia* was transferred to *A. gambiae*,³⁵ the bacteria only established infections in the cytoplasm of somatic cells and did not achieve the desired germ cell establishment, necessary for vertical transmission. This lack of establishment into germ cells is a concern if stable *Wolbachia* densities are to be maintained in *Anopheles* mosquitoes for purposes of malaria control programs.

However, recent studies in certain parts of Africa, Malaysia and Myanmar report that some *Anopheles* and *A. aegypti* are naturally infected with *Wolbachia*.^{33,36–38} The identification of indigenous

Table 1
Summary of *Wolbachia* strains that have been artificially established in different mosquito strains.

Mosquito Species	<i>Wolbachia</i> strain	Transfer method	Reference
<i>Ae. Albopictus</i>	wAlbA, wAlbB, wRi, wPip, wMel	Embryonic, egg cytoplasm	8–10
<i>Ae. Aegypti</i>	wAlbA, wAlbB, wMelPop, wMel	Embryonic, egg cytoplasm	11, 12
<i>Ae. Aegypti</i>	wAlbA, wAlbB, wMelPop	Adult, tissue	11, 13
<i>C. pipiens</i>	wPipMol	Embryonic, egg cytoplasm	14
<i>An. Gambiae</i>	wMelPop, wAlb	Adult, cell culture	15
<i>An. Stephensi</i>	wAlbB	Embryonic, egg cytoplasm	16

Wolbachia infections in *Anopheles*, as well as the observation of persistent transinfections in *A. stephensi* highlights the potential utility of *Wolbachia* as a means of malaria control.

The vertical transmission of *Wolbachia* infections in mosquitoes exhibits varying transmission rates. Some infections demonstrate near-perfect vertical transmission rates, while others have rates of below 80 %. The high maternal transmission rates suggest that relative *Wolbachia* densities are high in reproductive tissues.^{39,40} Further still, *Wolbachia* densities are at higher densities and show more variability in testes than the ovaries.³⁹ The higher *Wolbachia* densities in testes alter sperms hence bringing forth stable and strong CI effect. This CI effect can then reduce the viability of offspring produced when infected males mate with uninfected females.

Further still, horizontal gene transfer of *Wolbachia* is reported in mosquitoes.^{41,42} Co-feeding,^{43,44} cannibalism,⁴⁵ and larvae crowding⁴⁶ are some of the reported ways horizontal transfer may occur in insects. Horizontal transfer of *Wolbachia* can have important implications for the spread and maintenance of the bacteria in natural populations, as it can allow for the establishment of new infections in previously uninfected hosts. This phenomenon is exploited in laboratories to artificially infect mosquitoes with different *Wolbachia* species. However, the frequency and efficiency of horizontal transfer can vary depending on a variety of factors, including the specific *Wolbachia* strain and host species involved, as well as environmental conditions.

Wolbachia titer variances in mosquitoes influence both host reproductive phenotypes and pathogen transmission potential in the host.^{47,48} The presence of higher *Wolbachia* densities may be complex, since higher densities may favor *Wolbachia*-driven infection spread or host-driven infection control. Regardless of the scenario, there must be trade-off between *Wolbachia* densities and host fitness if *Wolbachia* mediated benefits are to be stable over time. The right densities form a basis for an efficient population suppression or replacement⁴⁹ or sufficient prevention of the release of mosquito transmitted pathogens from the mosquitoes' salivary glands.⁵⁰ Understanding the mechanisms and factors responsible for *Wolbachia* variations in mosquitoes is crucial in deploying *Wolbachia* for mosquito control programs.

3. Mosquito-*wolbachia*-pathogen interaction

The interaction between mosquitoes, *Wolbachia*, and pathogens is an active area of research. The presence of *Wolbachia* in mosquitoes has been found to inhibit the replication and transmission of certain pathogens, including dengue, Zika, and chikungunya viruses. This is due to *Wolbachia*'s ability to activate the mosquito's immune system and compete with the pathogen for resources, among other mechanisms. Additionally, *Wolbachia* can be used to suppress mosquito populations by manipulating the mosquito reproductive system through CI. However, the effectiveness of *Wolbachia* in reducing pathogen transmission can vary depending on the mosquito species, *Wolbachia* strain and density, and pathogen type. Nonetheless, the use of *Wolbachia* as a tool for controlling mosquito-borne diseases is a promising area of research.

3.1. *Wolbachia* density and reproductive manipulation

Cytoplasmic incompatibility (CI) is a phenomenon that occurs when *Wolbachia*-infected males' mate with uninfected females, resulting in reduced viability of the offspring. *Wolbachia*-induced CI is a powerful tool that has been used to control mosquito populations. CI is controlled by the cifA and cifB genes expressed in WO bacteriophage found in some *Wolbachia* strains ovaries.^{51,52} The mechanism involves the modification of sperm by gene cifA and cifB expressed in infected male testes, which results in the death of embryos that are produced when infected males mate with uninfected females. Rescue will only occur when one gene cifA is expressed in female ovaries.^{53,54}

Wolbachia-induced CI has several advantages over other methods of mosquito control. One advantage is that it is species-specific, and it is

self-sustaining. Despite its potential as a tool for mosquito control, there are some challenges associated with *Wolbachia*-induced CI. One challenge is the need to introduce and maintain *Wolbachia* in local mosquito populations⁵⁵ and the evolution of resistance to *Wolbachia*-induced CI.

The expression of *cifA* and *cifB* genes, shows variation among different mosquito species, even when those species are infected with the same strain of *Wolbachia*. This suggests that there may be species-specific factors that influence the expression of these genes and the ability of *Wolbachia* to induce CI in different mosquito populations. Mosquito age, sex and *Wolbachia* density are also cited as determinants of the *cifA/cifB* expression and CI strength.^{48,56,57}

The expression of *cifA* and *cifB*, has been shown to be correlated with the density of *Wolbachia* in mosquito tissues. Specifically, as the density of *Wolbachia* increases in mosquito tissues, the expression of *cifA* and *cifB* also increases. This correlation between *cifA/cifB* expression and *Wolbachia* density has important implications for understanding the mechanisms by which *Wolbachia* induces cytoplasmic incompatibility (CI) in mosquitoes. This suggests that, in areas where *Wolbachia* is present at low densities, it may be more difficult to induce CI and achieve effective mosquito control.

In general, CI strength tends to decrease as host males age.^{55,58} This variation is linked to *Wolbachia* density and appears to be *Wolbachia* strain-dependent. Studies have shown that in flies infected with wRi strains of *Wolbachia*,⁵⁸ the densities of the bacteria and the expression of *cif* genes in testes decrease with male age, leading to a decline in CI strength. In contrast, in flies with wMel infections, the densities of *Wolbachia* and *cif* expression increase with male age, even though CI strength declines.⁵⁹

Wolbachia can manipulate the thermal preferences and mating behavior of their hosts, with this influence being density-dependent. For instance, the mating behavior and temperature preference of mosquitoes can be influenced by *Wolbachia*. Mosquitoes infected with supergroup A prefer cooler environments, while those infected with Supergroup B prefer warmer environments.⁵⁸ Moreover, in male *Drosophila*, higher *Wolbachia* densities can lead to an increase in male mating behavior,⁵⁴ which may interfere with the goals of CI by restoring compatibility in uninfected females⁵⁵ and ultimately reducing *Wolbachia* densities in the subsequent population.

Overall, the variation in *cifA/cifB* expression and *Wolbachia* titers among mosquitoes highlights the need for a better understanding of the complex interactions between *Wolbachia* and its host mosquitoes. Ongoing research is focused on identifying the factors that influence *cifA/cifB* expression.

3.2. *Wolbachia* quantities and pathogen blockage

Wolbachia has been shown to block the transmission of pathogens from mosquito salivary glands, which reduces the proliferation of viruses and other pathogens in mosquitoes.^{60,61} The mechanism behind this phenomenon, known as *Wolbachia*-mediated pathogen blockage (WMPB), is not yet fully understood. However, studies suggest that it involves the activation of mosquito immune system pathways and upregulation of certain genes that suppress viral particles in mosquitoes.^{62–64} Resource competition^{65,66,67} and hosts cellular machinery,⁶⁸ have also been proposed as mechanisms regulating WMPB.

Majorly, *Wolbachia* provides protection against RNA viral infections to their hosts,⁶⁹ with strains such as wAlbB, wAlbA, wPip, wMel, and wAu^{70–72} providing pathogen protection to mosquitoes. However, some strains including wExel, wMel, wAlb among others, have been shown to enhance viral infections in their hosts.^{73–75} This may have implications for *Wolbachia* attributes and could be a threat to establishment of stable populations in the field.

While some studies suggest that *Wolbachia* inhibits the entry of pathogen particles during a blood meal,⁷⁶ others propose that pathogen transmission is inhibited from the blockage of the release of pathogens from mosquito salivary glands. Interestingly, these observations were

noted in higher *Wolbachia* densities within the host's somatic tissues, suggesting that WMPB and its strength correlate with bacterial quantities.^{68,77,78}

Therefore, the effectiveness of *Wolbachia* as a tool for controlling mosquito-borne diseases depends on the stability of blocking.⁷⁹ For effective WMPB, infection within the mosquito has to be present in adults before the pathogen is taken in and is not affected by induced changes in *Wolbachia* densities.

The density of *Wolbachia*, a bacterium that depends on mosquitoes for its nutritional needs, can be affected by the presence of other pathogens within an infected mosquito. Competition for resources and space with other pathogens can slow down the multiplication of *Wolbachia*.⁶⁵ Grant et al, identified *Asaia* as the bacterium responsible for inhibiting *Wolbachia* transmission in *Anopheles* mosquitoes.⁸⁰ They suggest that microbiome incompatibility may influence distribution of *Wolbachia* in arthropods. In addition, Rupinder et al, report that viral infections regulate *Wolbachia* levels in *Drosophila* due to their high tissue tropism, which may be related to competition for space.⁸¹ Therefore, before releasing *Wolbachia*-infected mosquitoes, it is important to have information on the current presence of pathogens in the area to prevent the loss of *Wolbachia* over time.

Despite the potential benefits of using *Wolbachia* as a tool for controlling mosquito-borne diseases, there are concerns about the stability of blocking,⁸² and the potential interference of naturally infected mosquitoes with transinfected released mosquitoes.^{83–85} Therefore, models representing coinfections for WMPB should be created and tested for their effectiveness under natural environmental conditions. Furthermore, understanding the ways in which *Wolbachia* density is modulated by the host or by the pathogen is crucial for achieving stable host–pathogen relationships and selecting appropriate pressure on the mosquito, *Wolbachia*, and pathogen.

3.3. Host factors

One of the key factors that influence *Wolbachia* density in mosquitoes is the host genotype.⁸⁶ Certain mosquito species are more permissive to *Wolbachia* infection than others, and some strains of the same species are more susceptible to infection than others.

For example, the *Wolbachia* density suppressor (*wds*) suppresses bacterial transmission from mother to embryo, which determines the host interspecific difference in titers.⁸⁷ Additionally, the *gurken* (*grk*) gene, which encodes a crucial axis determinant, has a cumulative, dosage-sensitive impact on *Wolbachia* growth and proliferation.⁸⁸ *Aae-miR-12*, a differentially expressed mosquito miRNA in *Wolbachia*-infected cells, affects *Wolbachia* density in the host cells.⁸⁹ The miRNA targets two *A. aegypti* DNA replication licensing (*MCM6*) and monocarboxylate transporter (*MCT1*) genes. This mosquito genes are essential in the regulation of *Wolbachia* titers within the mosquito gonads.

Mosquito conserved signaling pathways like the Insulin signaling pathway (*IS*) and Autophagy regulate presence of *Wolbachia* in mosquito.^{90–93} The decrease in insulin is the systemic signal that leads to increased autophagy through the PI3-Kinase and TOR nutrient sensing pathways that are both downstream of insulin and play a vital role in the inhibition of autophagy.^{94,95} Therefore, there is a possibility of an interplay within these signaling pathways in regulating the symbiont in its host.

Autophagy occurs at the tip of mosquito gonads, a hub that is established during embryonic development, and anchors both the somatic and germ line stem cells, and regulates stem cell division and differentiation.⁹⁶ Autophagy genes *Atg1* and *Atg8* and autophagy selective gene *ref(2)* are the major players in this process.⁹⁷ The role of Autophagy signaling in regulating *Wolbachia* density is sex dependent. In males, *Wolbachia* density is negatively regulated in the hub by *ref(2)* gene.^{98,99} Whereas in females, the overexpression of the two *Wolbachia* effector proteins, *CifA* and *CifB*, implicate *CifB*, that act in the same

pathway with Agt1.⁹⁹ This could mean that *Wolbachia* depends on autophagy signaling pathway to increase its titers in the female germline.

Mosquitoes express varying numbers of insulin-like peptides (ILPs) in different tissues, including the nervous system, fat body, midgut, and ovaries.¹⁰⁰ Specifically, ILP1, ILP3, and ILP8 are expressed in the brains of adult females and regulate egg production and metabolism.¹⁰¹ Overexpression of insulin receptors in the mosquito fat body increases mosquito lifespan, and ILP overexpression has been shown to upregulate the mosquito immune system by inducing the production of nitric oxide (NO).^{101,102} The presence of NO in the midguts of mosquitoes limits the proliferation of pathogens.^{103,104} Surprisingly, the knockdown of the insulin receptor in certain mosquitoes leads to complete inhibition of pathogen proliferation. However, how the insulin signaling pathway modulates *Wolbachia* densities remains unexplored.

4. Environmental factors affecting mosquito-*wolbachia*-pathogen interactions

Environmental factors play a crucial role in regulating the tripartite relationship between *Wolbachia*, host mosquito, and pathogen. Temperature and nutritional status are reported to be the major extrinsic factors influencing this relationship. These factors can affect the host, pathogen, or *Wolbachia*, thus directly or indirectly impacting the positive attributes of *Wolbachia* in controlling mosquito-transmitted diseases.

4.1. Temperature

Temperature plays a crucial role in determining the density of *Wolbachia*, a bacterium that infects mosquitoes and influences their ability to transmit diseases. Insects exhibit temperature-dependent traits such as reproduction,¹⁰⁵ survival,¹⁰⁶ symbiosis, and swarming,^{107–109} which affect the geographic range of mosquitoes and the epidemiology of the pathogens they carry.

Previous studies indicate that males are often sterilized by temperatures lower than their lethal limit,¹⁰⁵ resulting in a restriction of their geographic range. Conversely, high sporadic temperatures in mosquitoes can increase their ability to transmit viruses.^{110,111} Temperature also affects the association of mosquitoes with *Wolbachia*.^{83,112}

Just like mosquitoes, *Wolbachia* is sensitive to temperature changes and exhibits a positive temperature-dependent relationship.¹¹³ This can have effects on the strength of cytoplasmic incompatibility, *Wolbachia* mediated pathogen blockage and maternal transmission fidelity.

Higher temperatures can lead to an increase in the density of *Wolbachia* within their host population. This is due to the fact that higher temperatures increase the rate of bacterial replication, allowing *Wolbachia* to multiply more quickly.^{114,115} High temperatures activate the bacteriophage lytic cycle, leading to the production of virion particles that lyse *Wolbachia* cells, causing *Wolbachia* DNA degradation and reducing their densities.^{116,117} This process leads to an inverse CI strength in mosquitoes.¹¹⁷

However, the density of various *Wolbachia* strains changes in response to temperature. Specifically, wMel and wTcon strains experience a decrease in density with an increase in environmental temperature, while wAlbB strain remains constant under heat stress.^{24,25,26} This variation could potentially affect cross infections targeting CI and WMPB in a single mosquito species.

Temperature variations on the other hand affect *Wolbachia*-mediated pathogen blocking (WMPB) in mosquitoes. Plasmodium blockage in *An. Stephensi* infected with wAlbB and dengue blockage by wMel in *A. egypti*^{118,119} are some instances in which temperature contributed to variances in WMPB. Higher temperatures increase pathogen proliferation and virulence,¹²⁰ affecting the mosquito immune system and antagonizing the *Wolbachia*-mediated immune priming,¹²¹ which directly affects WMPB. Another factor that indirectly affects *Wolbachia*

densities is the temperature effects on the gut microbiota, which enhances their proliferation, outcompeting *Wolbachia* for necessary elements.¹²²

Although temperature variations are always associated with variations in humidity, there is no single study that has independently validated the effects of humidity on *Wolbachia* density. However, a study by Anton Strunov found that higher humidity (60 %) resulted in increased thermal preferences in *Wolbachia* positive *Drosophila* flies.¹²³ Such limited studies call for more research on other environmental factors including humidity, photoperiodicity, biological rhythm which may affect *Wolbachia* within its arthropod host.

The independent physiological requirements of the host and symbiont may create a fine line between their thermal tolerances, leading to conflicts. This can result in several scenarios, including genetic differentiation between infected and uninfected mosquitoes as they adapt to different environmental conditions, over- or under-replication of *Wolbachia* under different temperatures causing costs or benefits to the host, or selection against *Wolbachia* and its loss from the host population. With global climate change, temperature is a major factor affecting the competence of vectors, particularly mosquitoes, leading to spatial migrations and distribution. Therefore, further research is necessary to understand the importance of temperature on *Wolbachia*, host and response to climate change if it is to be used as a biological control tool in an ever-changing climate.

4.2. Host diet

Wolbachia is a bacterium that relies on its host's nutritional deposits for survival, as it lacks genes for amino acid and lipid biosynthesis.¹²⁴ This creates a competition between *Wolbachia*, the host, and any potential pathogens for resources within the mosquito. The density of *Wolbachia* in mosquitoes positively correlates with the availability of nutrients within its host.¹²⁵ Therefore, the absence or decrease in nutritional resources can reduce *Wolbachia* densities in the mosquito host, indirectly affecting cytoplasmic incompatibility (CI) and *Wolbachia*-mediated pathogen blocking (WMPB).

Mosquitoes feed on sugars and female mosquitoes require a blood meal to complete their reproductive cycle. This type of feeding contributes different diet compositions for mosquitoes that contribute to their physiology, immunology, and the chemical environment around *Wolbachia*.^{85,126–128} Whereas natural sugars increase *Wolbachia* titers in oocytes, yeasts are reported to reduce *Wolbachia* titers.^{129,130}

The amino acids from the blood meal are used by the fat body for the synthesis of yolk proteins that are packaged into oocytes.¹³¹ *Wolbachia* catabolizes these amino acids produced by the host. *Wolbachia* further enters the ovaries through the host's vitellogenin transovarial transportation system into the developing oocytes using the host's nutritive cords.^{132,133} This leads to the proliferation of *Wolbachia* in oocytes as they develop, resulting into reduced egg production.¹³⁴ Therefore, it's important to understand the nutritional requirements of *Wolbachia* and its interaction with the host and pathogen.

5. Maintaining a stable *wolbachia* symbiosis

Wolbachia has a great potential in control of mosquito borne infections. However, the success of this strategy depends on maintaining a stable *Wolbachia* symbiosis in mosquito populations in the field.^{61,86,135} A stable symbiosis is one in which the bacteria are present at high frequency in the insect population and are efficiently transmitted from parent to offspring.¹³⁶ In addition, efficient transmission of *Wolbachia* from parent to offspring is necessary to maintain the symbiosis over multiple generations.¹³⁷

One of the main challenges to maintaining a stable *Wolbachia* symbiosis is the potential for the bacteria to be lost from the population over time.²⁸ This can happen if the frequency of *Wolbachia*-infected individuals drops below a certain threshold, or if the bacteria are not

efficiently transmitted from parent to offspring. To prevent this from happening, it is necessary to continuously monitor the frequency of *Wolbachia*-infected individuals in the population and take steps to increase this frequency if it drops too low.

One approach is to release large numbers of *Wolbachia*-infected mosquitoes into the environment,^{138–140} which can help to quickly establish the bacteria in the population. Another approach is to use selective breeding to increase the frequency of *Wolbachia*-infected individuals over time.¹⁴¹ This can be done by selectively breeding mosquitoes that are more likely to carry and transmit the bacteria.

Currently, the density of *Wolbachia* in the field has been controlled using similar methods that have been employed to enhance the release of sterile mosquitoes. One such method involves monitoring the population dynamics of mosquitoes before, during, and after the release of *Wolbachia*-infected individuals.^{142,143} This monitoring process allows for estimating local mosquito population size and collecting environmental data such as temperature and rainfall. The information obtained from population size estimates and seasonal patterns is valuable in determining the appropriate timing and quantity of releases for both population suppression and disease control by *Wolbachia*.

In addition, ensuring an efficient sexing system that guarantees the absence of females in the released population is crucial. Accidentally released females can mate with the released males and reproduce, potentially reducing the density of *Wolbachia*.¹⁴⁴ Releasing infected mosquitoes in a population with reciprocally incompatible populations at one site can decrease the number of biting females. However, concurrent or sequential release of males belonging to two reciprocally incompatible lines should be avoided, as this approach could drastically reduce the possibility of establishing transinfected lines.¹⁴⁴

Furthermore, it is essential to assess the mating competitiveness of incompatible males under semi-field conditions. In a study conducted by Célestine et al, competitive assessments were carried out, revealing that *Wolbachia*-infected males successfully competed with wild males for mating opportunities with wild females.¹⁴³ In addition to these strategies, it is also important to address some of the potential challenges that can arise when using *Wolbachia*-mediated disease control strategies. For example, there is a risk that the bacteria could mutate or evolve over time,¹⁴⁵ potentially leading to reduced effectiveness of the control strategy. There is also a risk that the bacteria could spread to unintended hosts, leading to unintended consequences. To address these risks, it is necessary to carefully monitor the genetic makeup of the bacteria and to conduct rigorous risk assessments before implementing *Wolbachia*-mediated disease control strategies.

6. Conclusion

In conclusion, maintaining a stable *Wolbachia* symbiosis is crucial for the success of *Wolbachia*-mediated disease control strategies. This requires continuous monitoring of the frequency of *Wolbachia*-infected individuals in the population and taking steps to increase this frequency if necessary. It also requires addressing potential challenges and risks associated with these strategies. More studies should be designed to study the coinfections with different strains having different outcomes. With an ever-increasing climate change, further studies should be conducted on independent variables like humidity, photoperiod, heatwaves on *Wolbachia* density and how they impact on its use as a biological tool for mosquito control. With careful planning and monitoring, however, it is possible to maintain a stable *Wolbachia* symbiosis and achieve effective control of insect-borne diseases.

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John Roberts Padde: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Qingyu Lu:** Data curation, Methodology. **Yuang Long:** Formal analysis, Methodology. **Donghui Zhang:** Conceptualization, Supervision, Validation, Writing – review & editing. **Min Hou:** Methodology, Resources. **Lu Chen:** Formal analysis, Methodology. **Zhipeng Xu:** Formal analysis, Resources. **Lin Chen:** Resources, Supervision, Validation, Conceptualization, Writing – review & editing. **Minjun Ji:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

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