

Composition and functional roles of the gut microbiota in mosquitoes

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An estimated 3500 species of mosquitoes (family Culicidae) exist worldwide of which several are known vectors of pathogens that cause disease in humans and other vertebrates. Mosquitoes also host communities of microbes in their digestive tract that form a gut microbiota. Recent studies provide important insights on how mosquitoes acquire a gut microbiota and the community of microbes that are present. Results also indicate that the gut microbiota affects several aspects of mosquito biology. Altogether, these effects impact mosquito fitness with potential consequences for disease prevalence.

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Introduction

All mosquitoes are aquatic during their juvenile stages and terrestrial as adults [1]. Larvae primarily consume organic detritus, unicellular organisms and small invertebrates while adults of both sexes commonly feed on extrafloral nectaries [2,3]. Adult females also usually blood feed on vertebrates, which provides nutrients for egg production but can result in transmission of pathogens between hosts [1]. Studies from the early 1900s noted that larval and adult stage mosquitoes harbor communities of extracellular microbes in their digestive tract that form a gut microbiota [4,5*,6]. However, only in the last 10 years have these microbial communities and their roles in mosquito biology been more broadly studied. Results summarized in several recent reviews indicate that the gut microbiota of adult mosquitoes can both

positively and negatively affect vector competency, which refers to the ability of females to acquire, maintain, and transmit vertebrate pathogens [7–10]. This short summary focuses on how mosquitoes acquire a gut microbiota, community composition, and known effects on larval development and adult physiology.

Acquisition of a gut microbiota by mosquito larvae

Some insects directly acquire their gut microbiota from parents or other individuals, while others primarily acquire their gut microbiota from the environment [11,12]. Three lines of evidence indicate that mosquitoes predominantly acquire their gut microbiota anew each generation from the environment. First, experimental studies show that mosquito larvae hatch with no extracellular microbes in their gut [13**]. Second, studies of gut community composition indicate that most microbes identified in larvae overlap with the community of microbes that are present in their aquatic habitat [13***,14–17,18**]. Third, mosquitoes host highly variable gut communities [13***,14–17,18**], which would not be expected if these communities were acquired directly from parents or congeners. Exceptions to environmental acquisition are results showing that several types of bacteria are present in the reproductive tracts of adult mosquitoes, and that some of these bacteria are on the surface of eggs females lay [13***,15,19–25]. This can result in larvae directly acquiring these microbes by ingesting egg shell fragments at hatching or inoculation of the aquatic habitat where larvae feed. Several mosquito species host vertically transmitted intracellular bacteria in the genus *Wolbachia* and select other genera, which are thus present in eggs [7,24,26]. Certain viruses have also been shown to be vertically transmitted [27,28]. However, these organisms are not part of the extracellular community of microbes that constitute the gut microbiota.

Culture-based studies initially suggested that mosquito larvae expel their gut microbiota in a meconium at metamorphosis, and that adults emerge from the pupal stage with few or no gut microbes [29]. These results further suggested adults reacquire a gut microbiota by imbibing water from the larval habitat and/or feeding on resources like extrafloral nectaries. However, controlled experiments coupled with analysis of gut community composition provide strong evidence that *Aedes* and

Anopheles larvae transstadially transmit a portion of their gut microbiota to adults [13^{••},30,31]. Thereafter though, the adult gut microbiota can change through consumption of microbe-containing water, nectar or other food sources [32–34,35[•]]. Vertebrate blood usually contains few or no microbes, but several studies show that consumption of a blood meal persistently or transiently alters composition of the gut microbiota through alterations in redox status or metabolism [35[•],36,37,38[•],39,40,41]. Infection by different vector-borne pathogens can also affect the composition of the gut microbiota through unknown mechanisms [26,41–44].

Composition of the gut microbiota

Insights into the composition of the mosquito gut microbiota primarily derive from metagenomic sequencing studies. Most identified microbes in larval and adult stage mosquitoes are gram-negative aerobes or facultative anaerobes that preferentially belong to four phyla (Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria) [13^{••},14–17,18^{••},35[•],45[•],46,47[•],48]. Bacteria in these phyla are also common community members in insects from several other orders that acquire their gut microbiota from the environment [49], which suggests prevalence in part reflects their abundance in habitats insects frequent. Several bacteria identified as gut community members in mosquitoes have also been isolated and successfully cultured [13^{••},18^{••},22,31,50–53]. Unicellular eukaryotes identified as gut community members in different species of mosquitoes include fungi, algae, and Apicomplexa but thus far none of these organisms have been isolated from mosquitoes and cultured independently [43,48,54,55[•],56,57]. Sequence-based surveys also identify a number of viruses in mosquitoes [58,59]. Most belong to families with small RNA genomes such as the Flaviviridae which includes species that are mosquito-vectored vertebrate pathogens [59]. In contrast, the absence of bacteriophages in published studies suggest either viruses infecting bacteria in the gut are underrepresented or few bacteriophages infect bacteria present in the mosquito gut.

Excluding rare sequence reads, most studies indicate that bacterial diversity in the mosquito gut is low (200 species) when compared to vertebrates [12]. However, diversity estimates in mosquitoes are comparable to other holometabolous insects that acquire their gut microbiota from the environment [11,49]. Differences in bacterial species diversity have been reported between larval stadia of *Culex quinquefasciatus* [60]. Studies of *Aedes aegypti* further show that substantial numbers of bacteria in the gut die at the end of each instar prior to molting, which provides opportunity for altering gut community composition in the succeeding instar [61[•]]. Other patterns of note include that bacterial species diversity is consistently higher in mosquito larvae than adults [13^{••},15,18^{••},47[•]], and that

diversity is lower in laboratory reared versus field collected mosquitoes of the same species [13^{••},14,35[•]].

A large number of studies indicate that bacterial diversity differs substantially both between and within mosquito species as a function of collection location and other factors [14–17,18^{••},30,43,44,45[•],46,47[•],62,63,64]. On a local scale, factors implicated in affecting community composition include habitat preferences of different mosquito species and larval feeding behavior [18^{••},30,62]. Studies that have analyzed mosquito larvae from multiple sites that are in close proximity to one another report greater similarities in gut community composition among individuals of the same or different species from the same site and collection date than among individuals from different sites and collection dates [14,15,47[•]]. This strongly suggests the community of microbes present in aquatic habitats can vary considerably even when in close proximity to one another. It also means that larvae from a given site can harbor similar communities of gut microbes but adults collected from a given local area can exhibit high levels of variation due to heterogeneity between larval sites [14–16,35[•],45[•]]. In addition, while bacteria in particular phyla or classes are commonly found in mosquitoes, no evidence currently indicates that particular species of mosquitoes across their geographic range host a ‘core’ microbiota that always includes particular microbes [10,12]. Instead, current results indicate that environment dominates over genetics in shaping the gut microbiota of mosquitoes, which is a similar conclusion to studies of other insects and vertebrates that acquire their gut microbiota from the environment [49,65].

Functional roles of the gut microbiota in mosquito larvae

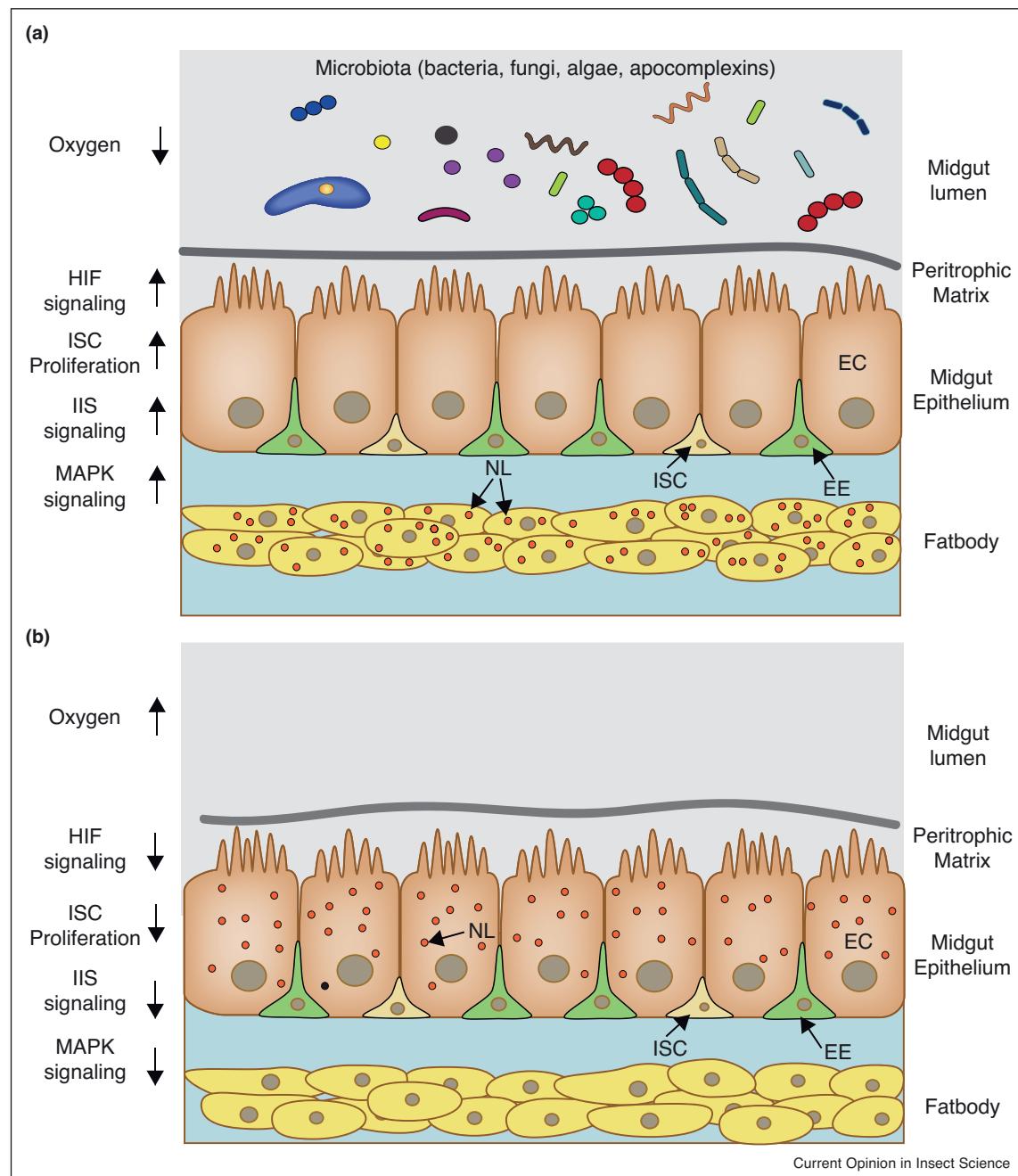
Several early studies reported that mosquito larvae exhibit higher mortality and/or delayed growth to the pupal stage when the abundance of microbes in the aquatic habitat is reduced [[47[•]],5[•],19]. More recently, increased mortality and delayed growth have also been reported when larvae are treated with antibiotics [66,67], whereas inoculating larvae with certain bacteria or yeast has been reported to promote growth [68,69]. These findings collectively suggest a positive role for the gut microbiota in development of mosquito larvae although the approaches used provide no insights into the mechanisms involved. Antibiotic treatment also does not fully eliminate the gut microbiota due to high levels of resistance in several bacterial community members and insensitivity of eukaryotic community members [47[•]].

Alternatively, surface sterilizing eggs produces axenic larvae with no gut microbiota, which can then be fed a sterile diet and/or inoculated with known microbes to produce gnotobiotic larvae [13^{••}]. This approach showed that axenic *Ae. aegypti* first instars consume a standard rearing diet like conventional (non-sterile) larvae but do

not grow which results in larvae dying after several days as first instars [13^{••}]. In contrast, *Escherichia coli* and several other species of bacteria identified as gut community members in different populations of *Ae. aegypti*

[13^{••},48] colonize the midgut of axenic larvae and rescue growth, whereas dead bacteria do not [13^{••}]. Axenic larvae from field collected populations of *Ae. aegypti* as well as several other species of mosquitoes further exhibit the

Figure 1



Schematic illustrating the known effects of the gut microbiota on larval growth and nutrient acquisition. **(a)** When living microbes are present in the midgut lumen, gut oxygen levels fall below 5%, HIF- α is stabilized with resulting HIF signaling stimulating: firstly, proliferation and differentiation of intestinal stem cells (ISCs) into endocrine cells (EE) and enterocytes (EC), secondly, insulin-insulin growth factor signaling (IIS), thirdly, mitogen activated kinase (MAPK) signaling, and finally, biosynthesis and transport of neutral lipids (NL) by midgut enterocytes (ECs) to the fat body. **(b)** When living microbes are absent from the midgut lumen, gut oxygen levels remain above 5%, which is associated with no stabilization of HIF- α and: firstly, greatly reduced ISC proliferation, secondly, no activation of IIS, thirdly, no activation of MAPK signaling, and finally, biosynthesis of neutral lipids in ECs but an absence of transport to the fat body.

same responses [13^{••},18^{••},47[•]], which altogether suggests several mosquitoes require living bacteria in their gut as larvae for growth and molting.

In considering how bacteria might promote growth, recent studies show that *Ae. aegypti* larvae inoculated with a mixed community of bacteria or wild-type *E. coli* exhibit markedly lower gut oxygen levels in the midgut (hypoxia) than axenic larvae or gnotobiotic larvae with bacteria defective for aerobic respiration [61[•]]. This suggested that bacteria in the midgut induce a gut hypoxia response which could function as a growth signal. Functional assays supporting this hypothesis include the finding that gut hypoxia activates hypoxia-induced transcription factors (HIFs) in *Ae. aegypti* larvae which leads to activation of the insulin/insulin growth factor pathway, select mitogen activated kinases and other processes with essential growth functions [61[•],70^{••}] (Figure 1). Transcriptome analysis also identifies several genes with functions in digestion and nutrient acquisition as additional targets that are potentially regulated by the gut microbiota or microbiota-induced hypoxia [71].

Axenic *Ae. aegypti* larvae were recently reported to grow into adults when fed a liver powder: yeast extract diet supplemented with heat-killed bacteria, which suggests other diets could obviate a requirement by mosquito larvae for living microbes under certain conditions [72]. In contrast, another study that bioassayed the same diets found no evidence axenic *Ae. aegypti* or *An. gambiae* larvae grow beyond the first instar but did find that several eukaryotic gut community members induce gut hypoxia, HIF signaling and larval growth if viable [73]. However, the preceding study and others also show that the proportion of larvae that develop into adults as well as the size, longevity, and fecundity of surviving adults varies among different bacteria and unicellular eukaryotes [18^{••},42,73–75]. Thus, microbes from diverse taxa can induce a gut hypoxia response and larval growth, whereas in the absence of a microbiota larvae do not grow or molt. In this respect the presence of a microbiota is more critical than its composition when compared to mosquitoes with no gut microbiota. However, community composition does affect several measures of mosquito fitness, which indicates the individual species of microbes or particular combinations of microbes benefit mosquito fitness more than others. Whether these differences reflect variation in the nutrients microbes provide in conjunction with the diet larvae are fed, the ability of certain microbes to facilitate digestion, or products that promote gut function or other activities is currently unknown.

Effects of the gut microbiota on midgut function in adult mosquitoes

In addition to affecting vector competence and fitness metrics like size and fecundity, a few studies indicate the gut microbiota also affects midgut function in adult

mosquitoes. These include evidence based on oral antibiotic treatment of adult *An. gambiae* that the gut microbiota affects immune gene expression in the midgut [50]. Antibiotic treatment of adult *An. coluzzii* further suggest the gut microbiota is required for peritrophic matrix formation around the blood bolus after blood feeding [41]. In turn, disabled peritrophic matrix formation allows bacteria to contact midgut cells, which could play a role in altered expression of immune genes and increased risk of systemic infection by gut microbes [41]. Other results implicate the gut microbiota in blood meal digestion either directly through the hemolytic activity of some community members or indirectly by affecting midgut function [76].

Conclusions

In addition to affecting vector competency, current results indicate the gut microbiota affects larval growth, adult fitness and select other traits that likely affect vector populations and disease prevalence. Results published in the last ten years substantially advance understanding of how mosquitoes acquire a gut microbiota and community composition but with a bias toward studies of bacteria. Going forward additional information is needed about the identity and diversity of unicellular eukaryotes in the mosquito gut as well as studies that begin to examine how particular networks of microbes interact, the stability of different communities and their effects on mosquito fitness. How mosquito diet, habitat and other factors interact to shape the composition of the gut microbiota is also largely unknown. While several recent papers indicate that the gut microbiota positively affects mosquito physiology, understanding of the underlying mechanisms remain understudied. To address this issue, more experimental data are needed that formally examine how the gut microbiota affects growth and other physiological processes, and the function of particular community members in these processes.

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