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Advances in Research on Mosquito Population Suppression Using Wolbachia-Induced Cytoplasmic Incompatibility

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Abstract

Wolbachia pipiens is an intracellular symbiotic bacterium widely found in arthropods and nematodes. Its symbiotic and mutualistic relationships with hosts have become a representative subject in symbiotic biology research. Recently, Wolbachia has also been studied for its application in controlling human and crop diseases. This review describes the natural infection of mosquitoes by Wolbachia and further summarizes research progress on suppressing mosquito populations through artificially induced Wolbachia infections. It elucidates the molecular mechanisms of cytoplasmic incompatibility induced by Wolbachia and outlines future directions for biological control of mosquito-borne diseases based on combined SIT (Sterile Insect Technique) and IIT (Incompatible Insect Technique) strategies. Additionally, the review highlights advancements and prospects in the study of controlling mosquito-borne diseases.

1. The diversity of Wolbachia, the natural infection situation in mosquitoes

1.1 Introduction and diversity of Wolbachia

WolbachWolbachia pipiens, genusmycetozoanThe α subphylum of Proteobacteria, Rickettsiaceae Ubaque is a genus of the Ubaque tribe, which was discovered by Hertig and Wolbach in 1924 Culex pipiens Culex pipiens was first found in the reproductive tissue. Wolbachia is one of the most widely distributed symbiotic bacteria in nature, in the order Coleoptera Diptera More than 10 orders including Hemiptera Hymenoptera Lepidoptera and Orthoptera have been found to carry Wolbachia with an estimated range of 1.5 to 5 million species of insects. Wolbachia is a vertically transmitted endosymbiotic bacterium characterized by maternal inheritance.

Wolbachia genetic diversity was initially described through the 16S gene [1] and the surface protein wsp gene [2]. However, the slow evolutionary rate of 16S and the extensive recombination of wsp could not meet the requirements for resolving the systematic classification of Wolbachia [3]. Therefore, XX authors proposed that a multi-locus sequence typing [MLST] system consisting of five or more conserved housekeeping genes could serve as the standard for Wolbachia classification [4]. MLST studies supported the discovery of new phylogenetic lineages and further classified Wolbachia into genetically distinct monophyletic lineages known as "supergroups" [5]. Recently, with the aid of genomic sequences, the utility of MLST sites has been found to be insufficient for Wolbachia typing compared to better performing single-copy sites [6]. To date, Wolbachia has been subdivided into at least 17 possible systematic supergroups [named A-F, H-Q, and S] [7,8]. Notably, the vast majority of Wolbachia genomic sequences come from strains within the A and B supergroups, with some supergroups represented by a single strain.

Supergroups A and B primarily occur in arthropods and often function as reproductive parasites [9,10] by manipulating host reproduction to enhance transmission through the maternal line. In contrast, supergroups C and D enhance the fertility and development of filarial worms through obligate mutualistic symbiotic relationships [11]. Supergroup E Wolbachia infects primitive wingless arthropods such as flea worms and springtails [12,10], although its systematic classification remains under investigation due to its clear distinction from other Wolbachia supergroups making its systematic classification uncertain [13]. Supergroup F is associated with arthropods [termites] and filarial worms [Mansonella ozzardi] [14]. Supergroups H, I, J, and K occur in termites [15], fleas [16], filarial worms [17], and spider mites [18], respectively. Supergroup L Wolbachia occurs in plant-parasitic nematodes Pratylenchus penetrans, while supergroup M occurs in aphids [19]. Supergroup S is found in pseudoscorpions [20], and the hypothesized supergroup T may occur in Cimex hemipterus bed bugs [21].

Although widely accepted, the methods used for classifying Wolbachia supergroups have little variation [2 2]. For example, the supergroup G Volbachia based on Australian spider wsp sequences [23] was later identified as belonging to supergroup B [24], while the supergroup R Wolbachia based on cave spider 16S rRNA and MLST genes [25] may belong to supergroup A [26]. In summary, our understanding of Wolbachia genetic diversity is still evolving, and the appearance of unresolved or single long branches in the Wolbachia tree requires more data and analysis.

1.2 Introduction to the infection of Wolbachia in mosquitoes

This section can list the cases of Anopheles mosquitoes, Culex mosquitoes and Aedes mosquitoes, and it is recommended to refer to the latest literature review [Wolbachia prevalence, diversity, and ability to induce cytoplasmic incompatibility in mosquitoes]. In addition, we can refer to this English literature review for the general framework of our review

2. Methods for artificially establishing Wolbachia infected

mosquitoes: adult injection and embryo injection

2.1 Embryo injection

In Anopheles stephensi, Wolbachia wAlbB exhibits perfect maternal transmission and the ability to induce high levels of cytoplasmic incompatibility. Naturally uninfected A. stephensi populations can be repeatedly inoculated with infected females to construct artificially infected Wolbachia laboratory mosquito populations. Additionally, wAlbB confers resistance to the human malaria parasite Plasmodium falciparum in mosquitoes. This review illustrates the process of constructing A. stephensi strains artificially infected with Anopheles albopictus wAlbB Wolbachia, detailing the method of embryonic microinjection for artificially establishing Wolbachia-infected mosquito populations.

2.2 Injection of adult mosquitoes

Experimental studies have shown that infected Wolbachia strains can be established in adult mosquitoes through injection, and this review focuses on injecting Wolbachia strains into Aedes aegypti to introduce the method of artificially establishing Wolbachia-infected adult mosquitoes. Unlike earlier methods, it is simpler and offers multiple advantages, including no need for specialized knowledge required for embryo microinjection. The drawback of this method is that due to indirect establishment in G0 germ cells, the number of Wolbachia is lower, resulting in a higher proportion of uninfected cells [28]. This finding contrasts sharply with embryo microinjection, where zygotic eggs are directly infected with Wolbachia in the pole cell region, leading to co-infection of Wolbachia and host embryos, particularly in germ-line tissues, potentially increasing the number of Wolbachia within germ tissues.

3. Phenomenon and principle of Wolbachia-induced CI

3.1 Phenomenon

Symbiotic bacteria Wolbachia are a class of bacteria that are widely present in the cytoplasm of arthropods and nematodes, inherited maternally. They can induce various reproductive phenotypes in hosts, including cytoplasmic incompatibility [cytoplasmic incompatibility, CI], parthenogenesis [parthenogenesis, PI], feminization [feminization], and male-killing, i.e., reproductive regulation [30]. In the reproductive regulation of hosts by Wolbachia, inducing cytoplasmic incompatibility [CI] is the most common method, with reports documented in Opisthokonta, Crustacea, Myriapoda species, and Arthropoda [31-34]. Within the same species, mating between infected males and uninfected females [unidirectional CI, unidirectional cytoplasmic incompatibility] or mating between males and females infected with different strains of Wolbachia [bidirectional CI, bidirectional incompatibility] often results in incompatibility phenomena [35]. In different populations, the expression of CI varies significantly. Cytoplasmic incompatibility often manifests as embryonic death and/or sex bias favoring males [36]. The first mating scenario belongs to unidirectional incompatibility [unidirectional CI], while the second mating scenario belongs to bidirectional incompatibility [bidirectional CI]. The effect of CI confers significant reproductive advantages to infected females within the population, allowing Wolbachia to rapidly

spread within the host populationExpansion [30].

3.2 Principle

Wolbachia-induced CI has been tested for controlling Aedes aegypti populations in invasive species in the United States and other regions, and has been used to control agricultural pests [37-43]. Recently, genomic elements involved in CI induction and "rescue" have been identified. These genes, collectively known as CI factors or Cifs, form alternative binary operons where the upstream gene is called cifA and the downstream gene is called cifB. These Cifs encoding sequences are further grouped by the proven cifB coding enzyme activity: the cidB gene encodes deubitylases [DUBs], which cleaves ubiquitin from target proteins, and the cinB gene encodes a nuclease. Although the exact molecular mechanisms remain elusive, co-expression of homologous genes cidA and cidB or cinA and cinB in the male reproductive system of Drosophila melanogaster induces CI-like male sterility. When cidA or cinA is expressed alone in this line, it is sufficient to rescue CI caused by the respective cifA-cifB gene pairs. Protein binding experiments and expression of CIF genes in yeast indicate that each CifA specifically binds its homologous CifB, which may be crucial for CI induction and/or "rescue." The nuclease activity of CinB and the deubiquitinating activity of CidB are essential for inducing CI in transgenic flies and producing toxic substances in yeast [44].

Before the discovery of CI factors, several different models had been proposed to explain the mechanisms of CI induction and "rescue," all falling under the broad "modification-rescue" paradigm [45-48]. In short, it is hypothesized that the modification activity of Wolbachia modifies sperm during spermatogenesis, while the "rescue" activity in infected oocytes reverses or nullifies the original sperm modification after fertilization. Uninfected oocytes cannot be normally fertilized by this mechanism, leading to ultimately no or very few offspring. Since the discovery of Cifs, some older models have been updated, and new models have been proposed to explain how the CIF protein functions in CI. A new descriptive model, known as the "2-by-1" model, suggests that male CI induction requires both CifA and CifB, while CifA expression in the female lineage is sufficient for rescue. This model summarizes observations from several transgenic fruit fly studies, showing that males require both homologous Cifs for CI induction, while females only need CifA for "rescue."

Two general types of CI functional models have been proposed, differing in the timing and location of CI induction modification and how CifA rescues viability. In the Host Modification [HM] model, which includes but is not limited to early "misstep," "titration-compensation," and "target guardian" models [49-53], Wolbachias Cifs [now referred to as CifA, CifB, or collectively] modify infected sperm in some way, leading to CI when the modified sperm fertilizes an uninfected Wolbachia egg. In the infected egg, the rescue factor [CifA] reverses or neutralizes sperm modification, restoring normal replication and nuclear division. The "toxin antidote" or TA model is very similar to the "lock and key" model, where CifB acts as a critical CI inducer, possibly through DUB [CidB] or nuclease [CinB] activity disrupting paternal chromosomal processing, leading to abnormal or delayed paternal chromatin condensation and separation, a hallmark of CI, typically occurring during the first fertilized mitosis. This process has two possible

mechanisms [54]: one is that Wolbachia produces toxin-specific inhibition of cyclin B expression within the male pronucleus, thereby inhibiting cyclin-dependent kinase 1 [CDK1] activity and delaying the time when the male pronucleus enters mitosisIt indicates that CDK1 kinase is an important regulator controlling the transition from interphase to mitotic phase of G2 [55]. CDK requires binding with cyclin B to exhibit kinase activity, and inhibition of cyclin B synthesis can suppress the activity of CDK1 kinase [56]. Active CDK1 kinase can phosphorylate histone H3, thereby promoting chromatin condensation near mitosis. In normal embryos, the activity of CDK1 kinase persists from pre-mitosis to post-mitosis. However, in CI embryos, CDK1 kinase in the female pronucleus becomes active from pre-mitosis but loses activity by post-mitosis, while CDK1 kinase in the male pronucleus remains active until metaphase and then loses activity by late mitosis [57]. The suppression of CDK1 kinase may be a key factor delaying chromosome condensation in male pronucleus. Another possible mechanism involves Wolbachia producing toxins that damage or delay DNA replication in male pronucleus, thereby activating G2/M cell cycle checkpoints and delaying entry into the first mitosis. Studies have shown that many Gram-negative bacteria can produce CDT toxins homologous to DNase1, which damage DNA and activate G2/M checkpoints, causing the cell cycle to arrest at G2 [58][59]. High levels of CifA in oocytes are proposed to inhibit the function of introduced CifB through homology-specific bindingThese HM models also use CifB as a key modifier in isolation. Compared to HM models, TA models explicitly involve the binding of maternal supply of CifA and paternal transmission of CifB in the rescue mechanism [44].

4. Key factors affecting population suppression of Wolabchia

The key to the population suppression strategy is the artificial establishment and maintenance of a two-way [or even multi-way] cytoplasmic incompatibility model [48], which requires the artificial establishment of incompatible mosquito strains.

4.1 Vertical transmission efficiency of Wolbachia

Wolbachia is primarily transmitted vertically from mother to offspring, and vertical transmission is the fundamental mode of Wolbachia transmission within the host species [60], meaning that Wolbachia is directly transmitted to offspring via oocytes. Duron et al. [59] used Pholcus phalangioides as experimental material to test the infection status of offspring from infected females, confirming that all offspring were infected, thus verifying that Wolbachia can be inherited through the maternal lineage. The reproductive areas of adult female flies are rich in Wolbachia throughout their entire adult life, ensuring high vertical transmission efficiency of Wolbachia. Studies have found that Wolbachia has a high vertical transmission efficiency in Drosophila melanogaster, with field transmission efficiencies of 97% in *Drosophila melanogaster* and *Drosophila simulans*, and laboratory transmission efficiencies reaching up to 100% [60,62]. Vertical transmission efficiency is a crucial factor influencing the prevalence of Wolbachia within populations [60]. In *Drosophila simulans* in Australia, wAu cannot induce cytoplasmic incompatibility, while Wolbachia in *Drosophila yakuba * can induce

cytoplasmic incompatibility, and such Wolbachia can stably exist within populations entirely due to 100% vertical transmission efficiency [63].

4.2 The intensity [level] of Wolbachia-induced CI

There are many factors that influence CI, including the hosts genetic background, Wolbachia strains, Wolbachia genotypes, symbiotic bacterial density [concentration, titer], male age, environmental factors, and the distribution of symbiotic bacteria in the hosts reproductive tissues. The hosts genetic background significantly affects the strength of Wolbachia-induced CI. wDm induces weaker CI [offspring embryonic mortality rate of 18%~32%] in Drosophila melanogaster, indicating incomplete CI; however, when wDm is introduced into Drosophila simulicola via microinjection, it can cause strong CI [offspring embryonic mortality rate>98%] [65], which is considered complete CI. Similarly, popcorn strains do not induce CI in Drosophila melanogaster but can induce strong CI when introduced into Drosophila simulicola [66].

An even more interesting phenomenon is: wCauA induces CI in the Mediterranean powdery mildew moth [Cadra cautella], but in the Mediterranean powdery mildew moth Ephestia kuehniella, Wolbachias reproductive regulation of the host becomes male induction [67]. Different strains of Wolbachia exhibit different reproductive regulatory phenotypes and can induce varying degrees of CI under the same host genetic background conditions. wSca induces male induction in the Chinese bollworm Ostrinia scapulalis, while wKue induces CI in the Mediterranean powdery mildew moth; when wSca is introduced into the Mediterranean powdery mildew moth, it exhibits male induction; when wKue is introduced into the Chinese bollworm, it exhibits CI [68]. Study 4.1 also indicates that within the populations of the two-spotted spider mite in Shanghai Minhang and Changsha, Liaoning, Wolbachia can cause high-intensity CI, while in the Xingcheng population of Liaoning, Wolbachia can induce moderate CI, whereas in the Xuzhou population of Jiangsu, Wolbachia cannot induce CI.

Previous studies have also found that poor nutritional conditions [69][70], multiple matings of male flies [71], and aging of male flies [62,72,73] can all reduce the level of CI. Yamada et al. [74] further found that the shorter the developmental time required for male black-bellied fruit flies infected with Wolbachia, the higher the degree of CI caused.

The Wolbachia symbiont-induced CI levels are closely related to the bacterial load in male insects [75-77]. Additionally, the density of Wolbachia within the host may require a specific threshold for the effect of Wolbachia-induced CI expression; when the density of Wolbachia is below this threshold, CI levels begin to decline, and when the density of Wolbachia exceeds this threshold, it may have no effect on CI levels, which could be influenced by the Wolbachia strain [69].

Studies on fruit flies have found that the density and distribution of Wolbachia in the reproductive tissues of male hosts are correlated with the degree of CI [77-80]. The number of Wolbachia in the testes of fruit flies and the number of infected seminal vesicles are positively correlated with CI, but this does not represent the situation for all species.

Therefore, the ability of Wolbachia to induce CI [complete CI or incomplete CI] will vary under different circumstances and conditions.

4.3 Host adaptability of Wolbachia mosquito strains

Wolbachia The adaptive regulatory effects on the host include influencing the hosts oogenesis, embryonic development, nutritional metabolism, and the expression of immune genes [81,82]. The selection of hosts is not limited to mosquito strains but also includes other similar organisms to facilitate a more comprehensive utilization of the obtained literature.

4.3.1 Wolbachia Suitability for host organisms

Wolbachia may affect the hosts biological fitness, including survival rates, growth and development rates, lifespan, and reproductive capacity. Extensive research has confirmed that Wolbachia influences the fitness and reproduction of the two-spotted spider mite [81,83,84]. Xie et al. [2011] found that under the combined effects of Wolbachia strains and host genotypes, the impact of Wolbachia on the reproductive capacity and developmental stages of Tetranychus. urticae shows population-specific differences.

Zhao et al. found that the CI phenomenon induced by Wolbachia is stronger in the endogenous population of Drosophila melanogaster, increasing the reproductive capacity of the host, but has no significant effect on the lifespan of females or mating competitiveness of males. Wolbachia may regulate sexual selection by influencing male mating strategies; in Drosophila melanogaster and Drosophila simulans, infected males mate faster than uninfected ones [85]. Studies have shown that this faster mating speed helps infected males quickly restore reproductive affinity with uninfected females, but uninfected males transfer more sperm per mating event, indicating different mating strategies between the two sexes [86]. Infection with Wolbachia leads to decreased hemocyte concentration in female Drosophila melanogaster, reduced peroxidase [peroxidase, PO] activity, and more severe hemolysis, ultimately resulting in shortened lifespan [87-88].

4.3.2 Wolbachia and host metabolism

Iron ions are crucial for insects, including the need for iron ions in the maturation and development of eggs. Brownlie et al. [2009] found that Wolbachia can maintain the dynamic iron balance in Drosophila melanogaster under iron stress, keeping the hosts reproductive level normal. Similarly, Kremer et al. [2009] discovered that iron excess significantly affects the development of Asobara tabida in bumblebees, inducing apoptosis in eggs during oogenesis, while Wolbachia can help regulate iron dynamics in bumblebees by downregulating ferritin expression. Studies also found that Wolbachia can influence iron metabolism in cells of both Drosophila simulans and Aedes aegypti [89]. The high affinity of Wolbachia for iron ions may be due to physiological needs of bacteria or by maintaining iron ion concentrations at a certain level to avoid the induction of reactive oxygen species and apoptosis, thereby ensuring bacterial survival.

When Aedes aegypti mosquitoes are infected with virulent wMelPop strains, the fertility and hatching success of the eggs significantly decrease. Studies have found that there is

amino acid competition between Wolbachia and Aedes aegypti. Supplementing amino acids in sheep blood before and after infection can significantly increase fertility and enhance the hatching success of eggs by 30% -40% [90].

The experiment also found that Wolbachia infection reduced cholesterol levels in mosquitoes by 15 to 25 percent, but feeding them with mouse blood did not improve fertility or egg viability, suggesting that cholesterol may not be the substance [88] that mosquitoes compete with symbionts for.

4.3.3 Wolbachia and host immunity

Wolbachia regulates the expression of host immune genes to provide protection for the host while ensuring its own survival [91]. The wMelPop-CLA strain transfected into Aedes. aegyp t i can help the host combat dengue virus [Dengue virus], chikungunya virus [Chikungunya virus] [avian Plasmodium] [92]. Transferring wMelPop into Culex quinquefasciatus also induces the expression of immune genes and inhibits the development of Plasmodium within the host. Wolbachia assists in combating viral infections in Drosophila melanogaster and Drosophila simulans [93].

Wolbachia infection induces oxidative stress in Aedes aegypti cell lines, and Aedes mosquitoes respond by expressing antioxidant genes. Studies have also found that Wolbachia infection reduces Drosophila melanogasters resistance to lead by restricting the production of lead-induced peroxides, thereby inhibiting the activation of immune pathways to protect itself from lead damage [94].

Although immune activation plays a certain role in virus resistance, it cannot explain all the effects. Experiments have found that Wolbachia strains capable of inducing immune activation responses in Aedes aegypti do not help Drosophila melanogaster resist bacteria and induce immune activation [95], and only exhibit weak antiviral capabilities [96], suggesting that immune activation may occur in newly established host-symbiotic relationships. Hughes et al. [2011] observed that when different strains of Wolbachia were transfected into Anopheles cells, the newly introduced Wolbachia significantly downregulated many transcripts related to immunity, stress response, and detoxification, which was inconsistent with results observed in other insects.

4.3.4 Other effects on the host

We analyzed other hosts different from mosquito strains. The parasitic wasps were naturally infected with three strains of Wolbachia, where wAtabl and wAtab2 only caused Cl, while wAtab3 is essential for oogenesis [97]. Kremer et al. [2012] compared the transcriptomes of parasitic wasps with different tissues [ovaries, whole-headed males] and physiological conditions [symbiosis, immune challenge], finding that Wolbachia may interfere with numerous biological processes, particularly in the regulation of reactive oxygen species. Chevalier et al. [2012] identified molecules C-type lectin 1 and 2 upregulated and downregulated in maleated Wolbachia strains of Drosophila, while C-type lectin 3 was not detected in the ovaries. C-type lectin 3 is downregulated in the immune tissues of female individuals infected with symbiotic bacteria, which may affect the hosts recognition of pathogens. Additionally, in the ovaries of A. vulgare infected with symbiotic bacteria, a kinesin-related gene was downregulated. In D. melanogaster,

kinesin-1 is involved in transporting wMel to the caudal part of oocytes. In A. vulgare, kinesin downregulation may limit Wolbachia movement in oocytes.

5. Field study progress of Wolbachia-induced CI control of mosquitoes [IIT]

5.1 Application of pure IIT

IIT, or incompatible insect technique, utilizes the CI induced by Wolbachia to control mosquito populations by releasing male mosquitoes infected with a wolbachia strain into a wild population that has not been infected with this wolbachia, aiming to suppress or even eliminate the population. Infected males and females produce offspring that cannot survive or cannot produce offspring; repeated cycles of introducing only infected males can achieve the goal of suppressing or eliminating the wild population, thereby reducing the risk of vector-borne disease transmission. [43,53,98,99] An ideal IIT should exhibit a 100% sterility rate when infected males mate with wild females, while ensuring that infected males have the same mating competitiveness as wild males. Several Wolbachia strains have met these experimental conditions in current experiments and have successfully been transduced into Aedes, Culex, and Anopheles populations. However, due to several shortcomings, IIT cannot yet be considered a long-term, sustainable strategy for mosquito population suppression: IIT requires frequent releases of large numbers of sex-selectively pure male populations, which consumes significant effort and resources, making it difficult to sustainably apply in many locations. If the released mosquito population includes female mosquitoes infected with Wolbachia, their offspring will also be infected with Wolbachia over timeContinuing this way, the Wolbachia bacteria that have been introduced would be unable to accomplish their task of suppressing population numbers and thus be abandoned. In the experimental case in Singapore, due to the extremely small number of infected female mosquitoes released, the goal of using wolbachia to suppress the population of Aedes aegypti failed [43].

5.2 SIT-IIT control of mosquitoes

The foundation of both SIT and IIT is to release processed male mosquitoes that carry sterility or lethal factors into the target population. In the absence of a powerful and efficient method for sex separation in Aedes aegypti, the combination of SIT and IIT has become a solution to compensate for technical deficiencies: in the experimental case in Singapore, technicians attempted to combine SIT with IIT by using low doses of radiation to ensure that a small number of infected female mosquitoes are sterile, aiming to address the issue of Wolbachia infection in females being passed on to offspring through maternal inheritance, thereby achieving population suppression [43].

Aedes albopictus is the primary vector for major human arboviral pathogens, including dengue fever, chikungunya, yellow fever, and Zika virus. In Guangzhou, China, the combined application of SIT and IIT was used in a small open trial concerning Aedes albopictus. Using Aedes albopictus with dual infection [wAlbA and wAlbB] as the subject, a triple-infection HC strain was established, where male mosquitoes in this strain can induce high levels of CI through appropriate hybridization, while female mosquitoes

can significantly reduce the transmission of arboviruses such as dengue and Zika. Additionally, it was observed that female mosquitoes in the HC strain can achieve sterility with extremely low doses of radiation. Due to the supplementation of SIT technology, although a small number of female mosquitoes were released, radiation treatment ensured their sterility, further reducing the viruss transmissibility; male mosquitoes, due to wPip [a third Wolbachia strain artificially introduced] and low-dose radiation, are completely sterile. On the other hand, experimental results showed that the radiation dose in the peripheral areas treated by radiation was 10% lower than that at the center, which may affect the sterility of treated female mosquitoes [100]. Before combining SIT and IIT in actual mosquito population suppression processes, we need to experimentally investigate the effectiveness of this low-dose radiation treatmentUnder the goal of mosquito sterility, whether it will affect the mating competitiveness of male mosquitoes infected with Wolbachia in the HC and GUA strains as well as their Wolbachia-induced CI [100]. Considering the combination of IIT and SIT, we should also take into account the impact of simultaneous Wolbachia infection and radiation treatment on host competitiveness

After experimental [101] research, some physiological traits of Mexican Aedes aegypti strains infected with wAlbB [including reproductive capacity, pupal size, and lifespan] have changed, while the Brazilian strains under the same infection condition did not exhibit these changes due to wAlbB infection. It can be concluded that the physiological characteristics and adaptive changes of mosquitoes after Wolbachia infection depend on the hosts genetic background. This finding can serve as a key factor in studying various physiological characteristics following Wolbachia transfection, including induced sterility, reproductive capacity, mating competitiveness, etc. Additionally, we should consider the impact of different research conditions such as the type of blood used, gene delivery methods [such as thoracic injection or ovary injection in adult mosquitoes], the generation of the mosquito strains used, and environmental and equipment types on these differences observed in the studies.

According to several recent studies, the combination of IIT and SIT is considered a safe and sustainable method for achieving population suppression of mosquito populations below the minimum density that can transmit diseases such as dengue fever and Zika virus. Experiment [101] demonstrated that both WB2-BRA and WB2-MEX strains of Aedes aegypti could achieve the goal of population suppression. A small-scale experiment conducted in Thailand to suppress Aedes aegypti populations using dual transfection with wAlbA and wAlbB also yielded successful results [102].

Due to the current lack of the most direct and efficient techniques for separating male and female mosquitoes, to achieve the safe and successful population suppression goal through the combination of SIT and IIT, we need to consider two key factors: one is the Wolbachia infection status and its ability to hinder virus transmission; the other is ensuring that the radiation treatment achieves complete sterility in female mosquitoes, avoiding the accidental release of fertile infected females [101].

6. Economic benefits of population suppression

Dengue fever and other mosquito-borne diseases pose significant challenges to healthcare systems and society. Currently, the widespread prevention and control of mosquito-borne diseases are limited to avoiding mosquito bites and vector control measures, primarily based on insecticides and community-participatory environmental management initiatives [103]. Treatment mainly involves supportive care, with a lack of licensed antiviral prophylactic or therapeutic treatments [104]. Of course, in addition to traditional vector control methods, innovative "technologies" for interrupting the transmission of mosquito-borne diseases are also in rapid development, including the release of Wolbachia-infected mosquito strains, which reduce the ability of Aedes aegypti to transmit dengue, Zika virus, chikungunya, and yellow fever [105,106]. Female mosquitoes infected with the bacterium can pass it on to their offspring, leading to vertical transmission of Wolbachia across generations. Increasing evidence suggests that large-scale deployment of Wolbachia-infected mosquitoes is effective in different regions, significantly reducing dengue incidence [107-109]. Given the challenges posed by mosquito-borne disease transmission to healthcare systems and society as a whole, public health officials must determine where to allocate scarce resources to manage these issues and response measures. Cost-effectiveness analysis [CEA] is typically used for allocating healthcare resources, selecting interventions based on incremental cost-effectiveness ratiosTo achieve the optimal allocation of resources [110], and to achieve the economic benefit of population suppression, that is, to obtain the highest blocking rate at the lowest cost

[1] Proportional release strategy

When developing a release strategy, the following issues need to be addressed:iWolbachia The threshold for successful diffusion to the entire mosquito population;iiWolbauchia Stable frequency threshold in mosquito populations;iii. Wolbachia The time required for mosquito populations to stabilize. Literature [111] first conducted a dynamic analysis of the system under extreme conditions and precisely provided the threshold for infection frequency: when replacing wild mosquito populations with those carrying Wolbachia, the initial release frequency of Wolbachia must be at least greater than this threshold. Meanwhile, theoretical analysis of the model showed that if the infected Wolbachia mosquitoes are in a fitness disadvantageous situation, when the infection does not alter lifespan, as long as the infection frequency remains strictly above the threshold [threshold value] for no less than the pre-reproduction period, the spread of Wolbachia will succeed. It also revealed a phenomenon: the minimum release amount of infected mosquitoes is sufficient and time-insensitive, while the waiting time almost increases linearly. Assuming a fixed time and a higher proportion of infected males compared to females, when the ratio increases appropriately, the waiting time decreases rapidly, but when the ratio increases further, the effect becomes less noticeable. Through the analysis of the time-lag model, it was found that the optimal release strategy occurs when the male-to-female infection ratio is 1:5.

[2] Comprehensive control strategy

In some field experiments, Wolbachia requires several months to become fixed in

mosquito populations [112]. During outbreaks of diseases transmitted by mosquitoes, it is difficult for a single Wolbachia control to alleviate this epidemic in a short period of time. Although pesticides are harmful to the environment, they can still be sprayed to kill flying mosquitoes. Integrating wolbachia control with pesticide control is known as integrated control strategies. Considering cost savings, pesticides are only sprayed when mosquito populations reach a certain economic threshold, which is the lowest density of mosquito populations and would cause economic losses. This is referred to as pulse state feedback control strategies [113]. Many studies have used mathematical models to develop control strategies for mosquito-borne diseases. However, most of these studies focus on a single control method. For example, Zheng et al. [2014] studied Wolbachia infection dynamics using delay differential equations. Endy et al. [2015] discussed the impact of introducing Wolbachia into mosquito populations on human dengue cases. In Yazhi Li and Xianning Lius mathematical model, they investigated the relevant technologies of integrated control strategies for mosquito control, noting that under complete replacement or before the solution of a single control model reaches a stable state, if the total number of mosquitoes exceeds the economic threshold,The total number of mosquitoes under integrated control is less than that under single control. Therefore, the integrated control strategy is superior to the single control strategy

7. Conclusions

The use of Wolbachia-based biological control strategies provides a relatively safer, more environmentally friendly, and sustainable approach for the long-term control of mosquito-borne diseases. Currently, many laboratory and field studies have successfully achieved the suppression and replacement of mosquito populations using Wolbachia-transferred organisms. Regarding population suppression, the combination f SIT and IIT is currently the safest and most sustainable method derived from experiments. With the continuous advancement of molecular biology techniques, the molecular mechanisms induced by Wolbachia such as CI will become more transparent, and the changes in mosquito adaptability and physiological characteristics will be clearer. Furthermore, with the further optimization of Wolbachia infection technology, the suppression effect will be significantly improved. At the same time, with the development of technologies that can achieve complete separation of males and females, the application of IIT will have broader prospects. Under the premise of optimizing infection methods and ensuring male-female separation, the economic cost of population suppression will be significantly reduced, and its feasibility will be greatly enhanced.

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