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REVIEW ARTICLE

Holobiont perspectives on tripartite interactions among microbiota, mosquitoes, and pathogens

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Mosquito-borne diseases like dengue and malaria cause a significant global health burden. Unfortunately, current insecticides and environmental control strategies aimed at the vectors of these diseases are only moderately effective in decreasing disease burden. Understanding and manipulating the interaction between the mosquito holobiont (i.e., mosquitoes and their resident microbiota) and the pathogens transmitted by these mosquitoes to humans and animals could help in developing new disease control strategies. Different microorganisms found in the mosquito's microbiota affect traits related to mosquito survival, development, and reproduction. Here, we review the physiological effects of essential microbes on their mosquito hosts; the interactions between the mosquito holobiont and mosquito-borne pathogen (MBP) infections, including microbiota-induced host immune activation and *Wolbachia*-mediated pathogen blocking (PB); and the effects of environmental factors and host regulation on the composition of the microbiota. Finally, we briefly overview future directions in holobiont studies, and how these may lead to new effective control strategies against mosquitoes and their transmitted diseases.

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INTRODUCTION

Mosquitoes transmit a variety of devastating mosquito-borne pathogens (MBPs), such as dengue virus (DENV), *Plasmodium*, Zika virus (ZIKV), West Nile virus, chikungunya virus (CHIKV), and Saint Louis encephalitis virus [1], by blood feeding on an infected host and then transmitting pathogens when mosquitoes feed on a new susceptible host. In recent decades, mosquitoes and the diseases they carry have expanded in distribution worldwide due to factors such as climate change, urbanization, international travel and trade, resulting in a heavy financial burdens for affected countries [2]. For instance, DENV, one of the mosquito-borne viruses (MBVs) mainly transmitted by *Aedes aegypti*, is estimated to cause 390 million infections annually, of which 96 million present with clinical symptoms [3]. According to the WHO's latest world malaria report, malaria infected over 229 million humans and caused more than 400,000 deaths in 2019. In 2020, there were an estimated 241 million new cases and 627,000 malaria-related deaths in 85 countries [4].

Due to the lack of efficient vaccines or drugs for mosquito-borne diseases, mosquito control remains the primary target for disease prevention. The most common methods are the use of insecticides or insecticide-treated bed nets. However, the overuse of chemical insecticides has led to increasingly severe resistance issues developing in mosquitoes as well as harmful effects on non-target organisms [5]. New novel, efficient, and economical strategies to control mosquito-borne diseases are needed urgently.

The term symbiosis is often used to describe the relationship between bacteria and their invertebrate hosts [6]. This relationship is often assumed to be beneficial to both parties, but symbiont-host interactions are often complex and range from being beneficial to being antagonistic. The term holobiont was proposed originally by Rosenberg to describe the dynamic relationship between corals and their symbiotic microbes, and later used to explain the interaction between hosts and all of their interacting resident partners (bacteria, fungi, viruses, and protists) more generally [7]. Mosquitoes are home to various microbes and, like corals, also represent a complex of interactions that represent a holobiont (Fig. 1), in which some partners (mosquitoes and their resident microbiota) are inseparable whereas other microbial components hitchhike along with resident microbes [8]. By understanding these interactions between holobiont members and MBPs, it may be possible to develop novel and targeted strategies for controlling mosquito-borne diseases that can eventually be applied in the field [9].

In this review, we focus on the tripartite interactions among microbiota, mosquitoes, and pathogens from a holobiont perspective. Firstly, we consider the impact of microbiota on mosquito hosts and explore interactions involving microbiota-mosquito-pathogen components. Secondly, we discuss the potential regulatory role of mosquito hosts and ecological factors on their microbiota and these interactions. Thirdly, we suggest future research directions that could lead to potential applications in control of mosquito-borne diseases.

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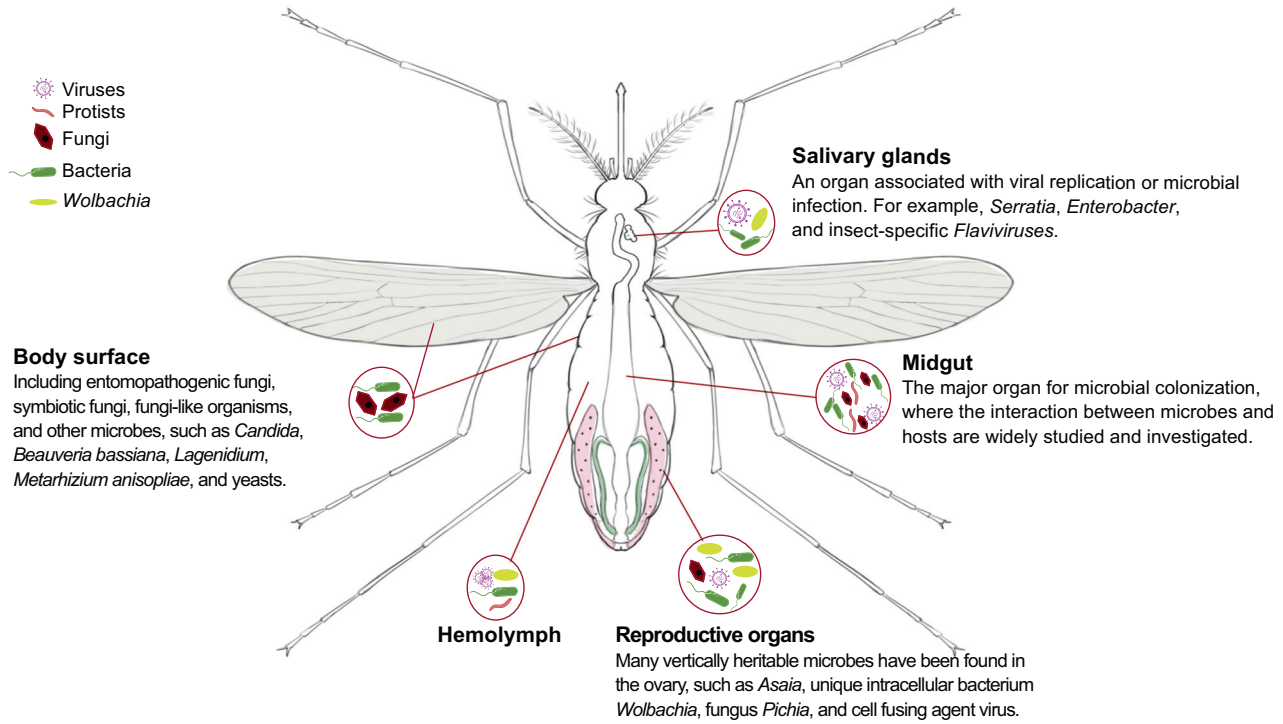

Fig. 1 Mosquito holobiont: the chimera of the mosquito, its microbiota, and interactions between them. The microbiota represents the complex of all microbes that live in or on the host body by mutualism or commensalism, including bacteria, fungi, viruses, and protists. They are generally considered to be non-pathogenic. Different mosquito populations and species can share similar microbiota, which may depend on various factors related to the host, microbiota, environmental factors, host-microbiota combinations, hologenomic variation, and other factors.

Mosquito microbiota modulate host physiology

Mosquitoes harbor a complex and diverse microbiota, including bacteria, fungi, viruses, protists, and other microbes. These microbes usually colonize different organs and tissues of mosquito, such as the gut, salivary glands, reproductive organs, and hemolymph. Of particular interest is the dynamic and diverse microbiota that assembles in the mosquito midgut, which serves as the organ for food digestion and plays a crucial role in the immune response. In recent decades, an increasing number of studies have focused on the effects of mosquito microbiota (especially gut-associated microbiota) on a host. These include (1) host physiology and life history, with diverse microbes such as *Comamonas, Chromobacterium violaceum*, *Klebsiella* sp., and *Aeromonas* sp. contributing to many aspects of the mosquito physiology and life history, such as lifespan, nutrition, mating choice, reproduction, development, fecundity, fertility, and metabolism; (2) pathogen defense, such as vector competence, immunity response, and pathogenicity; and (3) other host features, such as host insecticide resistance and thermotolerance (Table 1). However, given the high level of microbial diversity and the complex interactions between mosquitoes and their microbiota, significant knowledge gaps remain particularly around the underlying mechanisms that drive these effects [10]. Further work is needed to clearly understand the mechanisms between mosquito microbiota and their host phenotypes.

Mosquito-microbiota-*Plasmodium* interactions

Mosquitoes, like other insects, possess an innate immune system that directly responds to various pathogen invasions and differs from adaptive immunity. Of the three major signaling cascades of the mosquito's innate immune system [11], the Toll pathway mainly protects the host against fungi, viruses, and bacteria, while the Immune deficiency (IMD) and Janus kinase (JAK)-signal transducers (JAK-STAT) pathways are involved in responding to bacteria, malaria parasites, and viruses infections [12]. All three pathways contribute to *Plasmodium* resistance, as there is an overlap between antibacterial and antimalarial defense in mosquitoes [13]. Recently, it has been shown that mosquito microbiota interacts with mosquito immune processes, affecting *Plasmodium* infection by stimulating host immune signals or secreting effectors (Fig. 2A). For example, in *Anopheles* mosquitoes, silencing immune effector genes expressed by the bacterium *Serratia* Y1 rescued a protective effect against *Plasmodium* [14]. This finding indicates that the inhibitory effect of *Serratia* Y1 is achieved via the expression of mosquito immune genes. Similarly, a naturally sourced *Serratia ureilytica* (Su_YN1) strain induces a response in the host *Anopheles sinensis* against parasitic infection via secreting an antimalarial lipase [15]. *Asaia*, a symbiont of several mosquitoes, has been introduced into *An. stephensi* and shown to play an anti-malaria role by triggering the mosquito immune response [16]. Recent reviews have covered progress in understanding mosquito-microbiota-*Plasmodium* interactions and the development of a *Plasmodium* transmission-blocking strategy based on microbiota [17–19]. Multiple interactions between these microbes and their effectors on mosquito physiology and *Plasmodium* parasites pave the way for developing paratransgenesis (discussed below) to reduce vector competence.

Mosquito-microbiota-arbovirus interactions

Mosquito-borne viruses mainly comprise pathogenic mosquito-borne viruses (MBVs) and other non-pathogenic viruses (insect-specific viruses, ISVs). MBVs include DENV, ZIKV, Yellow Fever virus, and other viruses infecting humans and animals. ISVs do not directly infect vertebrates, but only replicate in insect hosts and may spread vertically within populations [20]. Mosquito-specific viruses (MSVs), such as the families *Flaviviridae* and *Bunyavirales*,

Table 1. Influence of specific microbes on mosquito physiology and pathogen transmission.

Mosquito species	Microbiota species	Locations	Functions	Refs
Aedes aegypti	*Wolbachia**	Malpighian tubules, ovaries, and testes	Life span, CI, PB effect, immune activation, inhibition parasites	[78, 85, 86]
	Serratia marcescens	Gut	Arboviruses infection promotion, colonization, and blood-feeding	[29, 87]
	Beauveria bassiana		Life span, PB effect	[88]
	Talaromyces		DENV infection promotion, blood-digesting	[89]
Aedes atropalpus	*Comamonas*	Gut	Development and oviposition	[90]
Aedes albopictus,	*Asaia**	Unknow	Immune activation and against *Plasmodium*	[16]
	*Wolbachia**	Ovaries, midguts, and salivary glands	CI	[91]
Aedes aegypti, Anopheles stephensi, Anopheles gambiae Anopheles coluzzii*	*Asaia*	Gut, ovaries, testes, and salivary glands	Life span, nutrition, development, immune activation, against *Plasmodium*, insecticide resistance	[16, 92, 93]
Anopheles gambiae	*Enterobacter*	Gut	Against *Plasmodium*	[67]
	Serratia marcescens		Immune activation	[79]
	Pantoea agglomerans		Against *Plasmodium*	[94]
	Penicillium chrysogenum		Immune inhibition, enhance *Plasmodium* infection	[95]
Anopheles stephensi	*Serratia ureilytica* Su_YN1	Gut	Against *Plasmodium* via secreting an antimalarial lipase	[15]
	Serratia marcescens		Agaist *Plasmodium*	[96]
	Beauveria bassiana	Gut, hemocoel	Life span	[97]
	Wickerhamomyces anomalus	Gut	Against *Plasmodium*	[98]
	*Wolbachia**	Midguts, salivary glands, fat bodies, and ovaries	PB, CI	[99]
Anopheles sinensis	*Serratia* Y1	Gut	Immune activation, inhibit *Plasmodium*	[14]
Anopheles coluzzii	*Chromobacterium violaceum*	Unknow	Life span, blood-feeding, reproduction	[100]
	Serratia	Gut	Insecticide resistance	[93]
Culex pipiens	*Klebsiella* sp.	Unknow	Nutrition, development, oviposition	[101]
	Aeromonas sp.	Unknow	Nutrition, development, oviposition	[101]
Culex quinquefasciatus	*Wolbachia**	Ovaries, and salivary glands	CI, PB	[35]

*Transinfection.
CI cytoplasmic incompatibility, *PB* pathogen blocking.

belong to a subgroup of ISVs [21]. Studies have shown that ISVs suppressed the replication of MBVs in co-infected mosquito cells. The first described example was a cell-fusing agent virus isolated from *Ae. aegypti* cell lines that can induce a cell-fusing phenotype in *Aedes albopictus* cells and inhibited MBV replication [22]. Recently, another mosquito ISV, Espirito Santo virus, has been shown to inhibit DENV replication and spread in *Ae. aegypti* or *Ae. albopictus* cell lines [23]. These results provide new insights and indicate that ISVs could be potential tools for controlling arboviruses through several mechanisms such as blocking the regulation of MBVs, providing the basis for the development of new vaccines.

The majority of viruses in mosquitoes are not arboviruses, with the relative abundance of arboviruses representing less than 1% of the total virome [24, 25]. In addition, the viral load varies among mosquito tissues, with a higher abundance of RNA viruses in the legs and salivary glands of mosquitoes [24, 25]. A growing number of studies support the notion of a core viral population in mosquitoes that can be transmitted vertically across multiple generations [26]. The composition, diversity, distribution, and function of new MBVs and ISVs needs further study to understand their potential role in preventing and controlling mosquito-borne diseases [27].

Several examples have emerged showing that the mosquito microbiota interacts with MBVs through the secretion of secondary metabolites (Fig. 2A). For instance, a midgut symbiotic bacterium of *Ae. aegypti*, *Chromobacterium* species Panama, prevents DENV attachment within the mosquito and degrades the viral envelope protein [28]. Conversely, *Serratia marcescens* facilitates the arboviral load in *Ae. aegypti* through the *Sm*Enhancin protein, increasing host susceptibility to DENV, as verified in wild populations lacking this bacterium [29]. Similarly, *Serratia odorifera* enhances the susceptibility of *Ae. aegypti* to DENV or CHIKV by secreting P40 proteins that bind to mitochondrial proteins in mosquito midgut cells [30]. Although these components of the microbiota may provide a way of blocking the transmission of MBVs, the specific mechanisms involved remain unknown. Until now, only the interaction between the intracellular bacterium *Wolbachia* and MBVs has been widely studied.

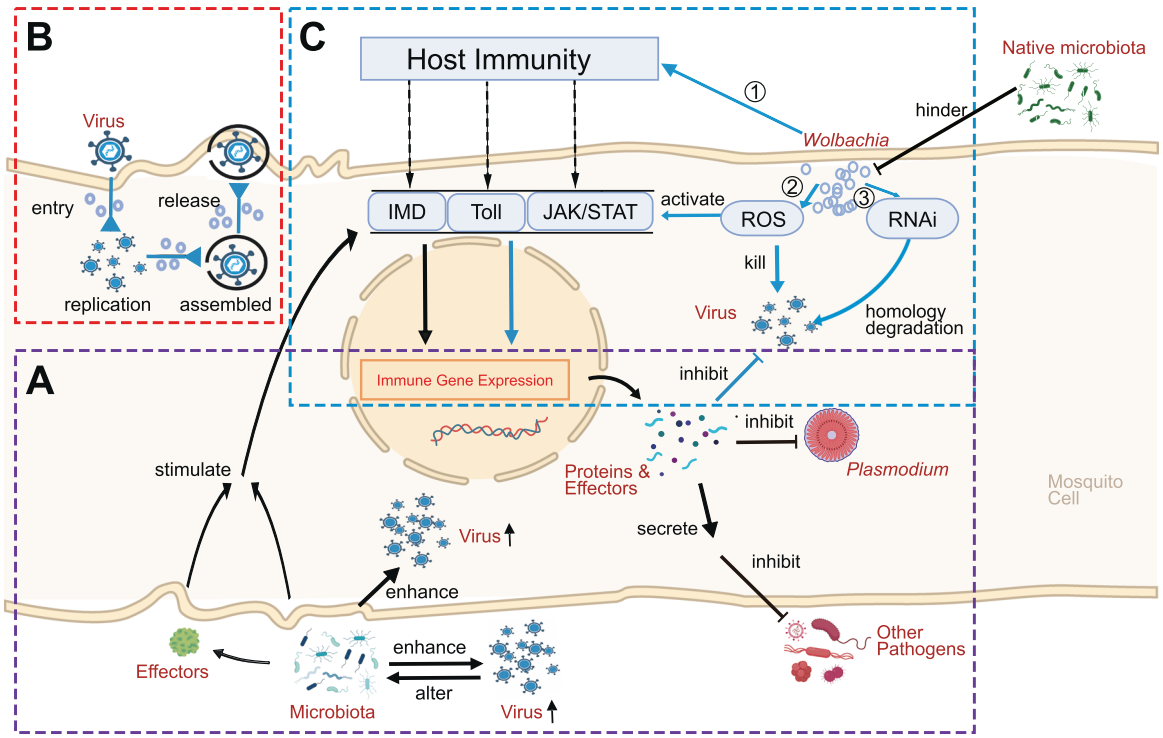

Fig. 2 Schematic diagram of the microbiota-mosquito-pathogen interactions. A The mosquito microbiota modulates mosquito pathogen infection by stimulating host immune signal regulation or secreting effectors, or by enhancing intracellular/extracellular viral infection through different mechanisms. Meanwhile, viral infection can alter the composition of the mosquito microbiota. **B** *Wolbachia* induces a PB effect by disrupting or competing for cellular cholesterol and lipid homeostasis in viral infection. **C** *Wolbachia* induces a PB effect by stimulating mosquito immunity. (1) *Wolbachia* activates the IMD, Toll, and JAK/STAT pathways to increase the expression of downstream effectors. (2) *Wolbachia* directly kills pathogens through the ROS pathway, which may participate in other immune processes. (3) *Wolbachia* induces homology degradation of viral RNA by the RNAi pathway. Blue arrows: the process of a *Wolbachia*-induced PB effect mediated through mosquito immunity; Blue arrows with a triangle (in box B): *Wolbachia*-induced PB in viral transmission. Black arrows: other microbiota-induced pathogen inhibition.

Wolbachia is an obligate endosymbiont that naturally infects various arthropods, including many mosquito species, such as *Ae. albopictus*, *Culex pipiens*, and *Culex quinquefasciatus* [31]. *Wolbachia* infection can affect multiple reproductive traits of their insect hosts, including the feminization of genetic males, induction of parthenogenesis, the killing of male progeny, and cytoplasmic incompatibility [32]. In mosquitoes, cytoplasmic incompatibility occurs in several genera [33], and female infertility occurs when females emerge from *Wolbachia*-infected quiescent eggs [34]. Transinfected *Wolbachia* block pathogen transmission and replication in mosquitoes, most notably the blockage of arboviruses in *Ae. aegypti* [35, 36]. This phenomenon, called pathogen-blocking (PB), has been widely studied due to its potential to generate (when combined with cytoplasmic incompatibility) a self-spreading mechanism that reduces disease burden (Fig. 2B) [37]. Because of these critical properties, *Wolbachia*-carrying mosquitoes can be released to suppress or modify the mosquito populations. Multiple cellular mechanisms of *Wolbachia*-induced PB have been reported, and here we mainly focus on the interactions between PB and the mosquito holobiont (*Wolbachia*, mosquito, and other symbionts).

Recent studies have revealed that the *Wolbachia*-induced PB effects in mosquitoes are related to the mosquito immune system (Fig. 2C). Infection with *Wolbachia* strongly activates the Toll pathway and regulates proteins involved in reactive oxygen species (ROS) production, leading to oxidative stress changes and inhibiting DENV proliferation in *Wolbachia*-infected mosquitoes by antimicrobial peptides [38]. *Wolbachia* also regulates the production of ROS-related proteins and antioxidant production, which subsequently reduces the ZIKV polyprotein in the presence of *Wolbachia* [39]. *Wolbachia* can utilize long non-coding RNAs of *Ae. aegypti* to activate the anti-Dengue Toll pathway through the ceRNA network that manipulates ROS abundance, suggesting potential interactions between Toll and ROS pathways [40]. In *Wolbachia*-infected *Drosophila*, ROS has also been shown as important for *Wolbachia*-mediated antiviral protection [41]. Regulation of the mosquito immune pathway genes may depend on host backgrounds, and is influenced by the microecology of the holobiont. For example, native microbes, such as those from the genus *Asaia*, can hinder the transmission and proliferation of *Wolbachia* by activating the host immune system [42]. This interaction between *Wolbachia* and the native microbiota of mosquitoes deserves further exploration because it may influence the development of stable transinfected *Wolbachia* and their spread in the field. In addition, the RNA interference (RNAi) pathway plays a key role in *Wolbachia*-induced antiviral defense. *Wolbachia* infection affects the expression of the RNAi pathway-related gene AGO1, and silencing AGO1 reduces miRNA expression and significantly inhibits the replication of DENV [43]. Furthermore, *Ae. aegypti* p400 regulates siRNA pathway activity and has antiviral activity against Semliki Forest virus (SFV), CHIKV, and Bunyamwera virus by controlling the expression levels of another RNAi pathway associated-gene, AGO2 [44], although this is not a key component in *Wolbachia* blocking of the Mayaro virus in *Ae. aegypti* [45]. However, RNAi may not be essential for *Wolbachia*-mediated PB in *Drosophila*, as it may not be tightly related to the expression and function of other proteins (such as Dicer 2) in the RNAi pathway [46].

Although *Wolbachia* often leads to changes in mosquito immunity and other signal pathways, mosquito immune activation

is not regarded as the sole mechanism involved in PB. In particular, competition between viruses and *Wolbachia* for essential intracellular resources may reduce viral replication, thus moderating PB in *Drosophila* and mosquitoes [47]. These diverse mechanisms may lead to a range of interactions between the mosquito holobiont and *Wolbachia* affecting *Wolbachia*-induced PB.

Wolbachia-mediated PB has inspired the development of novel strategies for controlling MBV based on a population replacement approach. In addition, releases of male *Wolbachia* that induce female sterility have been used to suppress populations. Large-scale field releases of *Wolbachia*-infected *Aedes* mosquitoes have now occurred in different countries, such as Australia, China, Brazil, Indonesia, Malaysia, and Vietnam, aimed at replacing existing populations with those infected by *Wolbachia* [37, 48–50]. The pathogen blocking efficiency of transinfected *Wolbachia* in *Ae. aegypti* has now been demonstrated in several releases. Population replacement by *w*Mel infected *Ae. aegypti* has proven effective for more than 10 years [37] and has led to a reduction of more than 60% in the incidence of DENV and CHIKV in human populations [51–53]. Other releases with *Ae. aegypti* transinfected with *w*AlbB *Wolbachia* sourced from *Ae. albopictus* have shown similarly promising effects [54]. *Wolbachia*-based field applications have shown that this approach can stably, efficiently, and continuously block the transmission of MBVs. Follow-up studies should focus on the efficient optimization of mosquito production to expand the scale of releases and continued monitoring to ensure that high *Wolbachia* frequencies and associated PB are maintained in populations without other adverse effects. By addressing these issues, the effectiveness and sustainability of *Wolbachia*-mediated PB as a novel strategy for controlling MBVs can be validated.

Genetically engineered microbiota-based tools to block disease transmission

An alternative strategy for blocking the transmission of MBPs, especially *Plasmodium*, is through paratransgenesis, which involves the expression of effector genes from mosquito resident symbiotic bacteria rather than the mosquitoes themselves. To date, quite a few microbes have been engineered to express effector molecules against pathogens, which have proven effective in blocking the transmission of MBPs. Recently, a bacterium strain, *Serratia marcescens* AS1, isolated from the *Anopheles* ovary, has been shown to stably colonize the mosquito midgut and reproductive organs and can be transmitted both horizontally and vertically. This strain has been engineered to inhibit the development of *Plasmodium falciparum* by expressing five effector genes $(MP2)_2$-scorpine-$(EPIP)_4$-Shiva1-$(SM2)_2$ [55]. Another engineered fusion effector protein secreted by the bacterium *Asaia* can inhibit the development of *Plasmodium berghei* in *Anopheles stephensi* mosquitoes [56]. Additionally, entomopathogenic fungi (*Metarhizium anisopliae*) can be engineered to express the antimalarial effector protein SM1-scorpine to block mosquito transmission with *Plasmodium* infection at high efficiency [57]. Viruses like *Densonucleosis* virus can be used as a paratransgenesis vector to express anti-*Plasmodium* genes or insect-specific toxins for mosquito control after being transmitted over generations [58].

Paratransgenesis is an exciting research area with potential to provide an ingenious way to control mosquitoes and reduce disease transmission, although it may be difficult to screen efficient effectors [59]. In the future, a combination of transgenesis and paratransgenesis may be feasible for controlling mosquito-borne diseases. However, releasing engineered bacteria into the environment may have unpredictable consequences. In addition to conducting appropriate scientific experiments and complying with regulatory regulations, a social license to operate and broader public and environmental health impacts must be considered before conducting field trials.

The acquisition and composition of mosquito microbiota: environmental effects and host regulation

Various intrinsic and extrinsic factors play a role in the acquisition and composition of the mosquito microbiota. Understanding the interaction between mosquito life stages and their environmental factors on the microbiota is necessary to clarify the relationship between mosquito hosts and their microbiota (Fig. 3). A mosquito's life cycle goes through aquatic and terrestrial stages, and its larvae and adults occupy different ecological niches. Mosquito larvae feed on detritus, microbes, and small invertebrates in the aquatic environment, and as a result, the microbiota of larvae is vulnerable to the aquatic environment and diet (Fig. 3A) [60]. It has been proposed that the mosquito larvae establish their initial microbial community from the aquatic environment, as there is a high similarity between bacterial populations in breeding sites and the composition of midgut microbiota in larvae, [61]. Similarly, the composition of adult microbiota can be affected by their habitat, which affects the dominant microbiota of each mosquito [62]. The microbial composition of field-captured mosquitoes from different environments can be diverse, highlighting the role of environmental factors in shaping microbiota formation [63]. Overall, these results suggest that the ecology of breeding sites determines the initial microbiota mosquitoes acquire from their environment.

The mosquito-related microbiota (mainly bacteria) depend on some nutrients introduced through a mosquito's diet for growth; thus, the nutritional composition of the food source may directly affect the composition of a mosquito's microbiota [64]. The mosquito's blood meal, in particular, can significantly affect their microbiota, with consequences that include the transmission of pathogens from a host mosquito to a human (Fig. 3B) [65, 66]. Recent studies have shown that blood meal effects on microbiota include (1) promoting the microbe (*Enterobacter* sp. Bacterium, *Esp_Z*) to proliferate and result in a 100–1000 fold expansion of the *Esp_Z* population [67]; (2) reshaping the community structure of intrinsic microbiota in mosquitoes [68, 69]; and (3) determining the diversity of mosquito microbiota; i.e., a rapid increase of bacterial abundance after blood-feeding is often accompanied by a decrease in bacterial diversity [61]. Blood-feeding, therefore, has a profound and long-lasting impact on the composition of mosquito microbiota.

Recent studies have shed light on the selective environment of the mosquito larval gut, which is known to play an essential role in regulating the initial composition of the larval microbiota by limiting it to a few aquatic microbes [70], strongly implying a role for host immunity in reshaping the microbiota (Fig. 3A). Host micro-ecosystem conditions, such as reflected by redox potential, pH, immune responses, and lytic enzymes, are thought to be responsible for establishing and maintaining the initial larval microbiota by preventing the growth of some bacteria and/or promoting others (Fig. 3A) [8, 70]. When the larvae go through the pupal stage, many microbes are excreted, but they can still be found in adults (Fig. 3C) [71], indicating that mosquito regulation of its microbiota has carryover effects from larvae to adults (Fig. 3C) [72]. More specifically, the microbial composition of adults relies on the larval/ pupal microbiota and trans-stadial transmission. In this process, the diversity and distribution in larval and adult microbial communities are not the same, reflecting a process of screening and filtering environmental microbes through specific host-mediated regulation, which then reshape and stabilize the microbial structure.

The changes in endogenous microbiota in mosquito hosts after blood feeding may be derived from the expression and regulation of heme peroxidase genes (Fig. 3B) [73]. Blood-feeding drastically reduced the expression of the Culicinae lineage-specific gene, *AsHPX2*, and promoted the growth of midgut bacteria [74]. Conversely, blood-feeding induced the high expression of an immunomodulatory peroxidase gene, *AsHPX15*, and promoted the

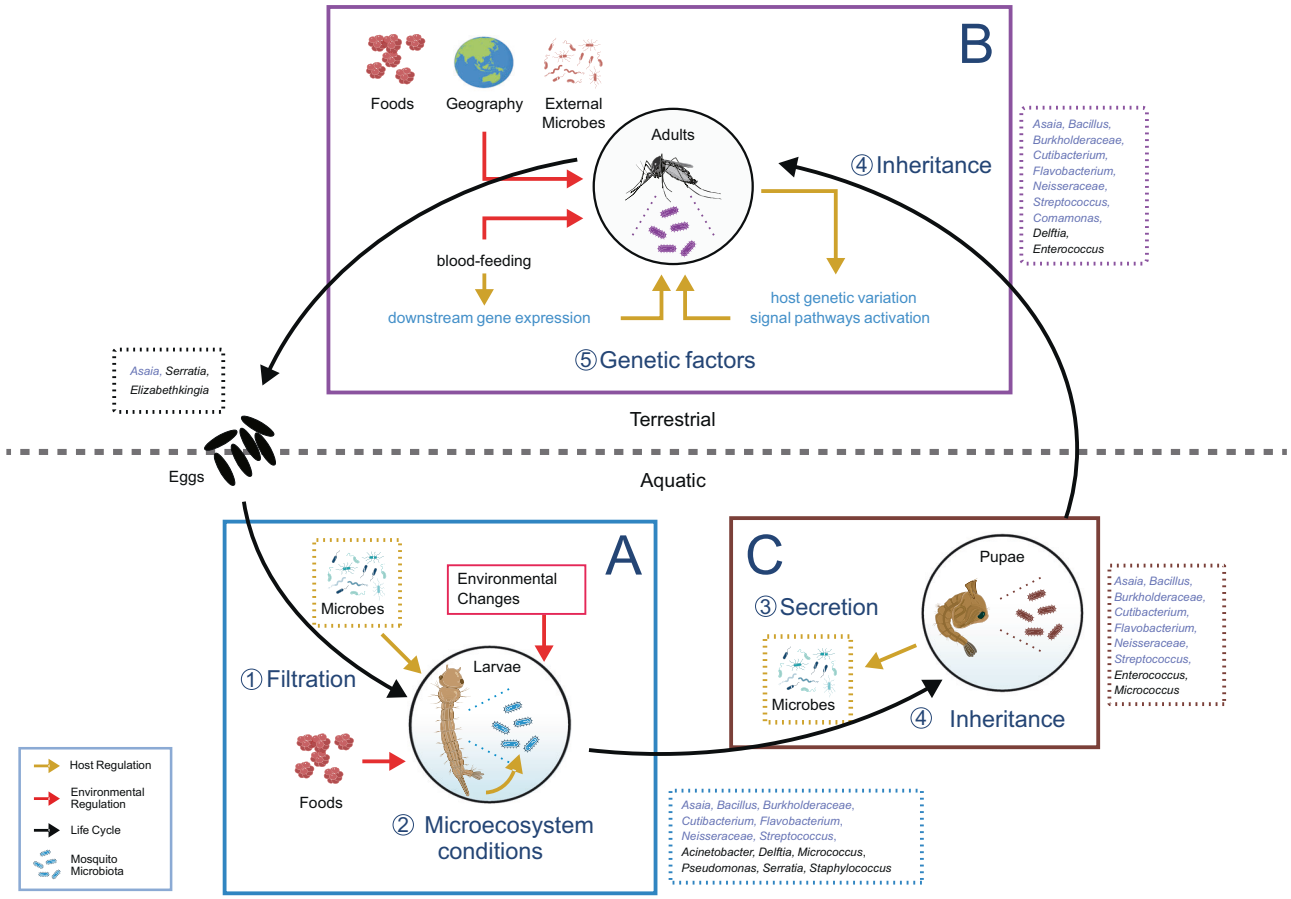

Fig. 3 Schematic representation of the acquisition and composition of mosquito microbiota. A Mosquito larval microbiota are determined by food resources, environmental changes, larval-mediated filtration, and their microhabitat conditions. **B** Mosquito adult microbiota are determined by various environmental factors or genetic factors, such as genetic variation in activation of different signal pathways. **C** Mosquito pupal microbiota are determined by larval secretion and/or inheritance from larvae. Dotted boxes with different colors: the mosquito microbes at different life stages identified at the genus level refer to some recently published studies, the bold purple font indicates the shared bacteria in larva, pupa, and adult stages, and the bold black font indicates the specific bacteria. The black arrows indicate mosquito life history. The red arrows indicate environmental factors regulating mosquito microbiota, and the yellow arrows indicate mosquito host-mediated regulation role on its microbiota.

growth of endogenous bacteria [75]. Although these two genes maintain the homeostasis of mosquito midgut microbes in different ways, the regulation of bacterial antagonistic (*AsHPX2*) and agonistic (*AsHPX15*) peroxidases promoted bacterial growth by creating a low-immunity area in the midgut [74].

In addition, mosquitoes can efficiently regulate the diversity and composition of bacteria by inducing the activation of diverse signal pathways (Fig. 3B) [76]. For instance, Hixson et al. found that mosquito hosts regulate the microbiota by activating immune defense after blood-feeding [77]. In *Ae. aegypti*, after blood-feeding, two lysozyme activity genes (*LYSC11* and *LYSC4*) in response to the ROS and bacterial density, two genes regulating IMD pathway activation (*PGRP-SC1* and *PGRPS4*), and two defensins genes (*DEFA* and *DEFD*) were induced and activated [77]. The expression of immune pathway-associated genes in mosquitoes, such as *REL1*, *REL2*, *PGRP*, *CAS*, and *CAP*, increased or reduced the titer of the *Wolbachia* *w*AlbB in transinfected *Ae. aegypti* [78], while allelic variation in the immune gene *FN3D* correlated with *S. marcescens* load and microbiota composition [79]. The expression of the gut-membrane-associated gene, *duox*, led to an increase in microbial load, suggesting that the ROS signaling pathway might participate in controlling microbial homeostasis in *Anopheles* [80]. The *thioester-containing protein 1* gene, a central component in the innate immune response of *Anopheles gambiae* to *Plasmodium* infection, is known to determine malarial infection in both wild-sourced and laboratory-sourced *An. gambiae* strains [81]. The amino acid metabolic signaling, especially the branched-chain amino acid degradation pathway, controls the colonization of midgut bacteria in different *Ae. aegypti* strains [82]. Although current technology is still insufficient to fully characterize the interactions and energy flow between mosquitoes and their microbiota, genes maintained by mosquito-specific microbiota that promote homeostasis are worthy of further study and may be useful in controlling the composition of mosquito microbiota to reduce mosquito vector competence.

In summary, mosquitoes inhabit a highly complex ecological environment. The interaction of multiple factors determines the composition of the mosquito's microbiome under natural conditions, especially through environmental factors affecting the microbiota taken up by the larval stages and their subsequent modification by host regulation.

Future directions

With an increasing incidence of mosquito-borne diseases worldwide, there is a need to develop more effective and safe ways (biotic and abiotic) and combine different approaches to manage mosquitoes and transmitted diseases (Fig. 4). Microbiota-based control strategies hold great promise and are already being applied in the case of *Wolbachia*-mediated

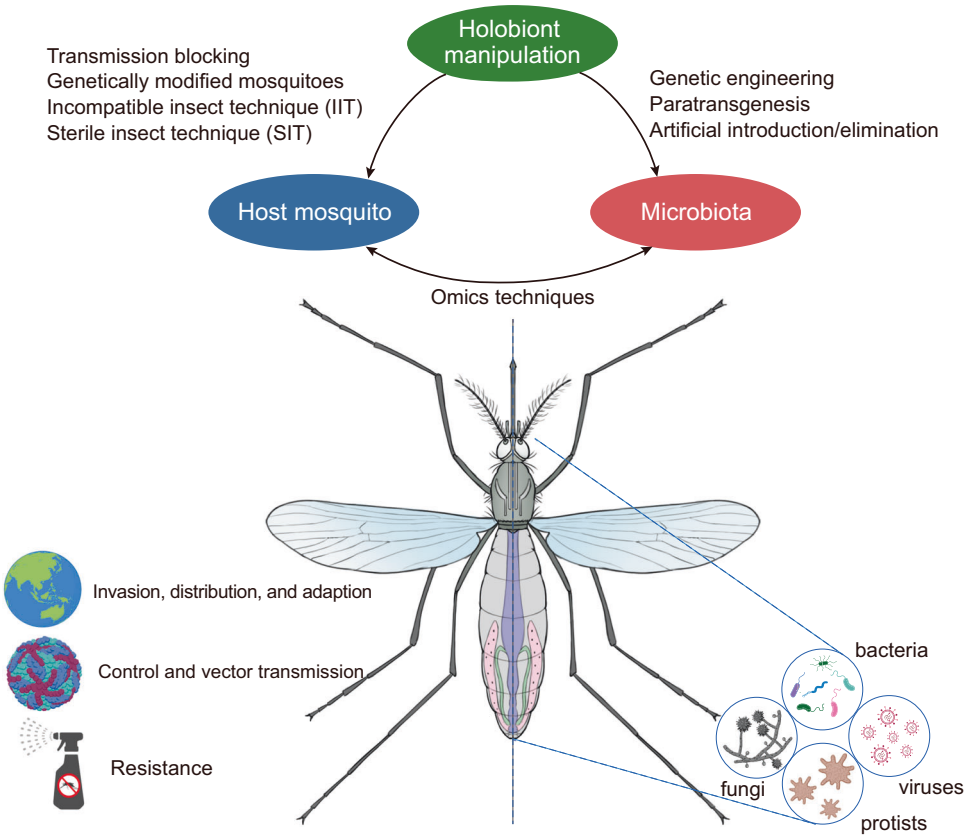

Fig. 4 Strategies of holobiont manipulation for mosquito control and pathogen transmission. We face increasing challenges from vector mosquitoes and mosquito-borne diseases, including rapid invasion by vectors leading to expanded distributions, high adaptive potential of host mosquitoes, challenges in outbreaks of new vectors, and the increasing resistance of mosquitoes to various pesticides and repellents. Mosquitoes and microorganisms (bacteria, fungi, viruses, and protists) interact as a holobiont. Holobiont manipulation provide new promising strategies for mosquito-borne diseases control. The application of omics techniques are providing new effective methods to help understand mechanisms underlying mosquito-microbiota interactions.

pathogen blocking as well as population suppression. Symbiont manipulation using paratransgenesis in *Anopheles* mosquitoes can effectively prevent the development of *Plasmodium*, and several promising candidates, such as the bacteria *Asaia* and *Serratia*, and the fungi *M. anisopliae* and *B. bassiana*, provide promising future applications for using symbiotic microbes in antimalarial field trials [15, 29]. More work on paratransgenesis is needed to assess the efficiency of introduced strains in *Aedes* and *Culex* mosquitoes.

At present, several *Wolbachia*-infected mosquitoes have been used to reduce the transmission of pathogens and control mosquito populations in the field with relatively good results, highlighting the effectiveness of this strategy in suppressing mosquito-borne diseases [83]. Despite being locally influential, the continuous release of *Wolbachia*-infected male mosquitoes for population suppression will always be challenging particularly due to the reinvasion of suppressed areas. For example, the accidental release of *Wolbachia*-infected females may rescue the suppressed population thereby represent a challenge for male-based mosquito population suppression strategies. Horizontal transmission to non-target species in treatment areas seems unlikely in *Wolbachia* replacement releases, but it remains important to monitor ecological and evolutionary changes following invasion by *Wolbachia*. For example, the loss of mitochondrial diversity, the possible adverse fitness effects of the infection, the stability of transinfections into natural populations, and any evolutionary change of pathogenic viruses in response to *Wolbachia* must be evaluated after *Wolbachia* invasions into natural communities. Therefore, future applications should pay particular attention to the possible adverse effects of symbiotically modified mosquitoes and manipulated bacterial strains.

In addition, the application of genetically engineered microbiota as another promising control strategy faces multiple issues, such as competition with indigenous microbiota, the possible transfer of manipulated DNA to other microorganisms, and the survival of manipulated organisms in natural systems. A comprehensive risk assessment process will be necessary before practical applications are realized, following similar assessments in *Wolbachia* [84]. It remains important to continue to explore other effective measures to reduce mosquito populations and block mosquito-borne pathogen transmission. Combining different approaches, both biotic and abiotic, may be necessary to achieve effective and sustainable management of mosquito populations and reduce the burden of mosquito-borne diseases worldwide.

Further investigation into the physiological and biochemical interactions of the functioning mosquito holobiont is necessary for a comprehensive understanding of the role of microbiota in the invasion and global distribution of host mosquitoes. The successful establishment of a mutualistic holobiont is essential for environmental adaptation in most insects, and microbiota likely plays a key role in this process. Moreover, the dynamic changes in microbe abundance make them able to respond readily to the selective environment, including human-linked selection pressures. Combined with high throughput -omics techniques, expression analysis of holobiont (microbiota and host) genomes would be helpful in developing a deeper understanding of interactions between hosts and microbiota. While current holobiont manipulation strategies via *Asaia*, *Serratia*, and other

dominant symbionts in mosquitoes have provided numerous novel insights into mosquito-borne disease control, other microbes with beneficial effects on reducing disease burden are likely to exist. Additional work is needed to explore the key components and gene pathways of host insects and microbiota assisting in controlling the mosquito-borne diseases. One silver bullet is likely insufficient for mosquito-borne disease control, and different approaches should be considered for their suitability based on local needs.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author, Guan-Hong Wang, upon reasonable request.

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AUTHOR CONTRIBUTIONS

All authors critically reviewed the manuscript and approved the final version for submission.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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