CellPress
REVIEWS

Review

Mosquito Microbiota and Implications for Disease Control

Han Gao,[1,2,4] Chunlai Cui,[1,2,4] Lili Wang,[1,2,4] Marcelo Jacobs-Lorena,[3,*] and Sibao Wang[1,2,*]

Mosquito-transmitted diseases account for about 500 000 deaths every year. Blocking these pathogens in the mosquito vector before they are transmitted to humans is an effective strategy to prevent mosquito-borne diseases. Like most higher organisms, mosquitoes harbor a highly diverse and dynamic microbial flora that can be explored for prevention of pathogen transmission. Here we review the structure and function of the mosquito microbiota, including bacteria, fungi, and viruses, and discuss the potential of using components of the microbiota to thwart pathogen transmission.

Highlights

Mosquitoes are vectors of pathogens that cause many fatal diseases; they harbor a diverse and dynamic microbial flora, including (prokaryotic) bacteria, (eukaryotic) fungi, and viruses.

The microbiota of the mosquito gut plays important roles in host physiology, such as blood digestion, development, fertility, and immunity.

The mosquito microbiota has promising applications for blocking pathogen transmission. Natural or engineered gut microbiota and *Wolbachia* have demonstrated the capacity to block transmission. Mosquito-pathogenic fungi and *Wolbachia* can be used to control mosquito populations.

Mosquito Microbiota and Mosquito-Borne Diseases

Epidemics such as malaria, dengue fever, yellow fever, Zika fever, and chikungunya fever, all transmitted by mosquitoes, account for around 350 million cases and about 500 000 deaths throughout the world each year [1]. As there is no efficient vaccine for most of these diseases [2], vector control remains one of the best strategies to prevent disease. Of concern, overuse of insecticides has caused widespread resistance [3,4], and novel disease-control strategies are urgently demanded. Mosquitoes harbor a highly diverse and dynamic microbial flora, collectively known as the **microbiota** (see Glossary), mostly in its **midgut** and on its surface (cuticle), but also in its somatic cells, crop, salivary glands, circulation system, and reproductive organs (Figure 1, Key Figure) [5]. Members of the **symbiotic microbiota** play a key role in mosquito physiology and immunity [6]. The microbiota of mosquitoes can significantly impact pathogen transmission, and has already displayed valuable potential to combat mosquito-borne diseases such as malaria, Zika fever, dengue fever, yellow fever, and other **vector-borne diseases** [7].

The composition and roles of the gut commensal microbiota in mosquitoes, and its influence on vector competence for malaria parasites and dengue viruses (DENVs), were recently reviewed by others [5,8–10]. In this review, we summarize research progress, made in the last two decades, of interactions between vector mosquitoes and their microbiota, including bacteria, viruses, mosquito-pathogenic fungi, and symbiotic fungi, emphasizing implications for disease control. We also address concerns toward future applications in the field.

Microbiota Composition and Dynamics in Pathogen-Transmitting Mosquitoes

Different Vector Mosquitoes and Diseases Transmitted

Disease-transmitting mosquitoes belong mainly to three genera – *Anopheles*, *Aedes*, and *Culex*. *Anopheles* transmits malaria and O'nyong-nyong fever [11,12]. **Arboviral** diseases, including dengue fever, chikungunya fever, West Nile fever, Zika fever, and yellow fever, are transmitted mostly by *Aedes* [13]. *Culex* transmits mainly filarial worms and West Nile Virus (WNV) [11]. When the female adult mosquito bites an infected person, pathogens – together with the blood – are taken into the mosquito midgut. The pathogens then infect or traverse the gut epithelial cells, enter the hemolymph, invade the salivary glands, and are then transmitted when the infected mosquito bites another person.

Composition of the Microbiota in Vector Mosquitoes

Prokaryotes

Symbiotic bacteria are the best studied symbionts of mosquitoes. The midgut is where most symbiotic bacteria are located. In both larval and adult stages, Gram-negative bacteria are the majority [8], with *Asaia*, *Acinetobacter*, *Aeromonas*, *Pantoea*, *Pseudomonas*, and *Serratia* being the most

[1]CAS key Laboratory of Insect Developmental and Evolutionary Biology, CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai 200032, China

[2]CAS Center for Excellence in Biotic Interactions, University of Chinese Academy of Sciences, Beijing 100049, China

[3]Department of Molecular Microbiology and Immunology, Malaria Research Institute, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA

[4]These authors contributed equally to this work.

*Correspondence: ljacob13@jhu.edu, sbwang@sibs.ac.cn

 https://doi.org/10.1016/j.pt.2019.12.001

Key Figure

Mosquitoes and Their Associated Pathogens and Microbes

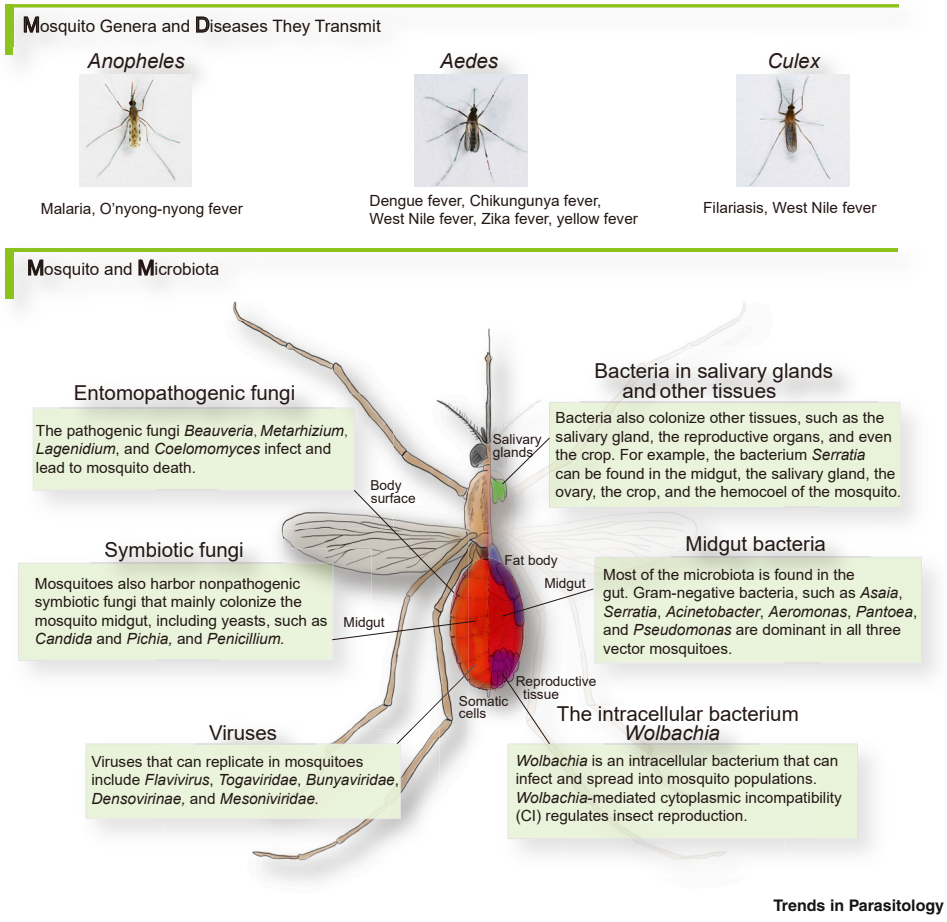


Figure 1. Mosquito species and their transmitted diseases are highly specialized, while they may share similar microbiota. Although the midgut harbors the majority of the microbiota, other tissues may also serve as microbe habitats.

common genera present in all vector mosquitoes [11,14,15]. Moreover, *Comamonas*, *Elizabethkingia*, *Enterobacter*, and *Klebsiella* are common in *Anopheles* [14]; *Sphingomonas*, *Cupriavidus*, and *Escherichia–Shigella* are common in *Aedes* [16]; and *Staphylococcus*, *Klebsiella*, and *Enterobacter* are dominant in *Culex* [11].

In addition to the midgut, symbiotic bacteria may also colonize other mosquito organs or tissues. For example, the common gut bacteria *Asaia*, *Serratia*, *Acinetobacter* and *Pseudomonas* can also colonize *Anopheles* and *Aedes* salivary glands and/or reproductive organs [5,14,17,18]. Bacteria of the genus *Serratia* can colonize *Aedes* crops [5]. Another notable prokaryotic genus which colonizes non-gut tissues is the **intracellular bacterium *Wolbachia***. *Wolbachia* can be vertically transmitted as it infects insect germ cells. Furthermore, many strains of *Wolbachia* induce **cytoplasmic incompatibility (CI)** to promote their spread among insect populations. Natural *Wolbachia* infection is common in over 50% of all insect species [19]. *Wolbachia* has been identified in *Culex pipiens*, *Aedes*

Glossary

Arbovirus: an arthropod-borne virus that shares a cycle of transmission between vertebrate hosts and hematophagous arthropod vectors.
Cytoplasmic incompatibility (CI): CI is manifested by embryonic lethality resulting from *Wolbachia*-infected males mating with uninfected females, whereas mating between infected males and infected females yields viable progeny. CI promotes *Wolbachia* spread into mosquito populations.
Effectors: proteins or noncoding RNAs (ncRNAs), produced by microbes, that block pathogens in vector mosquitoes. The microbes can be either natural or genetically modified.
Entomopathogenic fungi: fungal pathogens that infect insects, mainly through the cuticle, and proliferate in the hemolymph.
Intracellular bacteria: bacteria that invade and reside within their host cells. They can induce their uptake by host cells.
Microbiota: collectively, all the microbes that live in and on the host body; they comprise bacteria, archaea, viruses, fungi, and protozoans. Collectively, their genes are known as the 'microbiome'. The microbiota may associate with the host in a mutualistic/commensal or opportunistic/parasitic manner.
Midgut: the main digestive organ of invertebrates which digests a blood meal and assimilates nutrition. The midgut harbors a diverse microbial flora.
Paratransgenesis: a method used to genetically engineer symbiotic bacteria to produce antipathogen effector molecules.
Peritrophic matrix (PM): an extracellular layer, composed of chitin and glycoproteins, that lines the insect intestinal lumen. It is a physical barrier that protects the gut epithelium from the microbiota, pathogens, and blood.
Plasmodium: a protozoan pathogen and the causal agent of malaria. It infects its mammalian hosts via the bite of anopheline mosquitoes.
Symbiotic microbiota: microorganisms that colonize the gut of insects and mammals. They are essential to the health of the host

albopictus, and *Anopheles gambiae* in the wild [11]. It is present in various mosquito tissues, including reproductive organs, salivary glands, head, muscle, and Malpighian tubules [20]. Besides *Wolbachia*, *Spiroplasma* can also be found in hemolymph, hemocytes, thoracic flight muscle, and nerve cells, although there are not many reports on this genus [20].

Eukaryotes

Although not as well studied as prokaryotes, eukaryotic microorganisms such as fungi are part of the mosquito's microbiota. The **entomopathogenic fungi** *Beauveria* and *Metarhizium* infect the mosquito cuticle and proliferate in the hemolymph, causing progressive mosquito death [21]. Other parasitic fungi, such as *Lagenidium*, *Coelomomyces*, and *Culicinomyces* – that attack mosquito larvae and adults – are also used as mosquito biological control agents [22].

Mosquitoes also harbor nonpathogenic fungal microbiota; these fungi colonize mainly the mosquito midgut, but they can also be found in other tissues such as salivary glands and reproductive organs [5,23]. Mosquito symbiotic fungi are mainly yeasts such as *Candida* and *Pichia*. The filamentous fungus *Penicillium* has also been reported. These three genera have been found in *Anopheles* and *Aedes* [5,23,24].

Viruses

Mosquito-specific viruses can replicate only in mosquitoes and not in vertebrate cells. Previous studies have confirmed that a wide range of wild mosquito strains are infected with this type of virus. *Flavivirus* (cell-fusing agent virus, Kamiti River virus, and *Culex flavivirus*), Togaviridae, Bunyaviridae, Densovirinae, and Mesoniviridae are the main taxa that have been reported. Although artificial infection by these viruses is often pathogenic to naïve mosquitoes, it causes nonpathogenic persistent infection in the survivors, which means that viruses stay active in the mosquito and can be transmitted to their offspring [12].

Factors Shaping the Gut Microbiota in Vector Mosquitoes

The composition of symbiotic microbiota is highly dynamic throughout the mosquito's lifespan. Many factors, including developmental stages, living habitat, feeding habit, and even pathogen infection, can affect microbiota composition.

Mosquito larvae live in the water, so they obtain their gut microbiota mainly from their environment [5]. Changes in the breeding bacterial communities may impact the larval microbiota. For example, higher water temperature favors the growth of Betaproteobacteria, a common gut bacteria phylum, which is thought to be beneficial for the growth of *Anopheles* larvae [25,26]. Water contaminated by fertilizers rich in ammonium and phosphorus promotes the growth of microbes which can serve as a major source of nutrition for mosquito larvae [26]. Residual antibiotics in water also influence the mosquito larval microbiota as they reduce or eliminate certain bacterial taxa [5].

During pupation, the midgut microbiota is wrapped by the larval **peritrophic matrix** (**PM**) to form the meconium. The meconium is egested by the newly hatched adult mosquito, resulting in the loss of the majority of the gut microbiota [14,15]. Moll *et al.* [27] studied all three vector mosquito genera and found that the bacterial load is high in larvae, less in old larvae, increases in the pupa, and is very low in recently hatched adults. Wang *et al.* [28] found that gut bacterial diversity is higher in larvae than in adults. They also determined that cyanobacteria are the predominant gut bacteria in larvae and in the pupa, while Proteobacteria and Bacteroidetes dominate in adult guts, with Enterobacteriaceae and Flavobacteriaceae being the core taxa. Blood feeding also changes the gut microbiome composition of adults. Diversity decreases after a blood meal, while some specific taxa, such as Enterobacteriaceae, increase their representation [28–30].

Pathogen infection can affect the composition of the mosquito's microbiota. For instance, enteric bacteria are favored in ***Plasmodium***- or chikungunya virus (CHIKV)-infected mosquitoes [14,31]. Zika virus infection of *Aedes aegypti* results in increased representation of Rhodobacteraceae and

and play a role in nutrition, development, metabolism, pathogen resistance, and regulation of immune responses.
Vector-borne diseases: illnesses caused by pathogens transmitted by blood-feeding arthropods such as mosquitoes, ticks, and fleas.
Vector competence: refers to the capacity of a vector to transmit pathogens.
Wolbachia: a Gram-negative, maternally transmitted, endosymbiotic, intracellular bacterium that infects more than half of all arthropod species.

Desulfuromonadaceae [32]. In *Aedes triseriatus* and *Aedes japonicus*, bacterial numbers increase while fungal representation decreases in response to La Crosse virus infection [33].

The Influence of the Microbiota on Mosquito Physiology

Mosquito–microbiota interactions are complex. Mosquito factors can shape the composition and proliferation of the microbiota, and the microbiota contributes to the mosquito's food digestion, nutrition, growth, fertility, and immunity [34].

Influence of the Microbiota on Mosquito Nutrition

Female mosquitoes acquire nutrition essential for reproduction by feeding on vertebrate blood. Elimination of the female *Ae. aegypti* gut microbiota with antibiotics slows the digestion of ingested mouse blood; *Enterobacter* sp. and *Serratia* sp. residing in the mosquito midgut may have hemolytic activities that contribute to blood digestion [35]. In *Ae. albopictus*, *Acinetobacter baumannii* and *Acinetobacter johnsonii* improve blood protein digestion and nectar assimilation, respectively [36]. In addition to facilitating food digestion, the microbiota can serve as a food source. *Ae. aegypti* larvae that feed solely on *Saccharomyces cerevisiae* can develop into adults in a normal way [37]. During a 21-day feeding experiment, *Candida glabrata*, *Candida albicans*, *Candida pseudolambica*, and *Wickerhamomyces anomalus* isolated from *Culex theileri* and *Cx. pipiens* larvae can each separately serve as the sole source of nutrients for *Cx. pipiens* larval growth and pupation [38]. *Wolbachia* (*w*MelPop strain) infection impairs blood-feeding success in *Ae. aegypti* [39].

Influence of the Microbiota on Mosquito Development

The mosquito microbiota can influence the progress of mosquito development. In *Anopheles stephensi*, rifampicin-treated larvae showed developmental delay and asynchrony of later instars; supplying antibiotic-treated larvae with rifampicin-resistant *Asaia* could rescue larval development [40]. Live bacteria or eukaryotes are essential microorganisms for the development of *Ae. aegypti* larvae to adults [41,42], potentially due to a hypoxia signal in the mosquito gut induced by the microbiota [43,44]. However, a recent study shows that axenic *Ae. aegypti* larvae could complete their development to adulthood without live microbiota, suggesting that the main role of the microbiota is to supply nutrition essential for larval development [45]. Removing midgut bacteria shortens longevity of *An. stephensi* [46]. *Wolbachia* (*w*MelPop strain) infection also shortens the *Ae. aegypti* lifespan [47]. *Paraclostridium bifermentans* strains isolated in anopheline endemic areas produce a neurotoxin, named PMP1, which cleaves mosquito syntaxin and kills *Anopheles* mosquitoes [48].

Influence of the Microbiota on Mosquito Reproduction

The microbiota can modulate mosquito mating, preoviposition, and reproduction behavior. A change in the microbiota community composition and number can make anti-*Plasmodium* transgenic *An. stephensi* males more attractive mates to wild-type females [49]. Two strains of bacteria isolated from *Cx. pipiens*, *Klebsiella* sp. and *Aeromonas* sp., enhance oviposition [50]. The rearing water of *Ae. aegypti* larvae infected with *Candida pseudoglaebosa* enhances the attractiveness of oviposition sites [51]. In *Ae. aegypti*, antibiotic treatment reduces egg production [45]. Supplementation of germ-free *Ae. aegypti* with commensal bacteria *Paenibacillus*, *Chryseobacterium*, *Sphingobacterium*, *Aquitalea*, or *Comamonas* could restore mosquito fecundity. However, *Aedes atropalpus* benefits only from *Comamonas*, while the other microorganisms could not support egg production to equivalent levels as conventionally reared females [52].

Wolbachia can spread through many arthropod populations by a mechanism known as cytoplasmic incompatibility (CI) [53]. CI is manifested by embryonic lethality of progeny from *Wolbachia*-infected males mated to uninfected females, whereas mating infected males to infected females yields viable progeny [53]. The molecular basis for CI is not completely understood. Mosquito-borne *Wolbachia* (*w*Pip strain) Type IV Effector WD0830 interacts with the actin cytoskeleton to induce CI [54]. *Wolbachia* (*w*Pip strain) deubiquitylating enzyme (DUB), CidB and partner CidA, are involved in the CI mechanism [55]. Another *cin* operon that encodes a nuclease, CinB, and a second protein, CinA, also

appear to take part in CI in mosquitoes infected with *Wolbachia* (*w*Pip strain) [56]. Prophage WO genes from *Wolbachia* (*w*Mel strain) also participate in and enhance CI [57].

Influence of the Microbiota on Mosquito Physiology and Pathogen Infection

The mosquito gut microbiota proliferates by hundreds of fold for about 24 h after a blood meal [29]. This expansion of the mosquito microbiota could influence pathogen infection through diverse mechanisms. The microbiota may directly interact with mosquito pathogens or modulate pathogen infection by regulating the host mosquito immune defenses and nutrition status. The influence of the microbiota on mosquito physiology and pathogen transmission is summarized in Table 1. The mechanisms are discussed in the next section.

The Potential of the Mosquito Microbiota to Reduce Vector Competence

Much progress has been made in the last two decades in developing procedures to reduce the **vector competence** of mosquitoes (Figure 2).

Exploring the Natural Gut Microbiota to Reduce Vector Competence

The mosquito gut is a major 'immunity organ' that plays an important role in fighting pathogen infections [58]. Bacterial strains isolated from mosquito guts, in both laboratory-reared and field-caught mosquitoes, were evaluated for their potential to control pathogen transmission. Bacteria were able to modify the gut environment and inhibit the development of parasites either by inducing reactive oxygen species (ROS) or modulating the expression of mosquito immune genes. In most cases, the introduction of bacteria inhibits pathogens such as *Plasmodium*, while removal of gut microbiota with antibiotics increases the susceptibility of mosquitoes to infection [59]. For example, *Asaia* sp. was reported to activate antimicrobial peptide expression in *An. stephensi* [60]. The presence of the dominant commensal Enterobacteriaceae positively correlates with *Plasmodium* infection, indicating that the Enterobacteriaceae play a positive role in *Plasmodium falciparum* infection [61]. Interestingly, a recent study reported that a *Serratia marcescens* strain, isolated from a laboratory *Ae. aegypti* strain, facilitates arboviral infection [62]; this bacterium secretes a protein, named SmEnhancin, which digests gut membrane-bound mucins to enhance viral dissemination in mosquitoes. It is important to note that strain-specific activity exists even between bacteria from the same genera or even species, isolated from the same mosquito species. A previous study showed that different strains of *S. marcescens* species can induce different outcomes in *Plasmodium* infections [63]. A more recent study by Bai *et al.* showed that *S. marcescens* strain Y1, isolated from the gut of field-caught *Anopheles sinensis*, inhibits *Plasmodium* development by modulating the immunity-related *Plasmodium* **effector** genes such as *TEP1* and *FBN9* [64]. Interestingly, in this study, another isolated *S. marcescens* strain J1 had no *Plasmodium*-inhibiting effect.

While many bacterial strains have different activities relating to pathogen transmission by the mosquito, exploration of the mechanisms of the mode of action is just getting off the ground. Apart from the immunity-related mechanisms mentioned above, gut bacteria can also directly inhibit pathogen development in mosquitoes via their secretions. ROS, metabolites, small peptides, and proteins secreted by gut bacteria may directly influence the transmission and development of pathogens [65]. When cofeeding *P. falciparum* with the *Enterobacter* strain Esp_Z, isolated from wild *Anopheles arabiensis* mosquitoes, *Plasmodium* development was arrested by bacteria-generated ROS [66]. Many strains of *Serratia* spp. secrete serralysin proteins and prodigiosin, which have a pathogen-killing effect *in vitro* [67,68]. Prodigiosin is also a larvicidal agent against *Ae. aegypti* and *An. stephensi* [69]. Furthermore, blood ingestion and digestion unleash abundant nutrients, ions, proteins, heme, and lipids that may pose a strong stress on the gut microbiota, resulting in changes in its composition and activities. These factors should be considered when contemplating the introduction of a bacterial strain into a mosquito.

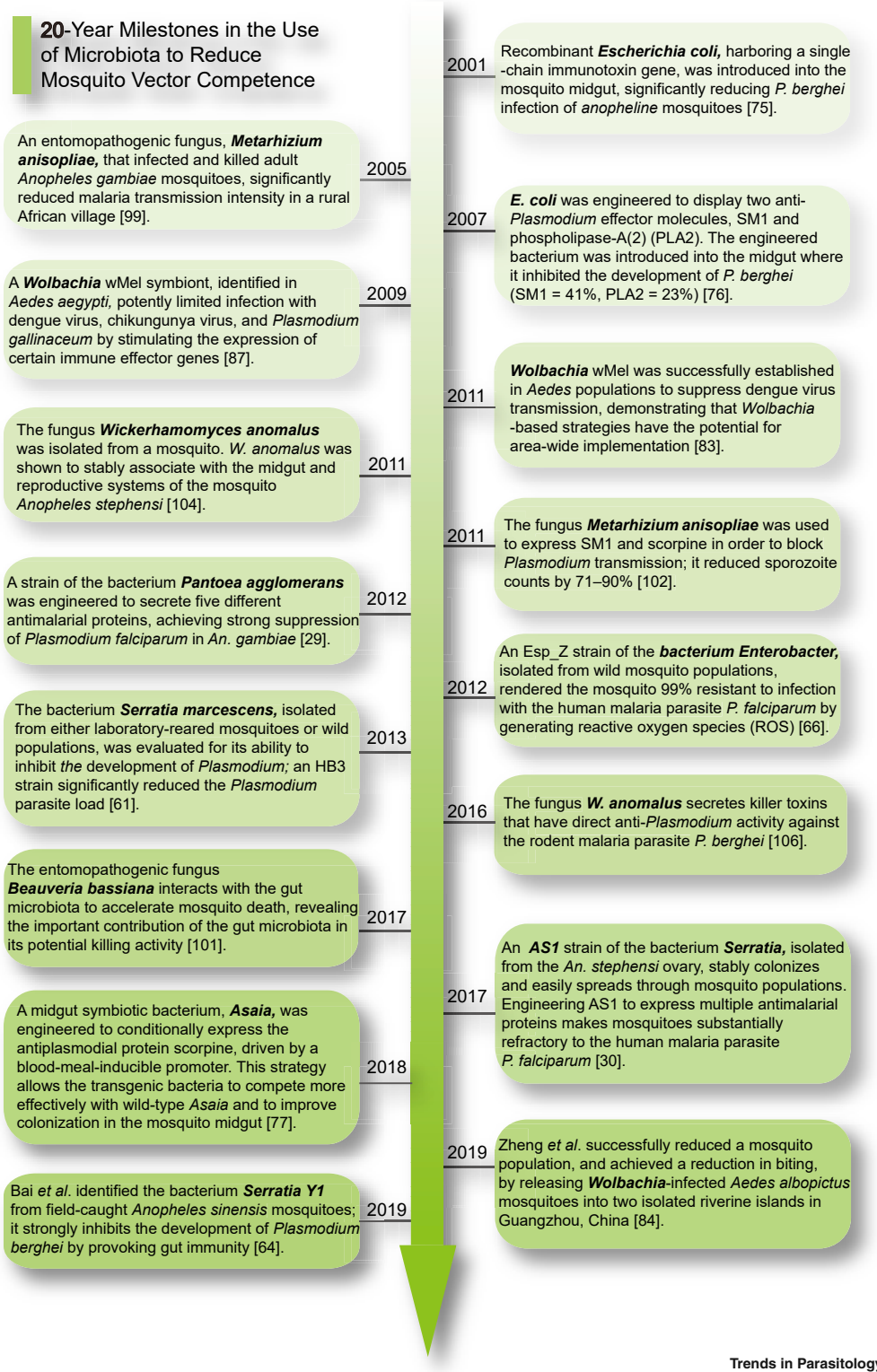
Another interesting perspective in using gut bacteria to inhibit specific pathogens comes from an understanding of the detailed physiological demands of the pathogens. Recently, Zhu *et al.* revealed that DENV acquisition by *Ae. aegypti* was inversely correlated with the iron concentration in serum

Table 1. Influence of the Microbiota on Mosquito Physiology and Pathogen Transmission[a]

Mosquito species	Microbiota	Function	Refs
Aedes aegypti	*Enterobacter* sp., *Serratia* sp.	Blood digestion	[35]
Aedes albopictus	*Acinetobacter baumannii*, *Acinetobacter johnsonii*	Blood digestion and nectar assimilation	[36]
Ae. aegypti, *Culex pipiens*	*Saccharomyces cerevisiae*	Nutrient source	[37,38]
Ae. aegypti	*Wolbachia* (wMelPop)	Blood-feeding success	[39]
Anopheles stephensi	*Asaia*	Larval development	[40]
Ae. aegypti	*Escherichia coli*	Larval development (hypoxia signal)	[43,44]
Ae. aegypti	*Wolbachia* (wMelPop)	Lifespan	[47]
Anopheles	*Paraclostridium bifermentans*	Kills mosquito (neurotoxin PMP1)	[48]
Cx. pipiens	*Klebsiella* sp., *Aeromonas* sp.	Attracts oviposition	[50]
Ae. aegypti	*Candida pseudoglaebosa*	Attracts oviposition	[51]
Ae. aegypti	*Paenibacillus*, *Chryseobacterium*, *Sphingobacterium*, *Aquitalea*, *Comamonas*	Fecundity	[52]
Aedes atropalpus	*Comamonas*	Fecundity	[52]
Anopheles gambiae	*Enterobacter (Esp_Z)*	Arrests *Plasmodium falciparum* (ROS)	[66]
Ae. aegypti	*Serratia marcescens*	Enhances dengue virus (SmEnhancin)	[62]
An. stephensi	*Wickerhamomyces anomalus*	Inhibits *Plasmodium berghei* (toxin)	[106]
An. stephensi	*Asaia* sp.	Activates antimicrobial peptides	[60]
An. stephensi	*Serratia marcescens* strain Y1	Inhibits *P. berghei*	[64]
Ae. aegypti	*Wolbachia* (wMelPop)	Inhibits DENV, YFV, CHIKV, ZIKV	[87,88,110]
An. stephensi	*Wolbachia* (wAlbB)	Represses *P. falciparum*	[111]
An. gambiae	*Wolbachia* (wMelPop and wAlbB)	Inhibits *P. falciparum*	[96]

[a]Abbreviations: CHIKV, chikungunya virus; DENV, dengue virus; ROS, reactive oxygen species; YFV, yellow fever virus; ZIKV, Zika virus.

from human donors [70]. This work implies that alterations in iron concentration can be used to interfere with pathogen transmission. Conversely, although host iron is required for *Plasmodium* parasite development, excess iron during blood digestion in the mosquito gut may be toxic to this pathogen [71]. Also, the iron compound ferric ammonium citrate (FAC) inhibits infections by many viruses, such as influenza A virus, HIV, Zika virus, and Enterovirus 71 (EV71) [72]. Therefore, gut bacteria manipulating the iron concentration and composition after a blood meal could help to develop new methods



Trends in Parasitology

(Figure legend at the bottom of the next page)

to block multiple pathogens in the mosquito. Gut bacteria can also cause pH fluctuation. In humans, gut commensals can prevent pathogen infection by altering host pH [73], and a similar phenomenon was also seen in insects [34]. Interestingly, *Plasmodium* gametocytes require a defined pH for activation and fertilization in the mosquito gut. Therefore, identifying gut bacteria that modulate gut pH has the potential to lead to the development of new methods to block pathogen transmission.

Engineering the Gut Microbiota to Reduce Vector Competence

Genetic engineering has been used to engineer symbiotic bacteria to produce antipathogen effector molecules (termed **paratransgenesis**). This approach was first tested to control transmission of *Trypanosoma cruzi* in 1997, when Durvasula *et al.* engineered an endosymbiont of *Rhodnius prolixus* to express Cecropin A, a naturally occurring pore-forming peptide lethal to the parasite [74]. Engineering symbiotic bacteria from the mosquito midgut to produce interfering factors has been explored as a promising way to fight various arthropod-borne human pathogens. In the earlier years, *Escherichia coli* was used to express either a single-chain immunotoxin, or compounds such as salivary gland and midgut peptide 1 (SM1) or phospholipase-A_2 (mostly targeting ookinetes) to block *Plasmodium* development in the mosquito midgut [75,76]. However, *E. coli* is not a mosquito symbiont and cannot persist in the mosquito gut. Moreover, using a single effector raises the concern of potential development of resistance by the pathogen. Wang *et al.* addressed these concerns by engineering a mosquito symbiotic *Pantoea agglomerans* strain to secrete five different antimalarial proteins at the same time, achieving strong suppression (up to 98%) of *Plasmodium* development [29]. However, forcing symbiotic microbes to constitutively express effectors may cause fitness cost to the microbe, leading to reduced effectiveness. A recent work by Shane *et al.* addressed such concern. In this work, the midgut symbiont *Asaia* was engineered to conditionally express the antiplasmodial protein scorpine, driven by a blood meal-inducible promoter, allowing the transgenic bacteria to compete more effectively with wild-type *Asaia* and improve gut colonization [77].

A central question in using gut microbiota to reduce vector competence is how to introduce the bacteria, and to ensure their persistence in mosquito field populations. This concern was recently addressed by Wang *et al.* who identified a new *Serratia* bacterial strain AS1 isolated from an *Anopheles* ovary. AS1 can stably colonize the mosquito midgut as well as its reproductive organs, and it can be transmitted vertically (from female to offspring), and horizontally (from males to females). These properties allow its fast and stable spread into mosquito populations. When engineered to express antimalarial compounds, AS1 strongly reduced mosquito competence for transmission of the human malaria parasite *P. falciparum* [30]. This advance provides a promising tool for driving mosquito pathogen refractoriness into the field.

Selection of proper effectors is key in paratransgenesis. In the fight against the malaria parasite, a variety of compounds for expression by gut bacteria were identified [78]. The repertoire for fighting viral infection of mosquitoes is much more restricted. Antiviral effectors are technically difficult to engineer for achieving a satisfactory expression level and delivery efficiency. Finding new delivery approaches may help to solve this problem in the future. Furthermore, concurrent infections of different pathogens are common in mosquitoes. For example, DENV and CHIKV cocirculation and coinfection in humans are frequent [79,80]; there are also reported cases of the presence of both CHIKV and *Plasmodium* in affected patients [81]. Therefore, finding ways to combat multiple pathogens at the same time is a desirable goal. In 2018, Yen *et al.* developed an miRNA-based approach which resulted in a dual-resistance phenotype in mosquitoes to DENV-3 and CHIKV viruses [82]. Perhaps it will be possible to engineer mosquito symbionts to block multiple pathogens in the future.

Figure 2. Recent Progress in the Use of the Microbiota to Reduce Mosquito Competence.
During the last two decades there have been efforts, using various microbes and effectors, to combat different pathogens in specific mosquito species; these studies are summarized in this figure. Certain trends can be seen, including the use of better symbionts, exploring stronger effectors, and designing more efficient expression-delivery methods. See [29,30,61,64,66,75–77,83,84,87,99,101,102,104,106].

The Intracellular Bacterium *Wolbachia*

In addition to the gut microbiota, mosquitoes also harbor microbes in other tissues. *Wolbachia* is an intracellular bacterium that infects most insect species, including mosquitoes. *Wolbachia*-mediated CI regulates insect reproduction and has been used to modify or reduce mosquito populations [53]. Population modification was demonstrated in the wild first in Australia in 2011 [83]. More recently, an *Ae. albopictus* field population was nearly eliminated in Guangzhou, China, by releasing *Wolbachia*-infected mosquitoes in an area-wide application [84].

Besides restricting mosquito populations, *Wolbachia* can also affect pathogen transmission in several ways. *Wolbachia* infection of *Ae. aegypti* alters blood meal excretion and delays oviposition without affecting trypsin activity [85]. Importantly, *Wolbachia* can affect viral replication via a combination of competition for host resources and activation of host immunity. In *Ae. aegypti*, *Wolbachia* (*w*MelPop strain) infection induces upregulation of the mosquito's innate immune response against filarial nematodes and pathogenic bacterial infection [86]. *Wolbachia w*Mel infection of *Ae. aegypti* has been reported to block mosquito-borne viruses, including DENV, CHIKV, yellow fever virus, and Zika virus, but not WNV [87–90]. Interestingly, the *w*MelPop strain of *Wolbachia* significantly reduced the replication of WNV in *Ae. aegypti* [90].

There are reports that *Wolbachia* may also facilitate transmission of certain viruses. Surprisingly, *Wolbachia w*Mel was reported to increase the mean and the variance in *Ae. aegypti* susceptibility to dengue infection when introgressed into Brazil and Vietnam genetic backgrounds [91]. *Wolbachia w*AlbB strain enhanced WNV infection in *Culex tarsalis* mosquitoes via downregulating the Toll immune pathway [92]. Recently, a *Wolbachia w*Pip strain was reported to enhance vertical transmission of *Cx. pipiens* densovirus (CpDV) when bacteria and viruses co-exist in ovaries of *Cx. pipiens* [93]. There is also a report showing no difference in prevalence of infection and viral load between *Wolbachia w*Flu-infected and -uninfected *Aedes fluviatilis* mosquitoes [94]. These bewildering results suggest that strain-specific effects of both the bacteria and vectors also exist in the case of *Wolbachia*.

It is worth mentioning that malaria-transmitting *Anopheles* mosquitoes are not usually naturally infected by *Wolbachia,* but infection can be accomplished in the laboratory [95]. The effects of *Wolbachia* introduced into *Anopheles* mosquitoes are not clear-cut and seem to be species-specific. In 2011, Hughes *et al.* characterized somatic infections of two *Wolbachia* strains (*w*MelPop and *w*AlbB) in *An. gambiae*. Both significantly inhibited *P. falciparum* oocyst levels in the mosquito midgut [96]. However, *Wolbachia* strain *w*AlbB enhanced *An. gambiae* infection by the rodent malaria parasite *Plasmodium berghei* [97]. Since *Plasmodium malariae*, *Plasmodium ovale*, *Plasmodium knowlesi*, and *Plasmodium vivax*, the four other human malaria parasites, are more closely related to rodent malaria parasites phylogenetically, it raises the possibility that *Wolbachia* infections would enhance transmission of these parasite species.

Unlike most gut bacteria, the intracellular bacterium *Wolbachia* cannot presently be engineered for paratransgenesis proposes. However, recently Reveillaud *et al.* identified a putative 9.2 kb circular plasmid – pWCP – carried by *Wolbachia* from field-caught *Cx. pipiens* in France, raising the possibility of future paratransgenesis with *Wolbachia* [98].

The Use of Fungi for the Control of Pathogen Transmission

Mosquito fungi can also be used to fight pathogen transmission, whether used directly or combined with paratransgenic approaches. Two fungi that can infect and spread in the mosquito population, *Beauveria bassiana* and *Metarhizium anisopliae*, have the advantage of being able to survive in the field for months in the form of spores, and of being able to infect various mosquito species [21,99]. These fungi infect mosquitoes through the cuticle and proliferate in the hemolymph. Fungus infection causes progressive mosquito death, so those fungi with high vector virulence (either selected or engineered to achieve this purpose) can be used as biopesticides to control mosquito populations [99]. Recently, it was shown that *B. bassiana* generated a cross-kingdom microRNA-like RNA (bba-milR1) that attenuates

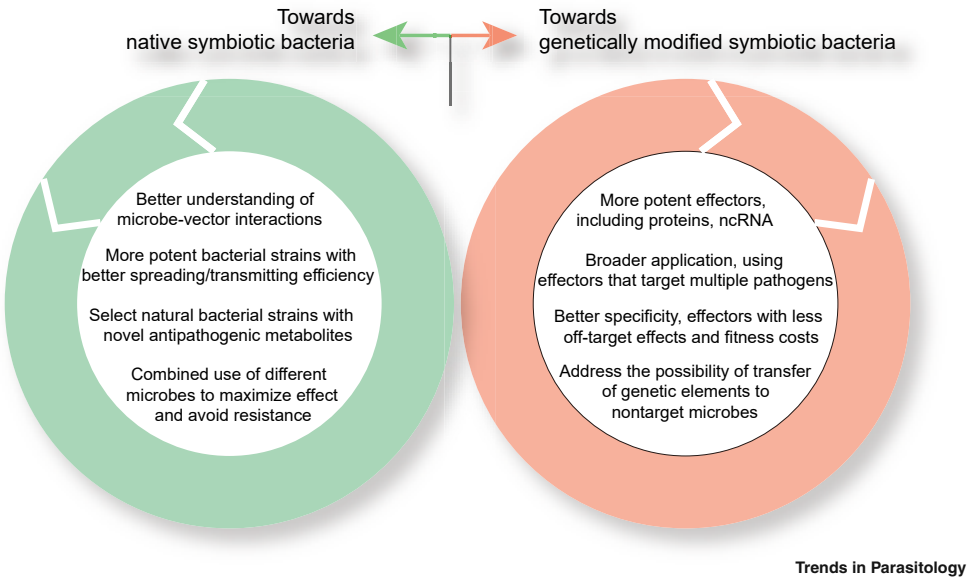


Figure 3. Prospects for the Use of the Mosquito Microbiota to Reduce Vector Competence.
While the two alternative strategies of using native or symbiotic bacteria to reduce mosquito competence address different challenges, they share common concerns such as improving inhibitory efficiency, avoiding fitness cost, and solving ecosafety concerns. Abbreviations: ncRNA, noncoding RNA.

mosquito immunity and accelerates insecticidal action [100]. Interestingly, mosquito fungi can manipulate the gut microbiota to accelerate vector mortality [101]. Moreover, fungi can also be engineered to express pathogen-killing effectors. In 2011, *M. anisopliae* was engineered to express SM1 and scorpine to block *Plasmodium* transmission, and reduced sporozoite counts by 71–90%, suggesting that mosquito fungi can be engineered as a powerful weapon for combating malaria [102].

Early studies indicated that the adult mosquito midgut environment is not compatible with fungal survival. The larval midgut may contain a few fungi as symbionts or even pathogens [103]. However, in 2011, the fungus *W. anomalus* was isolated from the midgut and reproductive system of different mosquito species, suggesting that symbiotic relationships between mosquitoes and fungi can truly exist in the gut [104]. *W. anomalus* acts via secretion of killer toxins (KTs). KTs have an enzymatic activity with broad-spectrum antimicrobial activities targeting the cell-wall glucan components of bacteria, yeasts, and protozoa [105]. The purified *W. anomalus* KT WaF17.12 acts against different developmental stages of the rodent malaria parasite *P. berghei* [106].

In recent years, with the help of high-throughput sequencing approaches, more mosquito midgut fungal communities have been characterized [33]. These studies indicate that some fungi, though in much smaller quantities, can survive the midgut environment and coexist with gut bacteria. These fungi can play important roles. A fungus – *Penicillium chrysogenum* – isolated from the gut of a field-caught *An. gambiae* mosquito, renders the mosquito more susceptible to *Plasmodium* infection via suppression of the mosquito's innate immune system [33]. More recently, a *Talaromyces* (Tsp_PR) fungus, isolated from the midgut of *Ae. aegypti*, was shown to enhance susceptibility to DENV by modulating gut trypsin activity [107]. These observations, together with the species- or strain-specific phenomenon in gut bacteria mentioned above, call for an in-depth study of microbiota–vector–pathogen interactions to find 'perfect symbionts' while avoiding the enhancement of other pathogen transmission.

Viruses in Pathogen Transmission Control

Mosquitoes carry many viruses, both mosquito-specific viruses lacking human pathogenicity and viruses pathogenic to humans. The densonucleosis viruses (DNVs) can infect arthropods, including

many mosquito species. Infection with DNVs is largely avirulent to mosquitoes [108]. In 2008, a DNV (AgDNV) was shown to infect *An. gambiae* and spread enhanced green fluorescent protein eGFP reporter gene into the mosquito population [109]. These properties indicate that DNVs can serve as an effective tool for a paratransgenic control strategy.

The use of mosquito-specific viruses to control pathogen transmission was largely conceptual until now. However, mosquito-specific viruses have interesting characteristics. First, mosquito-specific viruses are host-specific. Second, virus infection often results in spreading into mosquito populations. Viruses can have a broad impact on mosquito immunity. They compete with other microbes for nutrition and can even shape midgut bacterial and fungal microbiota [33]. However, their restrictive genome capacity severely restricts the size of potential effector genes and limits the use of viruses for pathogen control.

Concluding Remarks

Driving mosquito refractoriness to pathogens with microbiota has made much progress in the last two decades (Figure 2). Importantly, this strategy is also compatible with current mosquito-control tools (insecticides) and genetically modified mosquitoes. However, several key questions restrict this approach (see Outstanding Questions). Challenges for moving the use of gut microbiota to the field is summarized in Figure 3. A high priority should be given to address regulatory, ethical, and public acceptance issues. The insect gut provides favorable conditions for bacterial conjugation, so the possibility of gene transfer needs to be addressed. An alternative approach is to identify naturally occurring bacteria that naturally produce pathogen-limiting effectors.

Another consideration relates to the concurrent infections of different pathogens that can occur in mosquitoes. The targeting of one pathogen may facilitate the transmission of another. Thus, utilizing a symbiotic microbe with multipathogen inhibitory activities would be ideally sought. Finally, it is important to keep in mind that no tool is 100% effective. In the fight against mosquito-borne diseases, a 'magic bullet' does not exist. Diseases can be controlled only by the coordinated and simultaneous deployment of as many tools as possible.

Outstanding Questions

What are the underlying mechanisms by which the gut microbiota blocks pathogen transmission?

What roles does the gut microbiota play in commensalism?

How do some bacteria inhibit one pathogen in a mosquito species while facilitating infection of a different pathogen in a different mosquito vector?

What are the bases for mosquitoes to differentiate the commensal microbiota from pathogenic bacteria? By which mechanisms do mosquitoes support commensal microbiota and suppress harmful bacteria?

How can we increase the efficiency of microbial-based mosquito-borne disease control while ensuring biosafety?

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (grants 31830086, 31772534, 31830086, 31472044), the National Key R&D Program of China (2017YFD0200400, 2018YFA0900502), the Strategic Priority Research Program of Chinese Academy of Sciences (grant XDB11010500), the Key Research Program of the Chinese Academy of Sciences (KFZD-SW-219), National Institutes of Health (NIH) grant R01AI031478, and the Bloomberg Philanthropies.

References

1. WHO. (2017) *Global Vector Control Response (2017–2030)*, WHO
2. Ferguson, N.M. (2018) Challenges and opportunities in controlling mosquito-borne infections. *Nature* 559, 490–497
3. Ranson, H. and Lissenden, N. (2016) Insecticide resistance in African *Anopheles* mosquitoes: A worsening situation that needs urgent action to maintain malaria control. *Trends Parasitol.* 32, 187–196
4. Dusfour, I. *et al.* (2019) Management of insecticide resistance in the major *Aedes* vectors of arboviruses: Advances and challenges. *PLoS Negl. Trop. Dis.* 13, e0007615
5. Scolari, F. *et al.* (2019) *Aedes* spp. and their microbiota: a review. *Front. Microbiol.* 10, 2036
6. Douglas, A.E. (2014) The molecular basis of bacterial–insect symbiosis. *J. Mol. Biol.* 426, 3830–3837
7. Dennison, N.J. *et al.* (2014) The mosquito microbiota influences vector competence for human pathogens. *Curr. Opin. Insect Sci.* 3, 6–13
8. Strand, M.R. (2018) Composition and functional roles of the gut microbiota in mosquitoes. *Curr. Opin. Insect Sci.* 28, 59–65
9. Guegan, M. *et al.* (2018) The mosquito holobiont: fresh insight into mosquito–microbiota interactions. *Microbiome* 6, 49
10. Caragata, E.P. *et al.* (2019) Curious entanglements: interactions between mosquitoes, their microbiota, and arboviruses. *Curr. Opin. Virol.* 37, 26–36
11. Wilke, A.B. and Marrelli, M.T. (2015) Paratransgenesis: a promising new strategy for mosquito vector control. *Parasit. Vector* 8, 342
12. Kean, J. *et al.* (2015) Fighting arbovirus transmission: natural and engineered control of vector competence in *Aedes* mosquitoes. *Insects* 6, 236–278

13. Kraemer, M.U.G. *et al.* (2019) Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. *Nat. Microbiol.* 4, 854–863
14. Gendrin, M. and Christophides, G.K. (2013) The *Anopheles* mosquito microbiota and their impact on pathogen transmission. In *Anopheles Mosquitoes: New Insights into Malaria Vectors* (Manguin, S. ed), pp. 525–548, Intech
15. Wang, S. and Jacobs-Lorena, M. (2017) Chapter 13. Paratransgenesis applications: fighting malaria with engineered mosquito symbiotic bacteria. In *Arthropod Vector: Controller of Disease Transmission* (vol. 1) (Wikel, S.K. *et al.* eds), pp. 219–234, Academic Press
16. Mancini, M.V. *et al.* (2018) Estimating bacteria diversity in different organs of nine species of mosquito by next generation sequencing. *BMC Microbiol.* 18, 126
17. Sharma, P. *et al.* (2014) Salivary glands harbor more diverse microbial communities than gut in *Anopheles culicifacies*. *Parasit. Vectors* 7, 235
18. Tchioffo, M.T. *et al.* (2015) Dynamics of bacterial community composition in the malaria mosquito's epithelia. *Front. Microbiol.* 6, 1500
19. Sazama, E.J. *et al.* (2019) Bacterial endosymbionts are common among, but not necessarily within, insect species. *Environ. Entomol.* 48, 127–133
20. Jupatanakul, N. *et al.* (2014) The insect microbiome modulates vector competence for arboviruses. *Viruses* 6, 4294–4313
21. Bukhari, T. *et al.* (2011) Development of *Metarhizium anisopliae* and *Beauveria bassiana* formulations for control of malaria mosquito larvae. *Parasit. Vectors* 4, 23
22. Ernst-Jan Scholte, B.G.J.K. *et al.* (2004) Entomopathogenic fungi for mosquito control: a review. *J. Insect Sci.* 4, 19
23. Ricci, I. *et al.* (2012) Mosquito/microbiota interactions: from complex relationships to biotechnological perspectives. *Curr. Opin. Microbiol.* 15, 278–284
24. Romoli, O. and Gendrin, M. (2018) The tripartite interactions between the mosquito, its microbiota and *Plasmodium*. *Parasit. Vectors* 11, 200
25. Hörtnagl, P. *et al.* (2010) The bacterial community composition of the surface microlayer in a high mountain lake. *FEMS Microbiol. Ecol.* 73, 458–467
26. Onchuru, T.O. *et al.* (2016) Chemical parameters and bacterial communities associated with larval habitats of *Anopheles, Culex* and *Aedes* mosquitoes (Diptera: Culicidae) in western Kenya. *Int. J. Trop. Insect Sci.* 36, 146–160
27. Moll, R.M. *et al.* (2001) Meconial peritrophic membranes and the fate of midgut bacteria during mosquito (Diptera: Culicidae) metamorphosis. *J. Med. Entomol.* 38, 29–32
28. Wang, Y. *et al.* (2011) Dynamic gut microbiome across life history of the malaria mosquito *Anopheles gambiae* in Kenya. *PLoS One* 6, e24767
29. Wang, S. *et al.* (2012) Fighting malaria with engineered symbiotic bacteria from vector mosquitoes. *Proc. Natl. Acad. Sci. U. S. A.* 109, 12734–12739
30. Wang, S. *et al.* (2017) Driving mosquito refractoriness to *Plasmodium falciparum* with engineered symbiotic bacteria. *Science* 357, 1399–1402
31. Zouache, K. *et al.* (2012) Chikungunya virus impacts the diversity of symbiotic bacteria in mosquito vector. *Mol. Ecol.* 21, 2297–2309
32. Villegas, L.E.M. *et al.* (2018) Zika virus infection modulates the bacterial diversity associated with *Aedes aegypti* as revealed by metagenomic analysis. *PLoS One* 13, e0190352
33. Muturi, E.J. *et al.* (2016) Midgut fungal and bacterial microbiota of *Aedes triseriatus* and *Aedes japonicus* shift in response to La Crosse virus infection. *Mol. Ecol.* 25, 4075–4090
34. Engel, P. and Moran, N.A. (2013) The gut microbiota of insects – diversity in structure and function. *FEMS Microbiol. Rev.* 37, 699–735
35. Gaio Ade, O. *et al.* (2011) Contribution of midgut bacteria to blood digestion and egg production in *Aedes aegypti* (diptera: culicidae) (L.). *Parasit. Vectors* 4, 105
36. Minard, G. *et al.* (2013) Prevalence, genomic and metabolic profiles of *Acinetobacter* and *Asaia* associated with field-caught *Aedes albopictus* from Madagascar. *FEMS Microbiol. Ecol.* 83, 63–73
37. Sirot, L.K. *et al.* (2011) Towards a semen proteome of the dengue vector mosquito: protein identification and potential functions. *PLoS Negl. Trop. Dis.* 5, e989
38. Steyn, A. *et al.* (2016) Yeasts associated with *Culex pipiens* and *Culex theileri* mosquito larvae and the effect of selected yeast strains on the ontogeny of *Culex pipiens*. *Microb. Ecol.* 71, 747–760
39. Turley, A.P. *et al.* (2009) *Wolbachia* infection reduces blood-feeding success in the dengue fever mosquito, *Aedes aegypti*. *PLoS Negl. Trop. Dis.* 3, e516
40. Chouaia, B. *et al.* (2012) Delayed larval development in *Anopheles* mosquitoes deprived of *Asaia* bacterial symbionts. *BMC Microbiol.* 12 (*Suppl. 1*), S2
41. Coon, K.L. *et al.* (2014) Mosquitoes rely on their gut microbiota for development. *Mol. Ecol.* 23, 2727–2739
42. Valzania, L. *et al.* (2018) Both living bacteria and eukaryotes in the mosquito gut promote growth of larvae. *PLoS Negl. Trop. Dis.* 12, e0006638
43. Coon, K.L. *et al.* (2017) Bacteria-mediated hypoxia functions as a signal for mosquito development. *Proc. Natl. Acad. Sci. U. S. A.* 114, E5362–E5369
44. Valzania, L. *et al.* (2018) Hypoxia-induced transcription factor signaling is essential for larval growth of the mosquito *Aedes aegypti*. *Proc. Natl. Acad. Sci. U. S. A.* 115, 457–465
45. Correa, M.A. *et al.* (2018) Generation of axenic *Aedes aegypti* demonstrate live bacteria are not required for mosquito development. *Nat. Commun.* 9, 4464
46. Sharma, A. *et al.* (2013) Gut microbes influence fitness and malaria transmission potential of Asian malaria vector *Anopheles stephensi*. *Acta Trop.* 128, 41–47
47. McMeniman, C.J. *et al.* (2009) Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. *Science* 323, 141–144
48. Contreras, E. *et al.* (2019) A neurotoxin that specifically targets *Anopheles mosquitoes*. *Nat. Commun.* 10, 2869
49. Pike, A. *et al.* (2017) Changes in the microbiota cause genetically modified *Anopheles* to spread in a population. *Science* 357, 1396–1399
50. Díaz-Nieto, L.M. *et al.* (2016) *Culex pipiens* development is greatly influenced by native bacteria and exogenous yeast. *PLoS One* 11, e0153133
51. Reeves, W.K. (2004) Oviposition by *Aedes aegypti* (Diptera: Culicidae) in relation to conspecific larvae infected with internal symbiotes. *J. Vector Ecol.* 29, 159–163
52. Coon, K.L. *et al.* (2016) Gut bacteria differentially affect egg production in the anautogenous mosquito *Aedes aegypti* and facultatively autogenous mosquito *Aedes atropalpus* (Diptera: Culicidae). *Parasit. Vectors* 9, 375

53. Flores, H.A. and O'Neill, S.L. (2018) Controlling vector-borne diseases by releasing modified mosquitoes. *Nat. Rev. Microbiol.* 16, 508–518
54. Sheehan, K.B. *et al.* (2016) Identification and characterization of a candidate *Wolbachia pipientis* type IV effector that interacts with the actin cytoskeleton. *mBio* 7, 4
55. Beckmann, J.F. *et al.* (2017) A *Wolbachia* deubiquitylating enzyme induces cytoplasmic incompatibility. *Nat. Microbiol.* 2, 17007
56. Chen, H. *et al.* (2019) A *Wolbachia* nuclease and its binding partner provide a distinct mechanism for cytoplasmic incompatibility. *Proc. Natl. Acad. Sci. U. S. A.* 116, 22314–22321
57. LePage, D.P. *et al.* (2017) Prophage WO genes recapitulate and enhance *Wolbachia*-induced cytoplasmic incompatibility. *Nature* 543, 243–247
58. Saraiva, R.G. *et al.* (2016) Mosquito gut antiparasitic and antiviral immunity. *Dev. Comp. Immunol.* 64, 53–64
59. Dong, Y. *et al.* (2009) Implication of the mosquito midgut microbiota in the defense against malaria parasites. *PLoS Pathog.* 5, e1000423
60. Capone, A. *et al.* (2013) Interactions between *Asaia, Plasmodium* and *Anopheles*: new insights into mosquito symbiosis and implications in malaria symbiotic control. *Parasit. Vectors* 6, 182
61. Boissiere, A. *et al.* (2012) Midgut microbiota of the malaria mosquito vector *Anopheles gambiae* and interactions with *Plasmodium falciparum* infection. *PLoS Pathog.* 8, e1002742
62. Wu, P. *et al.* (2019) A gut commensal bacterium promotes mosquito permissiveness to arboviruses. *Cell Host Microbe* 25, 101–112.e5
63. Bando, H. *et al.* (2013) Intra-specific diversity of *Serratia marcescens* in *Anopheles* mosquito midgut defines *Plasmodium* transmission capacity. *Sci. Rep.* 3, 1641
64. Bai, L. *et al.* (2019) A gut symbiotic bacterium *Serratia marcescens* renders mosquito resistance to *Plasmodium* infection through activation of mosquito immune responses. *Front. Microbiol.* 10, 1580
65. Azambuja, P. *et al.* (2005) Gut microbiota and parasite transmission by insect vectors. *Trends Parasitol.* 21, 568–572
66. Cirimotich, C.M. *et al.* (2011) Natural microbe-mediated refractoriness to *Plasmodium* infection in *Anopheles gambiae*. *Science* 332, 855–858
67. Castro, A.J. (1967) Antimalarial activity of prodigiosin. *Nature* 213, 903–904
68. Welch, R.A. (1991) Pore-forming cytolysins of gram-negative bacteria. *Mol. Microbiol.* 5, 521–528
69. Patil, C.D. *et al.* (2011) Prodigiosin produced by *Serratia marcescens* NMCC46 as a mosquito larvicidal agent against *Aedes aegypti* and *Anopheles stephensi*. *Parasitol. Res.* 109, 1179–1187
70. Zhu, Y. *et al.* (2019) Host serum iron modulates dengue virus acquisition by mosquitoes. *Nat. Microbiol.* 4, 2405–2415
71. Portugal, S. *et al.* (2011) Superinfection in malaria: *Plasmodium* shows its iron will. *EMBO Rep.* 12, 1233–1242
72. Wang, H. *et al.* (2018) Antiviral effects of ferric ammonium citrate. *Cell Discov.* 4, 14
73. Kamada, N. *et al.* (2013) Control of pathogens and pathobionts by the gut microbiota. *Nat. Immunol.* 14, 685–690
74. Durvasula, R.V. *et al.* (1997) Prevention of insect-borne disease: an approach using transgenic symbiotic bacteria. *Proc. Natl. Acad. Sci. U. S. A.* 94, 3274–3278
75. Shigeto Yoshida, D.I. *et al.* (2001) Bacteria expressing single-chain immunotoxin inhibit malaria parasite development in mosquitoes. *Mol. Biochem. Parasit.* 113, 89–96
76. Riehle, M.A. *et al.* (2007) Using bacteria to express and display anti-*Plasmodium* molecules in the mosquito midgut. *Int. J. Parasitol.* 37, 595–603
77. Shane, J.L. *et al.* (2018) Blood meal-induced inhibition of vector-borne disease by transgenic microbiota. *Nat. Commun.* 9, 4127
78. Wang, S. and Jacobs-Lorena, M. (2013) Genetic approaches to interfere with malaria transmission by vector mosquitoes. *Trends Biotechnol.* 31, 185–193
79. Chahar, H.S. *et al.* (2009) Co-infections with chikungunya virus and dengue virus in Delhi, India. *Emerg. Infect. Dis.* 15, 1077–1080
80. Chipwaza, B. *et al.* (2014) Dengue and Chikungunya fever among viral diseases in outpatient febrile children in Kilosa district hospital, Tanzania. *PLoS Negl. Trop. Dis.* 8, e3335
81. Baba, M. *et al.* (2013) Evidence of arbovirus co-infection in suspected febrile malaria and typhoid patients in Nigeria. *J. Infect. Dev. Countries* 7, 51–59
82. Yen, P.S. *et al.* (2018) Synthetic miRNAs induce dual arboviral-resistance phenotypes in the vector mosquito *Aedes aegypti*. *Commun. Biol.* 1, 11
83. Hoffmann, A.A. *et al.* (2011) Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature* 476, 454–457
84. Zheng, X. *et al.* (2019) Incompatible and sterile insect techniques combined eliminate mosquitoes. *Nature* 572, 56–61
85. Pimenta de Oliveira, S. *et al.* (2017) *Wolbachia* infection in *Aedes aegypti* mosquitoes alters blood meal excretion and delays oviposition without affecting trypsin activity. *Insect Biochem. Molec.* 87, 65–74
86. Kambris, Z. *et al.* (2009) Immune activation by life-shortening *Wolbachia* and reduced filarial competence in mosquitoes. *Science* 326, 134–136
87. Moreira, L.A. *et al.* (2009) A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and *Plasmodium*. *Cell* 139, 1268–1278
88. van den Hurk, A.F. *et al.* (2012) Impact of *Wolbachia* on infection with Chikungunya and Yellow fever viruses in the mosquito vector *Aedes aegypti*. *PLoS Negl. Trop. Dis.* 6, e1892
89. Caragata, E.P. *et al.* (2016) Inhibition of Zika virus by *Wolbachia* in *Aedes aegypti*. *Microb. Cell* 3, 293–295
90. Hussain, M. *et al.* (2013) Effect of *Wolbachia* on replication of West Nile virus in a mosquito cell line and adult mosquitoes. *J. Virol.* 87, 851–858
91. King, J.G. *et al.* (2018) Variation in *Wolbachia* effects on *Aedes* mosquitoes as a determinant of invasiveness and vectorial capacity. *Nat. Commun.* 9, 1483
92. Dodson, B.L. *et al.* (2014) *Wolbachia* enhances West Nile virus (WNV) infection in the mosquito *Culex tarsalis*. *PLoS Negl. Trop. Dis.* 8, e2965
93. Altinli, M. *et al.* (2018) Sharing cells with *Wolbachia*: the transovarian vertical transmission of *Culex pipiens* densovirus. *Environ. Microbiol.* Published online December 25, 2018. https://doi.org/10.1111/1462-2920.14511
94. Silva, J.B.L. *et al.* (2017) *Wolbachia* and dengue virus infection in the mosquito *Aedes fluviatilis* (Diptera: Culicidae). *PLoS One* 12, e0181678
95. Hughes, G.L. and Rasgon, J.L. (2014) Transinfection: a method to investigate *Wolbachia*-host interactions and control arthropod-borne disease. *Insect Mol. Biol.* 23, 141–151
96. Hughes, G.L. *et al.* (2011) *Wolbachia* infections are virulent and inhibit the human malaria parasite

Plasmodium falciparum in *Anopheles gambiae*. *PLoS Pathog.* 7, e1002043
97. Hughes, G.L. *et al.* (2012) *Wolbachia* strain wAlbB enhances infection by the rodent malaria parasite *Plasmodium berghei* in *Anopheles gambiae* mosquitoes. *Appl. Environ. Microbiol.* 78, 1491–1495
98. Reveillaud, J. *et al.* (2019) The *Wolbachia* mobilome in *Culex pipiens* includes a putative plasmid. *Nat. Commun.* 10, 1051
99. Scholte, E.-J. *et al.* (2005) An entomopathogenic fungus for control of adult African malaria mosquitoes. *Science* 308, 1641–1642
100. Cui, C. *et al.* (2019) A fungal pathogen deploys a small silencing RNA that attenuates mosquito immunity and facilitates infection. *Nat. Commun.* 10, 4298
101. Wei, G. *et al.* (2017) Insect pathogenic fungus interacts with the gut microbiota to accelerate mosquito mortality. *Proc. Natl. Acad. Sci. U. S. A.* 114, 5994–5999
102. Fang, W. *et al.* (2011) Development of transgenic fungi that kill human malaria parasites in mosquitoes. *Science* 331, 1074–1077
103. Tajedin, L. *et al.* (2009) Study on fungal flora in the midgut of the larva and adult of the different populations of the malaria vector *Anopheles stephensi*. *Iran J. Arthropod-Bor.* 3, 36–40
104. Ricci, I. *et al.* (2011) The yeast *Wickerhamomyces anomalus* (*Pichia anomala*) inhabits the midgut and reproductive system of the Asian malaria vector *Anopheles stephensi*. *Environ. Microbiol.* 13, 911–921
105. Walker, G.M. (2011) *Pichia anomala*: cell physiology and biotechnology relative to other yeasts. *Antonie Leeuw. Int. J. G.* 99, 25–34
106. Cappelli, A. *et al.* (2019) Killer yeasts exert anti-plasmodial activities against the malaria parasite *Plasmodium berghei* in the vector mosquito *Anopheles stephensi* and in mice. *Parasit. Vectors* 12, 329
107. Anglero-Rodriguez, Y.I. *et al.* (2017) An *Aedes aegypti*-associated fungus increases susceptibility to dengue virus by modulating gut trypsin activity. *eLife* 6, e28844.
108. O'Neill, S.L. *et al.* (1995) Insect densoviruses may be widespread in mosquito cell lines. *J. Gen. Virol.* 76, 2067–2074
109. Ren, X. *et al.* (2008) Viral paratransgenesis in the malaria vector *Anopheles gambiae*. *PLoS Pathog.* 4, e1000135
110. Dutra, H.L. *et al.* (2016) *Wolbachia* blocks currently circulating Zika virus isolates in Brazilian *Aedes aegypti* mosquitoes. *Cell Host Microbe* 19, 771–774
111. Bian, G. *et al.* (2013) *Wolbachia* invades *Anopheles stephensi* populations and induces refractoriness to *Plasmodium* infection. *Science* 340, 748–751