



HAL
open science

The tripartite interactions between the mosquito, its microbiota and Plasmodium

Ottavia Romoli, Mathilde Gendrin

► **To cite this version:**

Ottavia Romoli, Mathilde Gendrin. The tripartite interactions between the mosquito, its microbiota and Plasmodium. *Parasites & Vectors*, 2018, 11 (1), pp.200. 10.1186/s13071-018-2784-x . pasteur-02919242

HAL Id: pasteur-02919242

<https://pasteur.hal.science/pasteur-02919242>

Submitted on 21 Aug 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

REVIEW

Open Access



The tripartite interactions between the mosquito, its microbiota and *Plasmodium*

Ottavia Romoli¹ and Mathilde Gendrin^{1,2*}

Abstract

The microbiota of *Anopheles* mosquitoes interferes with mosquito infection by *Plasmodium* and influences mosquito fitness, therefore affecting vectorial capacity. This natural barrier to malaria transmission has been regarded with growing interest in the last 20 years, as it may be a source of new transmission-blocking strategies. The last decade has seen tremendous progress in the functional characterisation of the tripartite interactions between the mosquito, its microbiota and *Plasmodium* parasites. In this review, we provide insights into the effects of the mosquito microbiota on *Plasmodium* infection and on mosquito physiology, and on how these aspects together influence vectorial capacity. We also discuss three current challenges in the field, namely the need for a more relevant microbiota composition in experimental mosquitoes involved in vector biology studies, for a better characterisation of the non-bacterial microbiota, and for further functional studies of the microbiota present outside the gut.

Keywords: Microbiota, *Anopheles*, *Plasmodium*, Experimental models, Vectorial transmission, Colonisation resistance

Background

Plasmodium malaria parasites are transmitted by *Anopheles* mosquitoes. Female mosquitoes become infected after taking a blood meal on humans carrying *Plasmodium* pre-sexual stages. Upon entering the gut lumen, parasites undergo sexual reproduction and differentiate into motile forms, ookinetes, within 24 hours. After crossing the gut epithelium, the ookinete develops into an oocyst which undergoes mitoses in the following week and releases sporozoites in the hemolymph. About 10–14 days after the infected blood meal, sporozoites reach the salivary glands. The mosquito then becomes infectious and will inject parasites to humans with its saliva during subsequent bites for the rest of its life.

Ingestion of an infectious blood meal will only result in malaria transmission if the parasite makes it through bottlenecks in the gut and salivary glands and if the mosquito bites humans after the extrinsic incubation period, the time needed for parasites to become infectious. The gut, salivary glands and reproductive organs are colonised by a dynamic microbial community

composed by bacteria, viruses and fungi, of which the bacterial part is the best characterised [1–3]. This microbiota impacts disease transmission by interfering with *Plasmodium* colonisation in the gut and by affecting different aspects of mosquito physiology, notably its lifespan.

As a consequence, microorganisms that colonise the mosquito are regarded as potential tools to reduce malaria transmission. They may be used to shorten the mosquito lifespan or to decrease *Plasmodium* infection rates, either *via* natural competition mechanisms [4, 5] or *via* the production of genetically introduced anti-*Plasmodium* molecules, so called paratransgenesis [6–8]. One of the main advantages of a strategy based on microbial colonisation is the potential for targeting several species of mosquitoes and *Plasmodium* at the same time.

During development, the mosquito acquires its microbiota from its mother's genitalia and from its larval and pupal breeding site. Some of these microorganisms are trans-stadially transmitted to the adult [9, 10], while others are acquired by adults when feeding on different substrates or during mating [11]. The microbiota population is particularly dynamic in the mosquito gut, where it drastically expands after a blood meal. More specifically, this proliferation is observed in the middle region of the gut, the midgut, where the blood is stored during

* Correspondence: mathilde.gendrin@pasteur.fr

¹Microbiota of Insect Vectors Group, Institut Pasteur de la Guyane, Cayenne, French Guiana, France

²Parasites and Insect Vectors Department, Institut Pasteur, Paris, France

digestion over a 2-day period and where the early stages of *Plasmodium* development take place. Here, we review the current knowledge on tripartite interactions between the mosquito, its microbiota and *Plasmodium* and we discuss current challenges of the field.

Effects of the microbiota on vectorial capacity

Since the midgut represents the first and main bottleneck of parasite development, the microbial community present in its lumen has a strong role in the first stages of *Plasmodium* infection. Several functional studies have investigated the specific role of the midgut microbiota on *Plasmodium* infection. These studies, performed on five *Anopheles* species and four *Plasmodium* species, point to an overall inhibitory effect of the microbiota on the parasite, independent of the species of *Anopheles* and *Plasmodium* (Table 1). The effect is, however, highly bacterial strain specific [4, 12–14]. Most bacteria showing an anti-parasitic effect are Gram-negative [12, 14].

Antiparasitic effects of the microbiota on *Plasmodium*

The microbiota interferes with *Plasmodium* colonisation of the mosquito gut through at least two mechanisms: (i) stimulation of the mosquito immune response; and (ii) production of metabolites directly impairing parasite survival (Fig. 1).

The fast multiplication of bacteria in the gut following a blood meal stimulates an immune response which is widely antimicrobial. At the level of the midgut epithelium, this immune response is largely due to the Immune-deficiency (Imd) pathway, which is induced upon detection of the bacterial cell wall peptidoglycan by Peptidoglycan Recognition Protein (PGRP) LC and positively regulated by PGRP-LA [15–17]. How the Imd pathway affects the parasite has not yet been characterised, but this pathway has been shown to induce *TEP1* expression, and *TEP1* has been reported to participate in the microbiota-dependent control of *Plasmodium* infection [18, 19]. Ookinete numbers are, however, already reduced in the gut epithelium in a microbiota-dependent manner, suggesting that microbiota-induced

Table 1 Effects of the microbiota or of specific bacterial species/strains on *Plasmodium* infection in different *Anopheles* mosquitoes in chronological order

Mosquito	Parasite	Inhibition	No inhibition	Reference
<i>An. stephensi</i> (colony)	<i>P. falciparum</i>	<i>Escherichia coli</i> H243 ^a ; <i>Pseudomonas aeruginosa</i> ^a ; <i>Ewingella americana</i> ^a	<i>Staphylococcus aureus</i> ^b ; <i>S. epidermidis</i> ^b ; <i>E. coli</i> HB101 ^a	[12]
<i>An. stephensi</i> (colony)	<i>P. falciparum</i>	<i>E. coli</i> HSS ^a ; <i>P. aeruginosa</i> ^a ; <i>Serratia marcescens</i> ^a ; <i>Xanthomonas malthophilia</i> ^a ; <i>Cedecea lapagei</i> ^a		[67]
<i>An. albimanus</i> (colony)	<i>P. vivax</i>	<i>S. marcescens</i> ^a ; <i>Enterobacter cloacae</i> ^a ; <i>Enterobacter amnigenus</i> 2 ^a		[4]
<i>An. gambiae</i> ^c (colony)	<i>P. falciparum</i>	Microbiota; Live and heat-inactivated mixture of <i>S. aureus</i> ^b + <i>E. coli</i> ^a		[5]
<i>An. gambiae</i> ^d , <i>An. coluzzii</i> (colonies)	<i>P. berghei</i>	<i>S. aureus</i> ^b ; <i>E. coli</i> ^a ; <i>E. cloacae</i> ^a		[15]
<i>An. gambiae</i> ^c , <i>An. stephensi</i> (colonies)	<i>P. falciparum</i>	<i>Enterobacter</i> sp. Zambia ^a	<i>Bacillus pumilus</i> ^b	[13]
<i>An. gambiae</i> ^d (colony)	<i>P. yoelii</i>		Microbiota	[68]
<i>An. gambiae</i> ^d (colony)	<i>P. falciparum</i>	Microbiota		
<i>An. coluzzii</i> (colony)	<i>P. falciparum</i>	<i>E. coli</i> ^a ; <i>S. marcescens</i> ^a ; <i>Pseudomonas sutzeri</i> ^a ; <i>Comamonas</i> spp. ^a ; <i>Enterobacter</i> spp. ^a ; <i>B. pumilus</i> ^b	<i>Acinetobacter septicus</i> ^a	[69]
<i>An. stephensi</i> (colony)	<i>P. berghei</i>	<i>S. marcescens</i> HB3 ^a	<i>S. marcescens</i> HB18 ^a	[14]
<i>An. dirus</i> (colony)	<i>P. yoelii</i>	Microbiota		[19]
<i>An. gambiae</i> ^c (colony)	<i>P. falciparum</i>	<i>Chromobacterium</i> sp. Csp_P ^a		[22]
<i>An. gambiae</i> ^c (colony)	<i>P. falciparum</i>	<i>P. putida</i> ^a ; <i>Pantoea</i> sp. ^a ; <i>S. marcescens</i> ^a		[16]
<i>An. gambiae</i> ^e (from field larvae)	<i>P. falciparum</i>	<i>Serratia</i> ^a ; <i>Methylobacterium</i> ^a		[3]

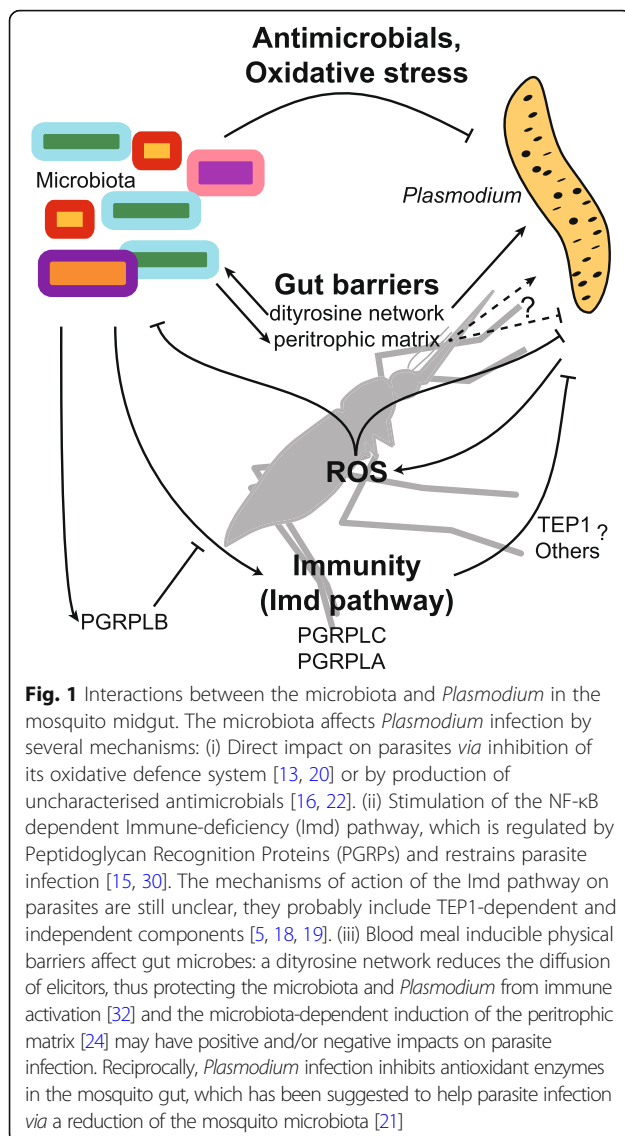
^aGram-negative bacteria

^bGram-positive bacteria

^cKeele strain, hybrid of *An. gambiae* and *An. coluzzii*

^dG3 strain, hybrid of *An. gambiae* and *An. coluzzii*

^eField-collected mosquitoes, not specified if *An. gambiae* or *An. coluzzii*



immunity also affects parasites before the action of the complement system [5].

Some microorganisms isolated from the mosquito gut also produce metabolites that directly affect *Plasmodium* and reduce its ability to infect the mosquito. *Enterobacter* Esp_Z is a Gram-negative bacterium isolated from the gut of *An. arabiensis* mosquitoes that was found to reduce *P. falciparum* ookinete, oocyst and sporozoite loads in *An. gambiae* *via* production of reactive oxygen species and/or inhibition of the oxidative defence system of the parasite [13, 20]. Interestingly, *Plasmodium* infection has been reported to reciprocally inhibit antioxidant enzymes in the mosquito gut. This may promote parasite infection *via* a reduction of the mosquito microbiota [21]. Some *Serratia marcescens* strains and *Chromobacterium* Csp_P initially isolated from the mosquito midgut reduce *P. falciparum* infection in *An. gambiae* and

show anti-parasitic activity *in vitro* by producing one or several uncharacterised metabolites [16, 22]. Moreover, the yeast *Wickerhamomyces anomalus* was isolated from the midgut of *An. stephensi* and found to produce a killing toxin with β -1,3-glucanase activity, which inhibits *P. berghei* ookinetes *in vitro* [23].

Besides these well-characterised effects of the microbiota on *Plasmodium*, some effects are indirectly suggested in the literature. For instance, the microbiota is also involved in the synthesis of the peritrophin matrix, a layer composed of chitin and proteins which surrounds the midgut epithelium after blood-feeding and protects the mosquito from the dissemination of midgut bacteria into the body cavity [24]. *Plasmodium* ookinetes secrete a chitinase required to cross the peritrophin matrix [25, 26], indicating that this barrier, if not impenetrable for malaria parasites, exerts at least some selection pressure on them. From this point-of-view, it may be a colonisation resistance mechanism induced by the microbiota against *Plasmodium*.

The microbiota may also nutritionally affect *Plasmodium* in the mosquito gut. Whole genome sequencing of bacterial strains of the mosquito microbiota identified genes involved in the digestion of macromolecules [27]. However, it is yet unknown whether this potential digestive role of the microbiota results in a benefit for the mosquito and for *Plasmodium* or in nutrient competition. In *Drosophila*, resistance to virus infection induced by endosymbionts is at least partly due to competition for cholesterol [28]. Nutrient availability is manipulated by *Plasmodium* in the mosquito gut through overexpression of digestive enzymes, suggesting that the high nutritional requirement of the parasite at its early developmental stages is a limiting factor for host colonisation [29].

Positive effects of the microbiota on *Plasmodium*

Several tolerance mechanisms avoid chronic activation of the immune system by gut microorganisms and participate in maintaining gut homeostasis, but they may also impair the mosquito immune defence against the parasite. Notably, PGRPLB is a negative regulator of the Imd pathway preventing systemic antimicrobial responses to the microbiota, which participates in a higher tolerance to *Plasmodium* infection [30]. Its expression is induced by the microbiota [24, 31]. Immunomodulatory Peroxidase (IMPer) and Dual oxidase (Duox) enzymes, induced by the blood meal in a microbiota-independent manner [24, 31], are involved in the formation of a dityrosine network between the gut epithelium and the peritrophin matrix [32]. This reduction in permeability protects both the microbiota and *Plasmodium* by preventing the activation of gut immunity. Whether the microbiota-induced peritrophin matrix [24] further reduces the diffusion of immune elicitors and is also

involved in tolerance mechanisms has not yet been characterised. Along these lines, antibiotic treatment leads to an increase in the diffusion of 4kD dextran molecules from the gut to the body cavity even in IMPer-silenced mosquitoes [32].

Finally, the microbiota is also reported to contribute to the nutrition of the mosquito and *Plasmodium*. After feeding *An. stephensi* with [¹⁴C]-glycine radiolabelled *Pseudomonas* isolated from the *Anopheles* microbiota, radioactivity is detectable not only in the whole mosquito body, but also in *P. berghei* oocysts and in disseminated sporozoites [33]. This may reflect a positive effect of the microbiota on proliferation within the oocyst.

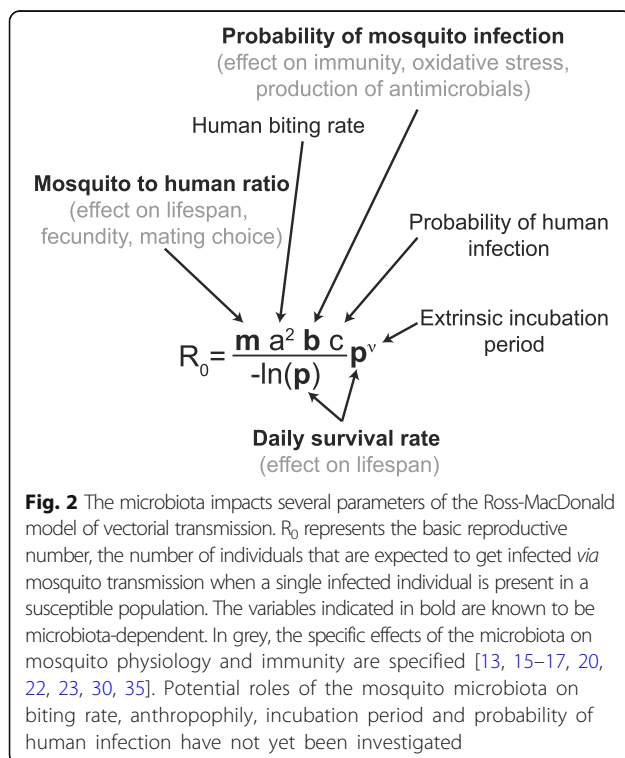
So far, antibiotic treatments and bacterial feeding treatment have reproducibly shown that the microbiota reduces infection by *Plasmodium* (Table 1), suggesting that even though some tolerance pathways and nutritional benefits exist, they only lessen the overall negative impact of the microbiota on *Plasmodium*.

Impact of the microbiota on mosquito fitness

A variety of factors influence parasite transmission by the mosquito. An estimation of their relative contribution to vectorial capacity is provided by the Ross-Macdonald model, which quantifies the basic reproductive number R_0 , i.e. the expected number of secondary infections from a single infected individual in a susceptible population (Fig. 2; [34]). It depends on the population sizes of mosquitoes and humans, the biting rate on humans, the

success of parasite infection, the incubation period of the parasite in the mosquito and the mosquito lifespan.

As shown in Fig. 2, the microbiota impacts R_0 via different aspects of mosquito fitness such as insect development, lifespan, fecundity and mating behaviour [10, 17, 35]. More specifically, the microbiota is fundamental for mosquito development: *Ae. aegypti* and *An. gambiae* larvae depleted of their gut microbes are unable to develop into adults [10]. A reduction in the proliferation of gut bacteria via a mild antibiotic treatment of the blood meal in *An. coluzzii* increases the mosquito fecundity and survival, meaning that the natural microbiota proliferation potentially decreases mosquito population size and time available for parasite transmission [17]. Some bacteria isolated from the midgut of *Anopheles* were shown to decrease mosquito lifespan when individually administered through sugar or blood meal while others have no effect [16, 20, 22]. Although the abundance and the effect of these single bacteria likely differ in physiological conditions, these studies suggest a composition-dependent negative impact of the gut microbiota on lifespan. A recent study reported a modification of the *An. stephensi* microbiota following manipulation of mosquito immunity and showed that this in turn influences reproduction behaviour, by increasing the chance of mating between mosquitoes harbouring a different microbiota [35]. All of these aspects may alter the probability of transmitting the *Plasmodium* parasite and thus have a strong impact on vector control.



Current challenges in the study of the mosquito microbiota

Working on mosquitoes with a representative microbiota composition

The *Anopheles* midgut hosts a simple, variable and dynamic microbial community whose composition mostly depends on environmental factors and individual history. More particularly, seasonality, diet, larval breeding site and blood-feeding history, but also host genetic identity, have a strong influence in shaping the midgut bacterial content [1, 3, 36–41]. The high variability of those factors in the field probably explains the high diversity of the mosquito microbiota composition between individuals, almost independently of the mosquito species [10, 36, 42–46]. Different studies suggest that the mosquito microbiota composition is not random, but whether a core bacterial community exists is still not clear. On one side, some bacterial genera are frequently found in *Anopheles* midguts. They are Gram-negative aerobic or facultative aerobic bacteria, mostly belonging to the families *Enterobacteriaceae* (*Serratia*, *Ewingella*, *Enterobacter* and *Klebsiella*), *Acetobacteraceae* (*Acetobacter* and *Asaia*) and *Flavobacteriaceae* (*Elizabethkingia* and *Chryseobacterium*) (reviewed in [47]). On the

other side, the bacterial diversity is relatively low, with four operational taxonomic units (OTUs) representing more than 90% of the midgut community and a poor composition overlap between individual mosquitoes [45]. This suggests that, rather than a core microbiota, several typical microbiota compositions, or enterotypes, may be defined in *Anopheles* mosquitoes. Such types may derive from individual history, environment and host genetics.

Remarkably, *Anopheles* reared in laboratory conditions showed a reduced bacterial diversity in their midgut with respect to field-collected ones, although the majority of bacteria found in laboratory mosquitoes were present in wild mosquitoes [1, 36, 43]. Functional studies on the interactions between the microbiota and *Plasmodium* have mostly been conducted on mosquitoes reared under controlled laboratory conditions (Table 1). The microbiota composition of laboratory-reared mosquitoes varies between insectaries due to local variations and differences in husbandry [48]. This variability could be one of the explanations for some discrepancies of results between laboratories in vector biology. Meanwhile, microbiota studies on field-caught mosquitoes identified correlations, with *Plasmodium* infection for instance,

but are elusive on the causes and consequences of these correlations. Notably, Straif et al. [49] found an association between Gram-positive bacteria and *P. falciparum* infection in field-collected *An. funestus*, while Boissière et al. [36] and Tchioffo et al. [3] found a positive correlation between parasite infection and *Enterobacteriaceae* or *S. marcescens* (Gram-negatives) abundance in *An. gambiae* after experimental infection of adults derived from field-collected larvae. These data point out the need for more appropriate microbiota models to perform functional studies on the tripartite relationship between the mosquito, the microbiota and *Plasmodium*. Several experimental setups may be favoured and are shown in Fig. 3 with their pros and cons. Choosing the most appropriate model to answer a scientific question, or combining the use of several models, may strengthen vector biology studies.

Further identification of the role of the non-bacterial microbiota

Despite the high number of studies on the midgut microbiota, our knowledge of its non-bacterial components is still poor. Metagenomic data on total mosquito RNA detected several viruses associated with wild-

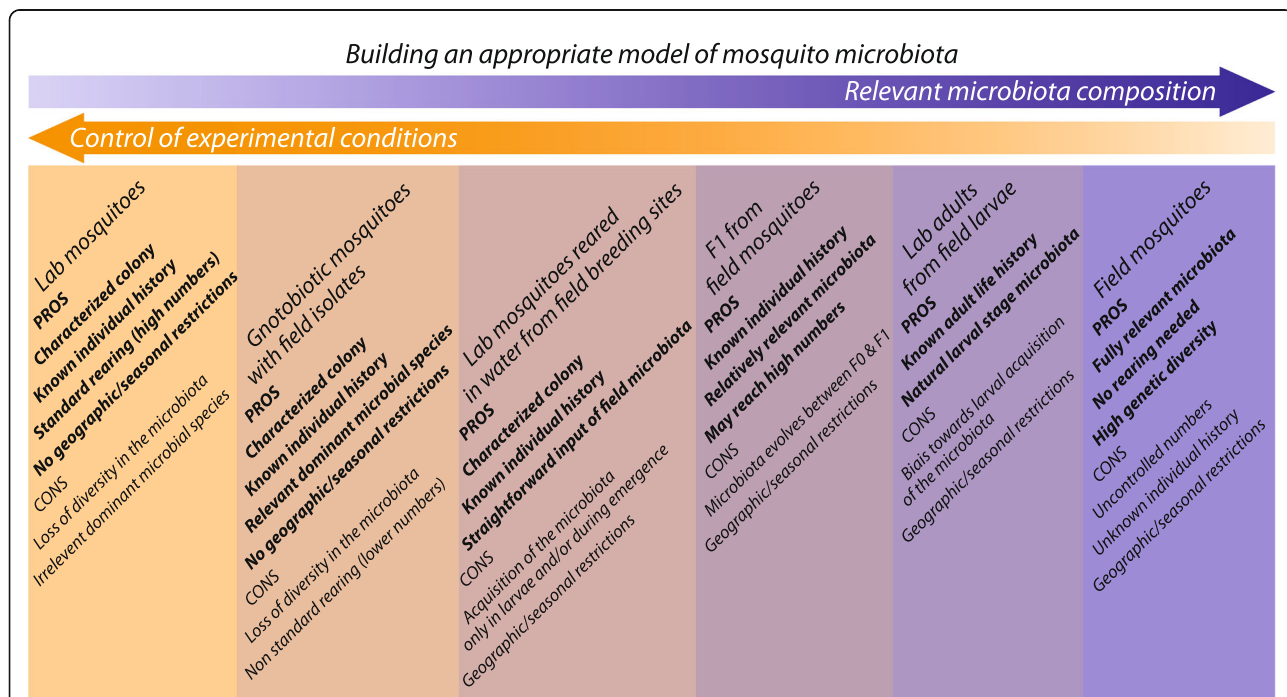


Fig. 3 Alternative models for the study of the mosquito and its microbiota. Besides laboratory conventionally-reared mosquitoes and field-collected mosquitoes, several other microbiota set ups are or may be used to study the mosquito biology. Field bacterial isolates may be used to obtain gnotobiotic mosquitoes, i.e. mosquitoes colonised by known strains of bacteria. Laboratory larvae may be reared in water collected from natural breeding sites, or a first generation of mosquitoes (F1) may be obtained from field collected individuals. Finally, adult mosquitoes may be raised in the laboratory from field collected larvae. None of these models is perfect but each may be more appropriate depending on the aims and requirements of each study. Combining several models may also strengthen experimental work and participate in increasing the reproducibility of results between laboratories

caught *Anopheles* [50, 51]. Similar data from other mosquitoes also catalogued some viral, bacterial and eukaryotic components of the microbiota [2], but cost and computational requirements limit the number of samples analysed.

The analysis of the eukaryotic microbiota by 18S rRNA sequencing is in part complicated by the amplification of DNA from the mosquito or from the blood source [52]. Several fungi belonging to the genera *Candida*, *Pichia* and *Penicillium* were isolated from *Anopheles* larvae and adults through standard microbiological techniques [53–55]. Two functional studies reported that specific fungi isolated from mosquitoes influence *Plasmodium* infectiveness either negatively [56] or positively [57]. However, we still lack an exhaustive investigation of the viral and eukaryotic components of the mosquito microbiota and of their interactions with *Plasmodium*.

Along these lines, the parasite itself may be considered as part of the microbial community, or pathobiome [58], harboured by the mosquito. In other insect-pathogen models, it has been shown that the invading microorganism can induce significant changes in the host microbiota composition to increase its infection success. For example, when the human pathogen *Anaplasma phagocytophilum* infects the tick *Ixodes scapularis*, it induces significant variations in the composition of the host gut microbial community. This correlates with pathogen-dependent induction of the *Ixodes* Antifreeze Glycoprotein IAFGP, which reduces biofilm formation and impairs the integrity of the peritrophic matrix, facilitating penetration of the pathogen into the tick body cavity [59]. Since similar interactions have not been described for the *Anopheles*-microbiota-*Plasmodium* system, further studies are required to clarify the role of the parasite in influencing the microbiota and host immunity.

Widen the knowledge on the microbiota in other tissues

The microbial communities colonising the mosquito salivary glands and reproductive organs are as relevant as those in the midgut for several aspects concerning *Plasmodium* transmission and mosquito ecology. Microorganisms present in the salivary glands are prone to interact with the parasite when the mosquito is becoming infectious and thus they might affect transmission efficiency, while microbes colonising the *Anopheles* genitalia may impact reproductive success, immunity, lifespan and microbiota composition of the offspring. The endosymbiont *Wolbachia* is found in half of the insect species, but had not been detected in any *Anopheles* species until recently. When artificially introduced in an *An. stephensi* strain, *Wolbachia* persists in mosquito ovaries, is vertically transmitted and increases the resistance against *P. falciparum* in laboratory conditions [60].

Wolbachia has now been found associated with around 10% of *Anopheles* in a wild population in Burkina Faso [61]. Its load was, however, much lower than that of other insect species, notably *Ae. albopictus* where *Wolbachia* was found to account for 99% of the 16S rRNA reads in whole mosquitoes [40, 61–63]. It was proposed that some members of the ovary microbiota, notably *Asaia*, interfere with *Wolbachia* colonisation and thus reduce its possible use against malaria [64, 65].

The microbiota of the ovaries and salivary glands has been investigated in *Anopheles* mosquitoes in few metagenomics studies [3, 37, 63, 66]. A recent study of the microbiota composition in midguts, salivary glands and ovaries suggests that individual history rather than the tissue shapes the mosquito microbiota, but still identified some differences in taxon abundance among the three tissues [3].

Functional studies on the role of the microorganisms colonising other tissues beyond the midgut will help to clarify several aspects of *Plasmodium* transmission and mosquito ecology. Moreover, the isolation of bacteria able to colonise the body of the mosquito without affecting its lifespan and to be efficiently vertically transmitted will identify new paratransgenesis candidates able to spread efficiently in the mosquito population. Recently, *Serratia* was identified in both ovaries and salivary glands of *Anopheles* mosquitoes and proposed for malaria control [3, 8].

Conclusions

In the past 20 years, the pivotal role of the mosquito microbiota in shaping *Plasmodium* infection and transmission has gradually emerged. However, the tripartite interaction between the mosquito, its microbiota and the parasite is a complex relationship that still needs further investigation. In general, the microbiota was found to reduce *Plasmodium* infection and to impact several physiological aspects of the mosquito, notably affecting its lifespan. Surprisingly, these effects induced by the microbiota were consistent almost irrespective of the *Anopheles* and *Plasmodium* species, suggesting that this tripartite interaction is a stable system in which each component plays a role. Although our knowledge on the mosquito microbiota is continuously expanding, several aspects have not been completely elucidated yet and represent the current challenges of this field. In particular, the non-bacterial component of the mosquito microbiota has not been investigated as extensively as the bacterial one, although viruses and eukaryotes might be as relevant as prokaryotes in limiting *Plasmodium* infection. Moreover, it is not clear whether the microbiota of the reproductive track or salivary glands impacts parasite transmission or mosquito fitness. Finally, most of the functional studies conducted on the mosquito

microbiota have been carried out on laboratory-reared insects, which are known to possess a different microbial community from that of field mosquitoes. Depending on the desired levels of control on experimental conditions and of relevance of microbiota composition, several experimental set ups may be used to improve the study of the mosquito microbiota.

Abbreviations

Duox: Dual oxidase; IAFGP: *Ixodes scapularis* antifreeze glycoprotein; Imd: Immune-deficiency; IMPer: Immunomodulatory peroxidase; OTU: Operational taxonomic unit; PGRP: Peptidoglycan recognition protein; ROS: Reactive oxygen species; TEP1: Thioester-containing Protein 1

Acknowledgements

The authors thank Faye Rodgers for proofreading of the manuscript.

Funding

This work is funded by the French Government's Investissement d'Avenir program, Laboratoire d'Excellence "Integrative Biology of Emerging Infectious Diseases" (grant no. ANR-10-LABX-62-IBEDI).

Availability of data and materials

Not applicable.

Authors' contributions

MG conceived the paper. MG and OR revised the current literature and wrote the paper. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 5 December 2017 Accepted: 6 March 2018

Published online: 20 March 2018

References

- Wang Y, Gilbreath TM 3rd, Kukutla P, Yan G, Xu J. Dynamic gut microbiome across life history of the malaria mosquito *Anopheles gambiae* in Kenya. *PLoS One*. 2011;6(9):e24767.
- Chandler JA, Liu RM, Bennett SN. RNA shotgun metagenomic sequencing of northern California (USA) mosquitoes uncovers viruses, bacteria, and fungi. *Front Microbiol*. 2015;6:185.
- Tchioffo MT, Boissiere A, Abate L, Nsango SE, Bayibeki AN, Awono-Ambene PH, et al. Dynamics of bacterial community composition in the malaria mosquito's epithelia. *Front Microbiol*. 2015;6:1500.
- Gonzalez-Ceron L, Santillan F, Rodriguez MH, Mendez D, Hernandez-Avila JE. Bacteria in midguts of field-collected *Anopheles albimanus* block *Plasmodium vivax* sporogonic development. *J Med Entomol*. 2003;40(3):371–4.
- Dong Y, Manfredini F, Dimopoulos G. Implication of the mosquito midgut microbiota in the defense against malaria parasites. *PLoS Pathog*. 2009;5(5):e1000423.
- Yoshida S, Ioka D, Matsuoka H, Endo H, Ishii A. Bacteria expressing single-chain immunotoxin inhibit malaria parasite development in mosquitoes. *Mol Biochem Parasitol*. 2001;113(1):89–96.
- Riehle MA, Moreira CK, Lampe D, Lauzon C, Jacobs-Lorena M. Using bacteria to express and display anti-*Plasmodium* molecules in the mosquito midgut. *Int J Parasitol*. 2007;37(6):595–603.
- Wang S, Dos-Santos ALA, Huang W, Liu KC, Oshaghi MA, Wei G, et al. Driving mosquito refractoriness to *Plasmodium falciparum* with engineered symbiotic bacteria. *Science*. 2017;357(6358):1399–402.
- Lindh JM, Borg-Karlson AK, Faye I. Transstadial and horizontal transfer of bacteria within a colony of *Anopheles gambiae* (Diptera: Culicidae) and oviposition response to bacteria-containing water. *Acta Trop*. 2008;107(3):242–50.
- Coon KL, Vogel KJ, Brown MR, Strand MR. Mosquitoes rely on their gut microbiota for development. *Mol Ecol*. 2014;23(11):2727–39.
- Favia G, Ricci I, Damiani C, Raddadi N, Crotti E, Marzorati M, et al. Bacteria of the genus *Asaia* stably associate with *Anopheles stephensi*, an Asian malarial mosquito vector. *Proc Natl Acad Sci USA*. 2007;104(21):9047–51.
- Pumpuni CB, Beier MS, Nataro JP, Guers LD, Davis JR. *Plasmodium falciparum*: inhibition of sporogonic development in *Anopheles stephensi* by Gram-negative bacteria. *Exp Parasitol*. 1993;77(2):195–9.
- Cirimotich CM, Dong Y, Clayton AM, Sandiford SL, Souza-Neto JA, Mulenga M, et al. Natural microbe-mediated refractoriness to *Plasmodium* infection in *Anopheles gambiae*. *Science*. 2011;332(6031):855–8.
- Bando H, Okado K, Guelbeogo WM, Badolo A, Aonuma H, Nelson B, et al. Intra-specific diversity of *Serratia marcescens* in *Anopheles* mosquito midgut defines *Plasmodium* transmission capacity. *Sci Rep*. 2013;3:1641.
- Meister S, Agianian B, Turlure F, Relogio A, Morlais I, Kafatos FC, et al. *Anopheles gambiae* PGRP-mediated defense against bacteria modulates infections with malaria parasites. *PLoS Pathog*. 2009;5(8):e1000542.
- Bahia AC, Dong Y, Blumberg BJ, Mlambo G, Tripathi A, BenMarzouk-Hidalgo OJ, et al. Exploring *Anopheles* gut bacteria for *Plasmodium* blocking activity. *Environ Microbiol*. 2014;16(9):2980–94.
- Gendrin M, Rodgers FH, Yerbanga RS, Ouedraogo JB, Basanez MG, Cohuet A, et al. Antibiotics in ingested human blood affect the mosquito microbiota and capacity to transmit malaria. *Nat Commun*. 2015;6:5921.
- Dong Y, Das S, Cirimotich C, Souza-Neto JA, McLean KJ, Dimopoulos G. Engineered *Anopheles* immunity to *Plasmodium* infection. *PLoS Pathog*. 2011;7(12):e1002458.
- Wang Y, Wang Y, Zhang J, Xu W, Zhang J, Huang FS. Ability of TEP1 in intestinal flora to modulate natural resistance of *Anopheles dirus*. *Exp Parasitol*. 2013;134(4):460–5.
- Dennison NJ, Saraiva RG, Cirimotich CM, Mlambo G, Mongodin EF, Dimopoulos G. Functional genomic analyses of *Enterobacter*, *Anopheles* and *Plasmodium* reciprocal interactions that impact vector competence. *Malar J*. 2016;15(1):425.
- Bahia AC, Oliveira JH, Kubota MS, Araujo HR, Lima JB, Rios-Velasquez CM, et al. The role of reactive oxygen species in *Anopheles aquasalis* response to *Plasmodium vivax* infection. *PLoS One*. 2013;8(2):e57014.
- Ramirez JL, Short SM, Bahia AC, Saraiva RG, Dong Y, Kang S, et al. *Chromobacterium Csp_P* reduces malaria and dengue infection in vector mosquitoes and has entomopathogenic and in vitro anti-pathogen activities. *PLoS Pathog*. 2014;10(10):e1004398.
- Valzano M, Cecarini V, Cappelli A, Capone A, Bozic J, Cuccioloni M, et al. A yeast strain associated to *Anopheles* mosquitoes produces a toxin able to kill malaria parasites. *Malar J*. 2016;15:21.
- Rodgers FH, Gendrin M, Wyer CAS, Christophides GK. Microbiota-induced peritrophic matrix regulates midgut homeostasis and prevents systemic infection of malaria vector mosquitoes. *PLoS Pathog*. 2017;13(5):e1006391.
- Huber M, Cabib E, Miller LH. Malaria parasite chitinase and penetration of the mosquito peritrophic membrane. *Proc Natl Acad Sci USA*. 1991;88(7):2807–10.
- Tsai YL, Hayward RE, Langer RC, Fidock DA, Vinetz JM. Disruption of *Plasmodium falciparum* chitinase markedly impairs parasite invasion of mosquito midgut. *Infect Immun*. 2001;69(6):4048–54.
- Kukutla P, Lindberg BG, Pei D, Rayl M, Yu W, Steritz M, et al. Insights from the genome annotation of *Elizabethkingia anophelis* from the malaria vector *Anopheles gambiae*. *PLoS One*. 2014;9(5):e97715.
- Caragata EP, Rances E, Hedges LM, Goffton AW, Johnson KN, O'Neill SL, et al. Dietary cholesterol modulates pathogen blocking by *Wolbachia*. *PLoS Pathog*. 2013;9(6):e1003459.
- Lavazec C, Boudin C, Lacroix R, Bonnet S, Diop A, Thiberge S, et al. Carboxypeptidases B of *Anopheles gambiae* as targets for a *Plasmodium falciparum* transmission-blocking vaccine. *Infect Immun*. 2007;75(4):1635–42.
- Gendrin M, Turlure F, Rodgers FH, Cohuet A, Morlais I, Christophides GK. The peptidoglycan recognition proteins PGRP-LA and PGRP-LB regulate *Anopheles*

- immunity to bacteria and affect infection by *Plasmodium*. *J Innate Immun*. 2017;9:333–42.
31. Vectorbase. RNA-Seq Experiment - Antibiotic treatment of midgut microbiota during blood feeding (Rodgers et al. 2017); 2017. <https://www.vectorbase.org/expression-browser/experiment/SRP106793>. Accessed 16 Nov 2017.
 32. Kumar S, Molina-Cruz A, Gupta L, Rodrigues J, Barillas-Mury C. A peroxidase/dual oxidase system modulates midgut epithelial immunity in *Anopheles gambiae*. *Science*. 2010;327(5973):1644–8.
 33. Jadin J. Role of bacteria in the digestive tube of insects, vectors of plasmodidae and trypanosomidae. *Ann Soc Belges Med Trop Parasitol Mycol*. 1967;47(4):331–42. (In French).
 34. Smith DL, Battle KE, Hay SI, Barker CM, Scott TW, FE MK. Ross, Macdonald, and a theory for the dynamics and control of mosquito-transmitted pathogens. *PLoS Pathog*. 2012;8(4):e1002588.
 35. Pike A, Dong Y, Dizaji NB, Gacita A, Mongodin EF, Dimopoulos G. Changes in the microbiota cause genetically modified *Anopheles* to spread in a population. *Science*. 2017;357(6358):1396–9.
 36. Boissiere A, Tchioffo MT, Bachar D, Abate L, Marie A, Nsango SE, et al. Midgut microbiota of the malaria mosquito vector *Anopheles gambiae* and interactions with *Plasmodium falciparum* infection. *PLoS Pathog*. 2012;8(5):e1002742.
 37. Gimonneau G, Tchioffo MT, Abate L, Boissiere A, Awono-Ambene PH, Nsango SE, et al. Composition of *Anopheles coluzzii* and *Anopheles gambiae* microbiota from larval to adult stages. *Infect Genet Evol*. 2014;28:715–24.
 38. Stathopoulos S, Neafsey DE, Lawniczak MK, Muskavitch MA, Christophides GK. Genetic dissection of *Anopheles gambiae* gut epithelial responses to *Serratia marcescens*. *PLoS Pathog*. 2014;10(3):e1003897.
 39. Akorli J, Gendrin M, Pels NA, Yeboah-Manu D, Christophides GK, Wilson MD. Seasonality and locality affect the diversity of *Anopheles gambiae* and *Anopheles coluzzii* midgut microbiota from Ghana. *PLoS One*. 2016;11(6):e0157529.
 40. Buck M, Nilsson LK, Brunius C, Dabire RK, Hopkins R, Terenius O. Bacterial associations reveal spatial population dynamics in *Anopheles gambiae* mosquitoes. *Sci Rep*. 2016;6:22806.
 41. Novakova E, Woodhams DC, Rodriguez-Ruano SM, Brucker RM, Leff JW, Maharaj A, et al. Mosquito microbiome dynamics, a background for prevalence and seasonality of West Nile Virus. *Front Microbiol*. 2017;8:526.
 42. Terenius O, de Oliveira CD, Pinheiro WD, Tadei WP, James AA, Marinotti O. 16S rRNA gene sequences from bacteria associated with adult *Anopheles darlingi* (Diptera: Culicidae) mosquitoes. *J Med Entomol*. 2008;45(1):172–5.
 43. Rani A, Sharma A, Rajagopal R, Adak T, Bhatnagar RK. Bacterial diversity analysis of larvae and adult midgut microflora using culture-dependent and culture-independent methods in lab-reared and field-collected *Anopheles stephensi* - an Asian malarial vector. *BMC Microbiol*. 2009;9:96.
 44. Dinparast Djadid N, Jazayeri H, Raz A, Favia G, Ricci I, Zakeri S. Identification of the midgut microbiota of *An. stephensi* and *An. maculipennis* for their application as a paratransgenic tool against malaria. *PLoS One*. 2011;6(12):e28484.
 45. Osei-Poku J, Mbogo CM, Palmer WJ, Jiggins FM. Deep sequencing reveals extensive variation in the gut microbiota of wild mosquitoes from Kenya. *Mol Ecol*. 2012;21(20):5138–50.
 46. Manguin S, Ngo CT, Tainchum KT, Juntarajumnong W, Chareonviriyaphap T, Michon AL, et al. Chapter 18. Bacterial biodiversity in midguts of *Anopheles* mosquitoes, malaria vectors in southeast Asia. In: Manguin S, editor. *Anopheles* mosquitoes - New insights into malaria vectors. InTech: Rijeka; 2013.
 47. Gendrin M, Christophides GK. Chapter 17. The *Anopheles* mosquito microbiota and their impact on pathogen transmission. In: Manguin S, *Anopheles* mosquitoes - New insights into malaria vectors. Rijeka: InTech; 2013.
 48. Linenberg I, Christophides GK, Gendrin M. Larval diet affects mosquito development and permissiveness to *Plasmodium* infection. *Sci Rep*. 2016;6:38230.
 49. Straif SC, Mbogo CN, Toure AM, Walker ED, Kaufman M, Toure YT, et al. Midgut bacteria in *Anopheles gambiae* and *An. funestus* (Diptera: Culicidae) from Kenya and Mali. *J Med Entomol*. 1998;35(3):222–6.
 50. Zhuang L, Zhang Z, An X, Fan H, Ma M, Anderson BD, et al. An efficient strategy of screening for pathogens in wild-caught ticks and mosquitoes by reusing small RNA deep sequencing data. *PLoS One*. 2014;9(3):e90831.
 51. Fauver JR, Grubaugh ND, Krajacich BJ, Weger-Lucarelli J, Lakin SM, Fakoli LS 3rd, et al. West African *Anopheles gambiae* mosquitoes harbor a taxonomically diverse virome including new insect-specific flaviviruses, mononegaviruses, and totiviruses. *Virology*. 2016;498:288–99.
 52. Belda E, Coulibaly B, Fofana A, Beavogui AH, Traore SF, Gohl DM, et al. Preferential suppression of *Anopheles gambiae* host sequences allows detection of the mosquito eukaryotic microbiome. *Sci Rep*. 2017;7(1):3241.
 53. da S Pereira E, de M Sarquis MI, Ferreira-Keppeler RL, Hamada N, Alencar YB. Filamentous fungi associated with mosquito larvae (Diptera: Culicidae) in municipalities of the Brazilian Amazon. *Neotrop Entomol*. 2009;38(3):352–9.
 54. Ricci I, Damiani C, Scuppa P, Mosca M, Crotti E, Rossi P, et al. The yeast *Wickerhamomyces anomalus* (*Pichia anomala*) inhabits the midgut and reproductive system of the Asian malaria vector *Anopheles stephensi*. *Environ Microbiol*. 2011;13(4):911–21.
 55. Bozic J, Capone A, Pediconi D, Mensah P, Cappelli A, Valzano M, et al. Mosquitoes can harbour yeasts of clinical significance and contribute to their environmental dissemination. *Environ Microbiol Rep*. 2017;9(5):642–8.
 56. Bargielowski I, Koella JC. A possible mechanism for the suppression of *Plasmodium berghei* development in the mosquito *Anopheles gambiae* by the microsporidian *Vavraia culicis*. *PLoS One*. 2009;4(3):e4676.
 57. Anglero-Rodriguez YI, Blumberg BJ, Dong Y, Sandiford SL, Pike A, Clayton AM, et al. A natural *Anopheles*-associated *Penicillium chrysogenum* enhances mosquito susceptibility to *Plasmodium* infection. *Sci Rep*. 2016;6:34084.
 58. Vayssier-Taussat M, Albina E, Citti C, Cosson JF, Jacques MA, Lebrun MH, et al. Shifting the paradigm from pathogens to pathobiome: new concepts in the light of meta-omics. *Front Cell Infect Microbiol*. 2014;4:29.
 59. Abraham NM, Liu L, Jutras BL, Yadav AK, Narasimhan S, Gopalakrishnan V, et al. Pathogen-mediated manipulation of arthropod microbiota to promote infection. *Proc Natl Acad Sci USA*. 2017;114(5):E781–90.
 60. Bian G, Joshi D, Dong Y, Lu P, Zhou G, Pan X, et al. *Wolbachia* invades *Anopheles stephensi* populations and induces refractoriness to *Plasmodium* infection. *Science*. 2013;340(6133):748–51.
 61. Baldini F, Segata N, Pompon J, Marcenac P, Shaw WR, Dabire RK, et al. Evidence of natural *Wolbachia* infections in field populations of *Anopheles gambiae*. *Nat Commun*. 2014;5:3985.
 62. Minard G, Tran FH, Dubost A, Tran-Van V, Mavingui P, Moro CV. Pyrosequencing 16S rRNA genes of bacteria associated with wild tiger mosquito *Aedes albopictus*: a pilot study. *Front Cell Infect Microbiol*. 2014;4:59.
 63. Segata N, Baldini F, Pompon J, Garrett WS, Truong DT, Dabire RK, et al. The reproductive tracts of two malaria vectors are populated by a core microbiome and by gender- and swarm-enriched microbial biomarkers. *Sci Rep*. 2016;6:24207.
 64. Hughes GL, Dodson BL, Johnson RM, Murdock CC, Tsujimoto H, Suzuki Y, et al. Native microbiome impedes vertical transmission of *Wolbachia* in *Anopheles* mosquitoes. *Proc Natl Acad Sci USA*. 2014;111(34):12498–503.
 65. Rossi P, Ricci I, Cappelli A, Damiani C, Ulissi U, Mancini MV, et al. Mutual exclusion of *Asaia* and *Wolbachia* in the reproductive organs of mosquito vectors. *Parasit Vectors*. 2015;8:278.
 66. Sharma P, Sharma S, Maurya RK, Das De T, Thomas T, Lata S, et al. Salivary glands harbor more diverse microbial communities than gut in *Anopheles culicifacies*. *Parasit Vectors*. 2014;7:235.
 67. Pumpuni CB, Demayo J, Kent M, Davis JR, Beier JC. Bacterial population dynamics in three anopheline species: the impact on *Plasmodium* sporogonic development. *Am J Trop Med Hyg*. 1996;54(2):214–8.
 68. Noden BH, Vaughan JA, Pumpuni CB, Beier JC. Mosquito ingestion of antibodies against mosquito midgut microbiota improves conversion of ookinetes to oocysts for *Plasmodium falciparum*, but not *P. yoelii*. *Parasitol Int*. 2011;60(4):440–6.
 69. Tchioffo MT, Boissiere A, Churcher TS, Abate L, Gimonneau G, Nsango SE, et al. Modulation of malaria infection in *Anopheles gambiae* mosquitoes exposed to natural midgut bacteria. *PLoS One*. 2013;8(12):e81663.