Review

Cell PRESS

*Feature Review*

# Microbiome influences on insect host vector competence

**Brian Weiss and Serap Aksoy**

Department of Epidemiology and Public Health, Yale School of Public Health, New Haven, CT 06520, USA

**Insect symbioses lack the complexity and diversity of those associated with higher eukaryotic hosts. Symbiotic microbiomes are beneficial to their insect hosts in many ways, including dietary supplementation, tolerance to environmental perturbations and maintenance and/or enhancement of host immune system homeostasis. Recent studies have also highlighted the importance of the microbiome in the context of host pathogen transmission processes. Here we provide an overview of the relationship between insect disease vectors, such as tsetse flies and mosquitoes, and their associated microbiome. Several mechanisms are discussed through which symbiotic microbes can influence the ability of their host to transmit pathogens, as well as potential disease control strategies that harness symbiotic microbes to reduce pathogen transmission through an insect vector.**

## Symbiotic associations are ubiquitous in nature

Soon after birth all mammals begin a lifelong association with a complex and diverse microbial community that resides on the skin, vaginal mucosa, gastrointestinal (GI) tract and in the mouth. Advances in PCR-based technologies have greatly expanded our knowledge of these microbial systems, which largely represent mutually beneficial associations. The human gastrointestinal tract alone harbors over 500 distinct microbial taxa comprising an estimated $10^{14}$ microbes, significantly exceeding the number of cells in the human body [1]. Because the majority of these microbes cannot be cultivated *in vitro*, the functional bases of these interactions are difficult to dissect and have remained largely elusive. Nevertheless, findings from experimental systems using animals reared under aseptic conditions indicate that proper maintenance of the microbial consortium is essential for mammalian nutrient breakdown. Furthermore, the absence of microbial fauna, or modifications in the composition of the consortium, can reduce the fitness of a host and lead to autoimmune disease states such as inflammatory bowel disease, rheumatoid arthritis or Type I diabetes (reviewed in [2]). A symbiont product, polysaccharide A, produced by the prominent human symbiont *Bacteroides fragilis* protects animals from experimental colitis induced by *Helicobacter hepaticus*, a commensal bacterium with pathogenic potential [3,4].

By contrast to higher eukaryotes, which are colonized by a multitude of commensal organisms representing members of five of the six kingdoms of life, insects harbor a significantly less diverse community of microbial symbionts. The reduced complexity of the insect microbiome enables investigations that aim to understand the contribution(s) individual symbionts make towards host physiological processes. Furthermore, the insect systems are easier to maintain than are the vertebrate models because generation times are relatively short and husbandry practices less costly. In particular in the case of *Drosophila*, extensive mutant lines exist for further functional characterization of loci of importance. In addition to being excellent model systems, many insects that harbor bacterial symbionts are also human disease vectors or agricultural pests. Given that many symbioses are indispensable for host physiology, understanding these relations are also of applied interest because they can lead to the development of more efficient insect control strategies. To this end, it has been possible to cultivate several insect commensal symbionts *in vitro*, and genetically modify and reintroduce the recombinant symbionts back into their native host. These 'paratransgenic' transformation systems are a promising alternative to those that involve direct modification of the host germline. Expression of anti-pathogen products by genetically modified insect symbionts could prevent the transmission of harmful agents to their eukaryotic hosts and thus provide a novel means of disease control [5–8].

## Insect symbiotic systems

Symbiotic associations have been described in insects that rely on nutritionally restricted diets, such as vertebrate blood (i.e. tsetse flies and triatome bugs), plant phloem (i.e. aphids and psyliids) or wood (i.e. termites and carpenter ants). Presumably these microbes supplement their hosts with nutrients that are limited or absent in their diet or that they cannot produce on their own. Such mutualistic symbioses involve bacteria (typically referred to as the primary symbionts) that are vertically transmitted and that are indispensable for their hosts to survive in unique or resource-limited environments. Phylogenetic reconstruction studies have indicated that these host–microbe relationships are often ancient, and presumably neither partner can live in the absence of the other. During the long co-evolutionary history with their hosts, primary symbiont genomes have undergone drastic size reductions, losing genes and pathways that are no longer necessary in the

*Corresponding author:* Aksoy, S. (serap.aksoy@yale.edu).

 1471-4922/$ – see front matter  doi:10.1016/j.pt.2011.05.001 

unique metabolic niches the hosts provide [9]. Among the well characterized primary symbionts are *Buchnera* from aphids [10], *Carsonella* from sharpshooters [11], *Blochmannia* from ants [12], SOPE from rice weevils [13,14] and *Wigglesworthia* from tsetse (Box 1) [15,16].

Many insects also harbor commensal microbes (referred to as secondary symbionts). These symbiotic associations apparently originated more recently and can be transient in nature. In some cases they are found to be dispensable for their hosts because not every individual in a population carries these microbes. Whereas primary symbionts are vertically transmitted from mother to progeny with high fidelity, secondary symbionts can be acquired through multiple means (i.e. vertically, horizontally or from the environment). Examples of secondary symbionts include *Hamiltonella defensa* in sap-feeding insects including aphids, psyllids and whiteflies [17] and *Sodalis glossinidius* in tsetse (Box 1) [18,19].

In addition to microbes with commensal and mutualistic associations, many insects also harbor maternally heritable parasitic microbes. The most extensively studied of these belong to the genus *Wolbachia* [20,21]. Several reproductive abnormalities are associated with *Wolbachia* infections, the most prominent being cytoplasmic incompatibility (CI). CI, which results in early embryo death, occurs when a *Wolbachia*-uninfected female mates with an infected male. Because infected females can successfully mate with males infected with the same strain of *Wolbachia* or with uninfected males, the *Wolbachia* infected female has a reproductive advantage, and the associated female genotypes can spread quickly through a population. CI has been proposed as a means of spreading desirable traits into populations, including genotypes or other maternally transmitted symbionts conferring disease resistance [22,23]. In addition to CI, certain *Wolbachia* strains have been found to reduce host lifespan. These strains are also of interest from an applied perspective because older adults typically contribute more to disease transmission, whereas younger individuals contribute to reproductive output [24]. Thus, skewing the age of the population towards younger individuals without reducing the reproductive output can provide an evolutionarily sustainable means of disease control. Finally, it has recently been observed that infections with certain strains of *Wolbachia* confer pathogen resistance traits to their hosts, presumably through host immune induction (as described below and reviewed in [25]).

Whereas insects with single diets of limited nutritional value have established associations with heritable primary and secondary symbionts, insects with multiple diets can harbor a wide range of environmentally acquired microbes. Both laboratory lines and field populations of *Anopheline* mosquitoes host a robust gut-associated microbiota that principally consists of Gram-negative (Gram$^-$) members of the family Enterobacteriaceae. For example, 16 bacterial species from 14 genera were identified from field populations of the predominant African malaria vectors *Anopheles gambiae* and *Anopheles funestus* [26]. Furthermore, laboratory populations of *A. gambiae* and *Anopheles stephensi* (the primary vector of human malaria in Asia) also

**Box 1. Tsetse and its associated microbiome**

Three endosymbionts with distinct phylogenetic and genomic characteristics have been described from tsetse from laboratory colonies (Figure 1). These symbioses reflect varying levels of host integration, and as such have a different impact on tsetse physiology [71,72]. Although the microbiome is highly restricted in laboratory maintained flies, analysis of tsetse in natural field populations indicates the presence of additional microbes, which are presumably environmentally acquired. The acquisition routes, persistence and the role(s) of these microbes on host physiology remain unknown [73–75]. Below are biological highlights of heritable the endosymbionts of tsetse.

***Wigglesworthia glossinidia***

- Obligate mutualist from the family Enterobacteriaceae [16,76].
- Phylogenetic analyses indicate that tsetse and *Wigglesworthia* have evolved concordantly for approximately 50–80 million years [77].
- Highly streamlined genome (700 Kb in size) exhibits exceptional A-T bias (82%) and contains no transposons or phage-related elements [15,78].
- Chromosome encodes several vitamin biosynthesis pathways that probably supplement the tsetse vertebrate blood-specific diet [15]. Female tsetse that lack *Wigglesworthia* are sterile [44].
- Resides intracellularly in tsetse bacteriome in the midgut and extracellularly in the female milk gland lumen [59]. The milk gland population is essential for transmission to progeny, whereas the bacteriome population is essential for fecundity [44].
- Must be present during larval maturation in order for the tsetse immune system to develop and function properly during adulthood (see text).

***Sodalis glossinidius***

- Facultative commensal from the family Enterobacteriaceae [19].
- Recent association with tsetse as *Sodalis* from different species are closely related [79]. Several other insects, including stinkbugs [80], hippoboscid flies [81], grain weevils [13,82] and slender pigeon lice [83] harbor endosymbionts that are closely related to *Sodalis*.
- Detected in all colony flies, but not present in all field populations of tsetse. Found both inter- and intracellularly in the midgut, muscle, fat body, milk gland and salivary glands of tsetse [84].
- Genome size (4.2 Mb) and chromosomal synteny indicates a close relationship to free-living enteric bacteria, including *Salmonella* and *Yersinia* [18,85].
- The genome of *Sodalis* has a low protein coding capacity (49%) and a high number of pseudogenes (972). Both of these characteristics are indicative of a transition from a free-living to endosymbiotic life style [85,86].
- *Sodalis* encodes three symbiosis islands (SSI) that share significant synteny and sequence homology with Type III secretion systems (TTSS) identified from pathogenic bacteria [85]. These SSIs presumably facilitate the *Sodalis* intracellular lifestyle [87–89].
- *Sodalis* can impact tsetse vector competence and longevity [90,91].
- Modifications associated with *Sodalis* outer membrane protein A could facilitate host tolerance to this symbiont [92].
- Field studies indicate a positive correlation between specific *Sodalis* genotypes and trypanosome infections in tsetse. However, flies that lack *Sodalis* are still competent for trypanosome infections [93–95].

***Wolbachia***

- Rickettsia-like bacterium found in many insect taxa, including tsetse [96].
- Infections with *Wolbachia* are restricted to tsetse reproductive tissues.
- Certain tsetse species harbor *Wolbachia* infections, and the field infection prevalence and the *Wolbachia* strains infecting different species in the different populations investigated varies [97].

harbor a wide variety of bacteria, the most abundant of which come from the genera *Asaia*, *Enterobacter*, *Mycobacterium*, *Sphingomonas*, *Serratia* and *Chryseobacterium* [27–29]. The dominant symbiont *Asaia* (an α-proteobacterium belonging to the family Acetobacteriaceae) has been observed in all of the developmental stages of wild and laboratory reared colonies of *Anopheline* as well as *Aedes* mosquitoes [30]. A study on the genetic variation present in the *Asaia* symbiont indicates the presence of various strains in four different mosquito species analyzed [31]. This symbiont has been detected in the midgut, salivary glands and reproductive organs of adult mosquitoes [30]. Fluorescent *in situ* hybridization (FISH) analysis on the reproductive tract of female *A. gambiae* showed a concentration of *Asaia* at the very periphery of the eggs, suggesting that transmission of *Asaia* from mother to offspring is probably mediated by a mechanism of egg smearing [30].

## Insect pathogen associations

In addition to carrying beneficial microbes that enhance their overall physiological functions, some insects also carry and transmit microbes that are pathogenic totheir mammalian or plant hosts (Table 1) [32]. Most of these insects (disease vectors of humans) utilize blood as a food source and typically acquire pathogens while feeding on infected hosts and then pass the disease agents on to other hosts during the course of subsequent meals. Once acquired by the insect host, viruses need to replicate, and protozoan pathogens and worms interact with different insect tissues and undergo several differentiation events before they can be transmitted to a subsequent host. This process of pathogen development in an insect can be significantly long and is known as the extrinsic incubation period. Insects that transmit disease to humans include tsetse flies (Diptera: Glossinidae), triatome bugs (Hemiptera: Triatominae) and sand flies (Diptera: Psychodidae), which transmit kinetoplastid parasites that cause human African trypanosomiasis (HAT), Chagas disease and leishmaniasis, respectively. In addition, lice (order Phthiraptera) can vector epidemic typhus and trench fever, and fleas (order Siphonaptera) transmit bacterial pathogens including the causative agent of bubonic plague. Finally, mosquitoes are prolific disease vectors that inhabit a broad geographic range and host a diverse array of pathogens including dengue, West Nile and Chickungunya viruses, filarial worms and *Plasmodium* parasites.

Interestingly, many vector insects exhibit an innate resistance to the pathogens they transmit. Both laboratory and field studies indicate that only a small proportion of insects that acquire pathogens actually allow for these organisms to establish successful infections for subsequent transmission to the next host. In fact, the majority of insects are capable of eliminating pathogens in their midgut shortly after acquisition in the bloodmeal. The genetic ability of an insect to transmit pathogens is measured in terms of its vector competence. An important component of vector competence is the proficiency of the immune responses of the host insect.

## Insect vector competence and a role for the microbiome

Multiple immunity pathways are implicated in vector competence, which results in insect host resistance to pathogen infection (for mosquito responses to malarial parasites, see [33]). An initial study that investigated gene expression responses of *A. gambiae* to microbial and malaria challenges by using cDNA microarrays constructed from an EST-clone collection noted that the response to malaria parasites partially overlapped with the response to Gram-positive ($^+$) and Gram$^-$ bacteria [34].Using a similar array approach, the mosquito response to the filarial worm has also been found to involve the induction of a large number of genes functioning in the innate immune pathways shown to respond to microbial challenge, although their role in parasite transmission remains to be confirmed [35].

In the case of tsetse flies, trypanosome infections induced innate immune responses that are typically involved in clearance of Gram$^-$ bacteria (i.e. the immune deficient (Imd) pathway) [36]. Trypanosome infection prevalence increased in flies when the expression of the Imd pathway regulator *relish* or the downstream expressed antimicrobial peptide (AMP) effector (*attacin*) were downregulated by an RNA interference (RNAi)-based reverse genetic analysis before subjecting flies to parasite infections [36,37]. In addition, the AMPs diptericin and attacin displayed trypanocidal activity both *in vitro* and *in vivo* in the midgut of the tsetse [38].

To understand the role of *Aedes aegypti* mosquito immune responses to dengue virus pathogen transmission, Xi *et al.* used high-throughput analysis of gene expression and an RNAi approach, reporting that another innate immune pathway (toll pathway) regulates viral resistance in mosquitoes [39]. Interestingly, the same study showed that regulation of genes in this immune pathway was also stimulated by natural gut microbiota. Furthermore, when mosquitoes were reared aseptically (in the absence of their

**Table 1. Insect model systems that harbor immunomodulatory bacterial symbionts**

| Insect host | Symbiotic bacteria (genera) | Pathogen/parasite | Refs. |
|---|---|---|---|
| Tsetse (*Glossina* spp.) | *Wigglesworthia, Sodalis, Wolbachia* | *Trypanosoma brucei* spp. | [15,16,18,19,44,51, 58,59,76,77] |
| 'Kissing' bug (*Triatomine* spp.) | *Rhodococcus, Serratia, Nocardia, Gordonia* | *Trypanosoma cruzi* | [100–103] |
| Mosquito (*Aedes, Anopheles, Culex* spp.) | *Asaia, Wolbachia, Serratia* | Protozoan parasites, filarial worms, viruses | [27–31] |
| Pea aphid (*Acyrthosiphon pisum*) | *Buchnera, Serratia, Hamiltonella* | N/A[a] (agricultural pest) | [10,17,48,49] |
| Grain weevil (*Sitophilus oryzae*) | SOPE (*Sitophilus oryzae* primary endosymbiont), *Wolbachia* | N/A (agricultural pest) | [13,14,53,54] |
| Carpenter ant (*Camponotus ligniperdus*) | *Blochmannia, Wolbachia* | N/A (agricultural pest) | [12] |

[a]N/A, not applicable.

endogenous bacterial flora), dengue virus was present in midguts at two-fold higher titers compared to wild type mosquitoes, implicating once again the microbial fauna in influencing levels of immune resistance.

Early studies on mosquitoes had noted a positive correlation between midgut microbiota and inhibition of *Plasmodium* sporozoite development. This phenomenon was demonstrated independently in two different mosquito vectors of *Plasmodium*. In one set of experiments *A. stephensi* adults were offered a *P. falciparum* gametocyte-enriched blood meal that also contained one of four distinct non-native Gram$^-$ bacteria or two distinct Gram$^+$ species. All of the Gram$^-$ bacteria tested were found to partially or completely inhibit oocyst development within the mosquito host. Conversely, the presence of neither Gram$^+$ bacteria resulted in an inhibitory phenotype [40]. A similar experiment was performed using aseptic *A. albimanus* adults and several bacteria that are found naturally in both wild type laboratory and field-captured populations. In this situation, the number of mosquitoes infected with oocysts, and oocyst density, was significantly lower in mosquitoes that received each of the bacteria separately as compared to controls that received no bacteria [41,42]. Although no physiological mechanism was proposed at the time, this study indicated that gut microbes have the potential to reduce the capacity of mosquitoes to vector *Plasmodium*. Recently, aseptic and septic adult *A. gambiae* were fed with *Plasmodium* gametocytes and subsequently monitored parasite infection status in each group [27]. Aseptic mosquitoes displayed an increased susceptibility to *Plasmodium* infection. This study also found that co-feeding mosquitoes bacteria and *P. falciparum* gametocytes resulted in lower than normal infection levels. Whereas ookinete numbers were the same in the midgut lumen of both mosquito lines, significantly more oocysts were found in the antibiotic treated aseptic mosquitoes. These results indicated that bacteria affect parasite viability before the oocyst formation. This can occur while the ookinete is in the midgut or while invading midgut epithelial cells. Global transcription profiling of septic and aseptic mosquitoes identified a significant subset of immune genes in the septic mosquitoes that were presumably upregulated by the microbial flora of the host. These immunity genes included several anti-*Plasmodium* factors [27,43]. Both expression and infection analyses suggest that the observed anti-*Plasmodium* effect is caused by the antimicrobial immune response of the mosquito, possibly through activation of basal immunity [33].

The commensal and obligate microbes of tsetse (*Sodalis* and *Wigglesworthia*, respectively) have also been implicated in trypanosome transmission in tsetse. There has been a correlation observed with the presence of the commensal symbiont *Sodalis* and in particular specific *Sodalis* genotypes and the presence of trypanosome infections in several natural tsetse populations. Also, in the absence of the obligate symbiont *Wigglesworthia* in the laboratory, flies have been found to be highly susceptible to parasite infections, as discussed below [44].

Microbiota can influence the vectorial competence of their hosts by means of direct interaction with parasites. This can occur through inhibitory bioactivity of secreted enzymes or toxins. Alternatively, microbiota can constrain pathogen development indirectly by inducing activity of the host immune system that in turn can clear the pathogenic microbes. Recent studies in several insect systems indicate that both direct and indirect microbiota-induced phenotypes can affect the capacity of an insect host to transmit pathogens (discussed in Box 2). In fact, both heritable symbionts and environmentally acquired commensal microfauna have been shown to influence this function (reviewed in [45,46]). Below we discuss examples of symbiont produced anti-pathogen products, symbiont induced host anti-pathogen products and host immune priming by resident gut fauna as well as by *Wolbachia*.

## Endosymbiont produced anti-pathogen products

Aphids harbor a beneficial facultative endosymbiont, Candidatus *Hamiltonella defensa* that can increase host survival following attack by parasitoid wasps [17]. Interestingly, different strains of *H. defensa* vary in the degree of protection they confer upon their aphid host. This symbiont-mediated protective phenotype depends on the presence of a lysogenic bacteriophage (APSE) present in certain symbiont host strains [47]. Aphids that host *H. defensa* infected with APSE are significantly more resistant to parasitoid wasps than are their counterparts that host

### Box 2. Manipulating the microbiome to modulate insect host vector competence

Endosymbionts are being investigated for their ability to decrease host vector competence. One promising symbiont-based strategy currently under development is called 'paratransgenesis.' This procedure involves isolating and genetically modifying symbiotic bacteria to express an anti-pathogen molecule. The recombinant symbionts are then reintroduced into their host, where they subsequently increase host resistance to pathogens. Three disease vector systems where this approach has been applied are described below.

- Tsetse: the tsetse commensal symbiont *Sodalis* has been genetically modified *in vitro* to express a marker gene, and then returned to fertile females where they are subsequently passed on to future generations (reviewed in [5]). *Sodalis* exhibits a natural resistance to several trypanocidal molecules, including tsetse antimicrobial peptides. Expression of these molecules by *Sodalis* can result in parasite resistance in themidgut of tsetse [38,98,99].
- Triatome bugs: triatomines that transmit the causative agent of Chagas disease (*Trypanosoma cruzi*) harbor genetically modifiable, gut-associated symbionts that are horizontally transmitted via copharagy [100]. These symbionts can be cultured and genetically modified [100–102]. Host recolonization with genetically modified symbionts that express anti-parasitic molecules subsequently blocks parasite transmission [103].
- Mosquito: anopheline mosquitoes form stable associations with bacteria from the genus *Asaia* that can be used for paratransgenesis [30]. Stable infections can be established in multiple epidemiologically relevant tissues when female mosquitoes were reconstituted with recombinant bacteria [28,104].

Research is currently under way to improve the efficiency of paratransgenesis by optimizing several variables. These include:(i) screening for other commensal symbionts that are stably associated with insect disease vectors, (ii) identifying novel anti-pathogen effector molecules that can be expressed in symbionts, (iii) engineering expression constructs that encode efficacious promoters and secretion signals, and (iv) establishing a mechanism to drive paratransgenic bacteria into field-based insect populations.

APSE-uninfected symbionts [48]. In laboratory studies, phage loss occurs repeatedly in *H. defensa*-infected aphid clonal lines. In each instance this phenomenon results in increased host susceptibility to parasitism [47]. APSE-mediated protection results when eukaryote-targeted toxins are expressed from the bacteriophage genome [48,49]. Thus, mobile genetic elements can code for ecologically important traits including defense against parasitoids. This characteristic can endow endosymbionts with direct benefits that extend to their animal hosts.

### Symbiont induced host anti-pathogen products

How the beneficial microbiome can evade the immune responses of their host is actively being investigated, a phenomenon referred to as host tolerance. One suggested mechanism leading to host tolerance involves symbiont suppression of host immune responses as a form of self-protection. An alternative possible mechanism is the absence of molecular signals on symbiont cell surfaces that are typically recognized by insect hosts as foreign following exposure to microbes. One molecular signal that mediates host recognition of bacteria is the peptidoglycan (PGN) structure present in bacterial membranes. A family of host PGN recognition proteins (PGRPs) have been identified with PGN binding activity and include pattern recognition receptors (PRRs), which serve as the initial component of signal transduction pathways that result in immune activation following exposure [7]. For example, in insects, the Imd pathway has been shown to respond to the presence of $Gram^-$ bacterial PGN and results in the production of a battery of antimicrobial peptides. Given that the symbiotic microbes also have PGN as part of their cell structures, it has been of interest if and how these microbes can evade host recognition and/or subsequent immune damage.

In *Drosophila*, one of the PGRPs, PGRP-LB, has been shown to exhibit amidase catalytic activity to degrade PGN [50]. In this way, metabolically costly insect immune pathways are not activated in response to environmental microbes present at low concentrations. In the presence of a persistent infection, however, the immune responses are activated when the level of the endogenous PGRP-LB is exhausted. Interestingly in tsetse, PGRP-LB is expressed at high levels in the *Wigglesworthia* symbiont-containing midgut bacteriome organ [51]. Furthermore, the level of *pgrp-lb* expression increases as a function of host age and *Wigglesworthia* density [52]. This positive correlation between *Wigglesworthia* density and host *pgrp-lb* expression levels was also demonstrated in natural field populations [51]. In the weevil, *Sitophilus zeamais*, which also has mutualistic symbionts, *pgrp-lb* expression was also detected in the bacteriome organ and found to be upregulated in the nymphal phase during a time when the symbionts are released from host cells [53,54]. Thus, symbiont density regulation through the action of catalytic PGRPs could be a general mechanism that insects use to downregulate their immune responses, which could otherwise be damaging to their symbiotic fauna. In fact, when *pgrp-lb* levels were reduced through the use of RNAi, the density of *Wigglesworthia* was significantly reduced. This decrease in *Wigglesworthia* numbers probably resulted from the activation of the Imd pathway, and induced synthesis of AMPs in tsetse as symbiont numbers were restored when both PGRP-LB and Imd pathway functions were downregulated [51]. Thus, host PGRP-LB expression appears to be essential to protect *Wigglesworthia* from damage by the responses of its host.

In addition to protecting the indispensable symbiosis with *Wigglesworthia*, PGRP-LB also plays a role in parasite transmission in tsetse, because flies with diminished *pgrp-lb* levels were found to exhibit increased susceptibility to trypanosomes [51]. It remains to be seen, however, if tsetse PGRP-LB could have an anti-protozoal function. One PGRP in *Drosophila* and all PGRPs in humans have direct antibacterial properties [55]. Thus PGRP-LB could have a dual function in tsetse. First, PGRP-LB expressed in response to *Wigglesworthia* might protect the mutualistic symbiosis of tsetse flies by negatively modulating the activity of the Imd pathway, which when induced can be harmful to this bacterium. Second, high PGRP-LB levels benefit tsetse by preventing the establishment of trypanosome infections, which in turn negatively impact host reproductive fitness and decrease fecundity by increasing the larval development period [56].

#### *Immune priming by resident gut fauna*

Recent studies indicate that *A. gambiae* immune response to malaria infection involves an intimate association between the presence of microbiota in the gut and hemocytes in the hemolymph [57]. Hemocytes, which are functionally homologous to mammalian macrophages, are an essential component of insect cellular immunity. Recent research demonstrated that when mosquitoes are pre-exposed to infection with *Plasmodium* ookinetes they subsequently exhibit enhanced immunity to a second parasite challenge [57]. During establishment of the initial infection, malaria ookinetes irreversibly compromise physical barriers that otherwise prevent bacteria normally found only in the gut lumen from directly interacting with host epithelial cells. When this atypical encounter occurs, hemocytes that are attached to the basal surface of host epithelial cells, as well as those circulating freely in the hemolymph, differentiate into a subtype (granulocytes) that enhances host immunity to the second challenge with malaria parasites. This 'priming' of the host immune system was only effective when endogenous midgut bacteria were present in the system and did not occur in aseptic mosquitoes.

Tsetse flies rely on their symbiotic microbiota for proper immune function development [58]. During the unique viviparous mode of reproduction of their host, the symbionts are vertically transmitted from mother to developing intrauterine larvae via milk gland secretions (Figure 1). In adult tsetse, two distinct populations of obligate *Wigglesworthia* are present. The first population is intracellular within the tsetse bacteriome organ, whereas the second population is extracellular in the milk gland organ [44,59]. Treatment of pregnant females with the antibiotic ampicillin results in the clearance of the second population, extracellular in the milk, whereas the intracellular bacteriome-associated *Wigglesworthia* are left undisturbed. Thus, immature progeny of ampicillin-treated females ($Gmm^{Wgm^-}$) lack *Wigglesworthia* throughout intrauterine development and later as adults [44]. Adult

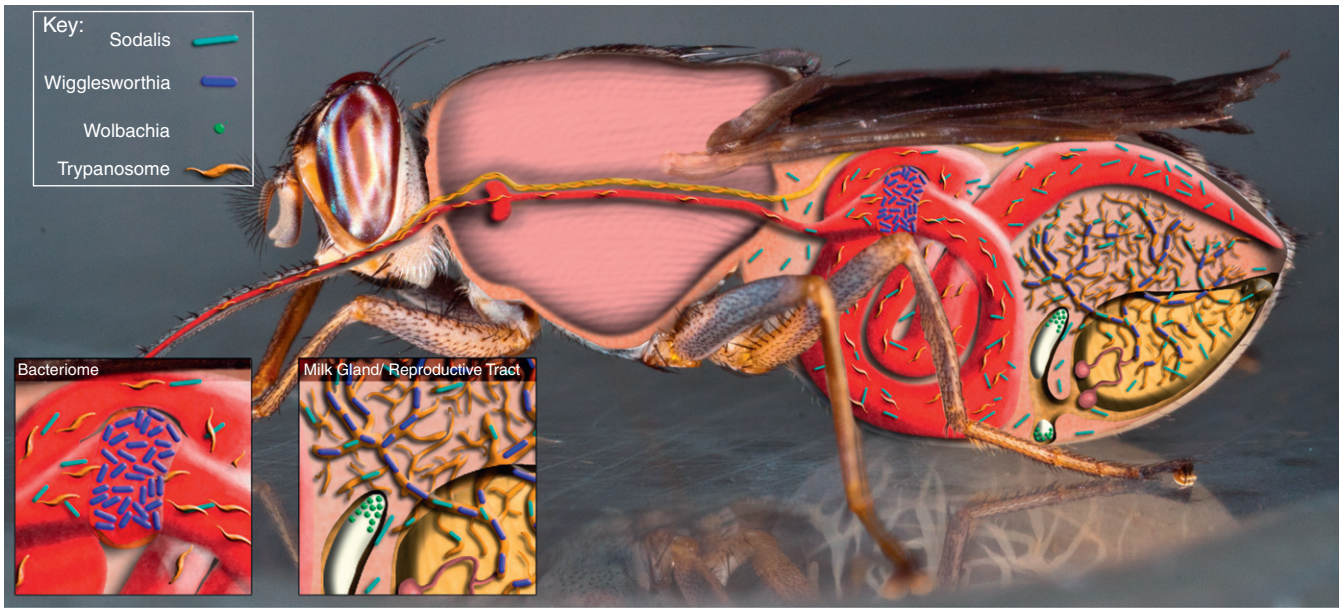


**Figure 1**. Tsetse female with its symbionts *Sodalis, Wigglesworthia* and *Wolbachia* and the parasite African trypanosome.The cartoon shows the major organs where the symbionts are located. During its transmission in the fly, the trypanosome resides in the midgut and then in the salivary glands of the fly. In the midgut the trypanosome is in close proximity to *Wigglesworthia* housed in the bacteriome organ and *Sodalis* found in the midgut. Thus anti-pathogenic products expressed by *Sodalis* or induced by *Wigglesworthia* can have an adverse effect on trypanosome transmission (shown by inset labeled bacteriome). The symbionts *Sodalis* and *Wigglesworthia* are maternally transmitted to the intrauterine progeny in the milk, whereas *Wolbachia* is transovarially transmitted (shown by inset labeled milk gland/reproductive tract). Contributed by Geoffrey Attardo.

*Gmm*$^{Wgm-}$ exhibit a highly immunocompromised phenotype compared to their wildtype counterparts. In fact, the challenge of *Gmm*$^{Wgm-}$individuals with *Escherichia coli* results in bacterial sepsis and death, whereas wild type flies eliminate the same infection and survive. Furthermore, when challenged with trypanosomes, gut infections are established in the majority of *Gmm*$^{Wgm-}$ flies, whereas wild type flies are highly resistant and can clear parasite infections effectively [44,60]. Interestingly, *Wigglesworthia* does not directly enhance immunity in wild type individuals, because elimination of *Wigglesworthia* from adult flies via antibiotic tetracycline treatment does not result in an immune compromised phenotype. Instead, this obligate must be present during the larval immature stages in order for the tsetse immune system to develop and function properly during adulthood [58]. Gene expression studies indicate that adult *Gmm*$^{Wgm-}$ exhibit highly depleted humoral and cellular immune responses.This condition is characterized by reduced expression of genes that encode AMPs (cecropin and attacin), hemocyte-mediated processes (thioester-containing proteins 2 and 4 and prophenoloxidase) and signal-mediating molecules (inducible nitric oxide synthase) following challenge with bacteria. Furthermore, *Gmm*$^{Wgm-}$ adults house a reduced population of sessile and circulating hemocytes. This phenotype might result from a significant decrease in larval expression of *serpent* and *lozenge*, both of which are transcription factors regulating the process of early hemocyte differentiation[58]. The specific physiological mechanism(s) underlying *Wigglesworthia*-induced maturation of tsetse immune system development, and the implications this has on host vector competence, remains to be determined.

### *Immune priming by Wolbachia*

Until recently, it was thought that *Wolbachia* infections of insects were largely parasitic and had invaded host populations by manipulating the reproduction of their hosts to increase their transmission through the female germline. However, new studies are suggesting that *Wolbachia* infections in *Drosophila* can confer resistance to viruses and therefore act as mutualists [25]. Initial work with *Drosophila* showed that certain *Wolbachia* infections reduced virus proliferation of *Drosophila* C, cricket paralysis, Nora and Flock House viruses and delayed mortality in flies [61–63]. Subsequently, a similar pathogen blocking phenomenon was shown to affect *A. aegypti* mosquitoes. Transinfection of *A. aegypti* with the life-shortening *Wolbachia* strain *wMelPop* resulted in reduced transmission of medically important pathogens, including dengue and Chikungunya viruses, *Plasmodium gallinaceum* (cause of avian malaria) and *Brugia pahangi* (rodent filarial nematodes) [61,62,64–66]. Replication of dengue virus following either ingestion or intrathoracic injection was almost completely inhibited in *Wolbachia* infected mosquitoes. Similarly,*Wolbachia* infections reduced the number of mosquitoes infected with *P. gallinaceum* by about 50% and lowered oocyte numbers significantly [66]. *Wolbachia* infections in *A. aegypti* also significantly reduced the prevalence and mean number of infective third stage filarial nematodes by more than two-fold compared to uninfected controls [64]. This inhibition of nematode development has been hypothesized to result at least partially from a significant *Wolbachia*-induced upregulation of immune genes that encode antimicrobial peptides (cecropin and defensin), thioester containing proteins (TEP) and C-type lectins, as shown by microarray analysis

[66]. *Wolbachia* infections naturally associated with *Culex quinquefasciatus* have also been found to influence West Nile virus transmission. *Wolbachia*-infected *Culex* mosquitoes were found to produce lower virus titers and had two to three-fold lower rates of virus transmission compared to mosquitoes lacking *Wolbachia* [67]. Stable infection of an *A. gambiae* cell line with *wMelPop* caused increased expression of malaria-related immune genes, indicating that host resistance to parasite infection can be regulated by symbiont-induced immunity. Although it has not been possible to establish stable infection of *A. gambiae* mosquitoes with *wMelPop*, transient somatic infections can be established by intrathoracic inoculation. This procedure induces the expression of several host immune-related genes, including LRIM1 and TEP1 that have been shown to influence the development of malaria parasites. *Plasmodium* infection intensity in mosquitoes that received this treatment was thereupon significantly reduced in comparison to uninfected cohorts [68]. By contrast, in a different study where gene expression of *Wolbachia* infected mosquito cells was analyzed by Affymetrix GeneChip microarray, a significant downregulation of many immune, stress and detoxification-related transcripts were noted [69]. It will remain to be seen if successful *Wolbachia* infections can be established in *Anopheline* mosquitoes where they confer a pathogen resistance phenotype. Thus, in several different insect systems in the laboratory and in natural populations, infections with specific *Wolbachia* strains apparently can confer resistance to the pathogens typically transmitted by the same hosts. This pathogen blocking process appears to involve induced expression of host immune responses against the pathogens. It is important to understand the fitness cost of such *Wolbachia* infections on their hosts, in particular on fecundity for field applications. Ability to harness *Wolbachia* conferred pathogen blocking coupled with CI outcomes provides a novel approach for disease control where *Wolbachia* infected, pathogen resistant insects can be spread via CI and replace the susceptible populations [70].

**Box 3. Future directions**

This review summarizes our current knowledge on the relationship between insect symbionts and host disease vector competence. Below we highlight future research avenues that will contribute to the development of microbiome-based strategies for the control of insect-transmitted diseases.

(i) Characterize the microbiome of natural vector populations, with special emphasis placed on evaluating differences between infected and pathogen resistant insects. This research could lead to the identification of symbiotic microbes that naturally confer resistance to pathogens.

(ii) Identify *Wolbachia* endosymbiont strains that are capable of modifying host immune competence and/or fitness as a novel means of vector control.

(iii) Ecological studies must be performed to better understand host–symbiont infection dynamics as a function of space and time and varying environmental conditions. This information is essential for the success of downstream field applications.

(iv) Large-cage studies, followed by open field releases, are necessary to access the ecological stability of modified insects in natural habitats. Although technical aspects associated with these methodologies are advancing, these programs now need to address societal challenges such as regulatory approval and ethical acceptance. One such study is ongoing in Australia for control of the dengue vectors (http://www.eliminatedengue.org/en/HOME.aspx).

(v) Despite challenges, these self-sustainable and cost-effective programs can provide unprecedented opportunities for control of tropical disease, for which limited funds are available.

## Concluding remarks

In this paper, we reviewed major trends on symbiotic microbes that are associated with different insects, including those that are disease vectors. Evidence to date indicates that these beneficial microorganisms are indispensable for nutrient provisioning and for proper host immune system maturation during development. We also discussed vector control strategies (currently under development) that use insect symbionts to 'artificially' boost the immune system of their host. The goal of these methods is to reduce the dissemination of pathogenic microbes. In Box 3 several future research directions are suggested that will enhance the ability to exploit insect symbiotic bacteria for the purpose of reducing disease transmission.

### Acknowledgements

We are grateful to our laboratory members for their contributions to the work presented on the tsetse system. We thank Geoffrey Attardo for the artwork provided in this manuscript.

### References

1 Eckburg, P.B. *et al.* (2005) Diversity of the human intestinal microbial flora. *Science* 308, 1635
2 Round, J.L. *et al.* (2010) Coordination of tolerogenic immune responses by the commensal microbiota. *J. Autoimmun.* 34, J220
3 Mazmanian, S.K. (2008) Capsular polysaccharides of symbiotic bacteria modulate immune responses during experimental colitis. *J. Pediatr. Gastroenterol. Nutr.* 46 (Suppl. 1), E11
4 Mazmanian, S.K. *et al.* (2008) A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* 453, 620
5 Rio, R.V. *et al.* (2004) Strategies of the home-team: symbioses exploited for vector-borne disease control. *Trends Microbiol.* 12, 325
6 Beard, C.B. *et al.* (2001) Bacterial symbiosis and paratransgenic control of vector-borne Chagas disease. *Int. J. Parasitol.* 31, 621
7 Gross, R. *et al.* (2009) Immunity and symbiosis. *Mol. Microbiol.* 73, 751
8 Bextine, B. *et al.* (2004) Delivery of a genetically marked *Alcaligenes* sp. to the glassy-winged sharpshooter for use in a paratransgenic control strategy. *Curr. Microbiol.* 48, 327
9 Wernegreen, J.J. (2002) Genome evolution in bacterial endosymbionts of insects. *Nat. Rev. Genet.* 3, 850
10 Shigenobu, S. *et al.* (2000) Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera* sp. APS. *Nature* 407, 81
11 Nakabachi, A. *et al.* (2006) The 160-kilobase genome of the bacterial endosymbiont *Carsonella*. *Science* 314, 267
12 Degnanm, P.H. *et al.* (2005) Genome sequence of *Blochmannia pennsylvanicus* indicates parallel evolutionary trends among bacterial mutualists of insects. *Genome Res.* 15, 1023
13 Heddi, A. *et al.* (1999) Four intracellular genomes direct weevil biology: nuclear, mitochondrial, principal endosymbiont, and *Wolbachia*. *Proc. Natl. Acad. Sci. U.S.A.* 96, 6814
14 Gil, R. *et al.* (2008) Massive presence of insertion sequences in the genome of SOPE, the primary endosymbiont of the rice weevil *Sitophilus oryzae*. *Int. Microbiol.* 11, 41
15 Akman, L. *et al.* (2002) Genome sequence of the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia*. *Nat. Genet.* 32, 402
16 Aksoy, S. (1995) *Wigglesworthia* gen. nov. and *Wigglesworthia glossinidia* sp. nov., taxa consisting of the mycetocyte-associated, primary endosymbionts of tsetse flies. *Int. J. Syst. Bacteriol.* 45, 848
17 Degnan, P.H. *et al.* (2009) *Hamiltonella defensa*, genome evolution of protective bacterial endosymbiont from pathogenic ancestors. *Proc. Natl. Acad. Sci. U.S.A.* 106, 9063

18 Akman, L. *et al.* (2001) Genome size determination and coding capacity of *Sodalis glossinidius*, an enteric symbiont of tsetse flies, as revealed by hybridization to *Escherichia coli* gene arrays. *J. Bacteriol.* 183, 4517
19 Dale, C. and Maudlin, I. (1999) *Sodalis* gen. nov. and *Sodalis glossinidius* sp. nov., a microaerophilic secondary endosymbiont of the tsetse fly *Glossina morsitans morsitans*. *Int. J. Syst. Bacteriol.* 49 (Pt 1), 267
20 Werren, J.H. (1997) Biology of *Wolbachia*. *Annu. Rev. Entomol.* 42, 587
21 Werren, J.H. *et al.* (1995) Evolution and phylogeny of *Wolbachia* - reproductive arasites of arthropods. *Proc. R. Soc. Lond. B. Biol. Sci.* 261, 55
22 Sinkins, S. and O'Neill, S. (2000) Wolbachia as a vehicle to modify insect populations. In *Insect Transgenesis* (Handler, A. and James, A., eds), pp. 271–287, CRC Press
23 Sinkins, S.P. and Gould, F. (2006) Gene drive systems for insect disease vectors. *Nat. Rev. Genet.* 7, 427
24 Cook, P.E. *et al.* (2008) Modifying insect population age structure to control vector-borne disease. *Adv. Exp. Med. Biol.* 627, 126
25 Cook, P.E. and McGraw, E.A. (2010) *Wolbachia pipientis*: an expanding bag of tricks to explore for disease control. *Trends Parasitol.* 26, 373
26 Lindh, J.M. *et al.* (2005) 16S rRNA gene-based identification of midgut bacteria from field-caught *Anopheles gambiae* sensu lato and *A. funestus* mosquitoes reveals new species related to known insect symbionts. *Appl. Environ. Microbiol.* 71, 7217
27 Dong, Y. *et al.* (2009) Implication of the mosquito midgut microbiota in the defense against malaria parasites. *PLoS Pathog.* 5, e1000423
28 Favia, G. *et al.* (2007) Bacteria of the genus *Asaia* stably associate with *Anopheles stephensi*, an Asian malarial mosquito vector. *Proc. Natl. Acad. Sci. U.S.A.* 104, 9047
29 Zouache, K. *et al.* (2011) Bacterial diversity of field-caught mosquitoes, *Aedes albopictus* and *Aedes aegypti*, from different geographic regions of Madagascar. *FEMS Microbiol. Ecol.* 75, 377–389
30 Damiani, C. *et al.* (2010) Mosquito-bacteria symbiosis: the case of *Anopheles gambiae* and Asaia. *Microb. Ecol.* 60, 644
31 Chouaia, B. *et al.* (2010) Molecular evidence for multiple infections as revealed by typing of *Asaia* bacterial symbionts of four mosquito species. *Appl. Environ. Microbiol.* 76, 7444
32 Hill, C.A. *et al.* (2005) Arthropod-borne diseases: vector control in the genomics era. *Nat. Rev. Microbiol.* 3, 262
33 Cirimotich, C.M. *et al.* (2010) Mosquito immune defenses against *Plasmodium* infection. *Dev. Comp. Immunol.* 34, 387
34 Dimopoulos, G. *et al.* (2002) Genome expression analysis of *Anopheles gambiae*: responses to injury, bacterial challenge, and malaria infection. *Proc. Natl. Acad. Sci. U.S.A.* 99, 8814
35 Erickson, S.M. *et al.* (2009) Mosquito infection responses to developing filarial worms. *PLoS Neglected Trop. Dis.* 3, e529
36 Hao, Z. *et al.* (2001) Tsetse immune responses and trypanosome transmission: implications for the development of tsetse-based strategies to reduce trypanosomiasis. *Proc. Natl. Acad. Sci. U.S.A.* 98, 12648
37 Hu, C. and Aksoy, S. (2006) Innate immune responses regulate trypanosome parasite infection of the tsetse fly *Glossina morsitans morsitans*. *Mol. Microbiol.* 60, 1194
38 Hu, Y. and Aksoy, S. (2005) An antimicrobial peptide with trypanocidal activity characterized from *Glossina morsitans morsitans*. *Insect Biochem. Mol. Biol.* 35, 105
39 Xi, Z. *et al.* (2008) The *Aedes aegypti* toll pathway controls dengue virus infection. *PLoS Pathog.* 4, e1000098
40 Pumpuni, C.B. *et al.* (1993) *Plasmodium falciparum*: inhibition of sporogonic development in *Anopheles stephensi* by gram-negative bacteria. *Exp. Parasitol.* 77, 195
41 Pumpuni, C.B. *et al.* (1996) Bacterial population dynamics in three anopheline species: the impact on *Plasmodium* sporogonic development. *Am. J. Trop. Med. Hyg.* 54, 214
42 Gonzalez-Ceron, L. *et al.* (2003) Bacteria in midguts of field-collected *Anopheles albimanus* block *Plasmodium vivax* sporogonic development. *J. Med. Entomol.* 40, 371
43 Dong, Y. *et al.* (2006) *Anopheles gambiae* immune responses to human and rodent *Plasmodium* parasite species. *PLoS Pathog.* 2, e52
44 Pais, R. *et al.* (2008) The obligate mutualist *Wigglesworthia glossinidia* influences reproduction, digestion, and immunity processes of its host, the tsetse fly. *Appl. Environ. Microbiol.* 74, 5965
45 Azambuja, P. *et al.* (2005) Gut microbiota and parasite transmission by insect vectors. *Trends Parasitol.* 21, 568
46 Dillon, R.J. and Dillon, V.M. (2004) The gut bacteria of insects: nonpathogenic interactions. *Annu. Rev. Entomol.* 49, 71
47 Oliver, K.M. *et al.* (2009) Bacteriophages encode factors required for protection in a symbiotic mutualism. *Science* 325, 992
48 Degnan, P.H. and Moran, N.A. (2008) Evolutionary genetics of a defensive facultative symbiont of insects: exchange of toxin-encoding bacteriophage. *Mol. Ecol.* 17, 916
49 Degnan, P.H. and Moran, N.A. (2008) Diverse phage-encoded toxins in a protective insect endosymbiont. *Appl. Environ. Microbiol.* 74, 6782
50 Royet, J. and Dziarski, R. (2007) Peptidoglycan recognition proteins: pleiotropic sensors and effectors of antimicrobial defences. *Nat. Rev. Microbiol.* 5, 264
51 Wang, J. *et al.* (2009) Interactions between mutualist *Wigglesworthia* and tsetse peptidoglycan recognition protein (PGRP-LB) influence trypanosome transmission. *Proc. Natl. Acad. Sci. U.S.A.* 106, 12133
52 Rio, R.V. *et al.* (2006) Dynamics of multiple symbiont density regulation during host development: tsetse fly and its microbial flora. *Proc. Biol. Sci.* 273, 805
53 Anselme, C. *et al.* (2006) Host PGRP gene expression and bacterial release in endosymbiosis of the weevil *Sitophilus zeamais*. *Appl. Environ. Microbiol.* 72, 6766
54 Anselme, C. *et al.* (2008) Identification of the weevil immune genes and their expression in the bacteriome tissue. *BMC Biol.* 6, 43
55 Dziarski, R. and Gupta, D. (2006) Mammalian PGRPs: novel antibacterial proteins. *Cell. Microbiol.* 8, 1059
56 Hu, C. *et al.* (2008) Infections with immunogenic trypanosomes reduce tsetse reproductive fitness: potential impact of different parasite strains on vector population structure. *PLoS Negl. Trop. Dis.* 2, e192
57 Rodrigues, J. *et al.* (2010) Hemocyte differentiation mediates innate immune memory in *Anopheles gambiae* mosquitoes. *Science* 329, 1353
58 Weiss, B.L. *et al.* (2011) Tsetse immune system maturation requires the presence of obligate symbionts in larvae. *PLoS Biol* 9, e1000619
59 Attardo, G.M. *et al.* (2008) Analysis of milk gland structure and function in *Glossina morsitans*: milk protein production, symbiont populations and fecundity. *J. Insect Physiol.* 54, 1236
60 Aksoy, S. *et al.* (2003) Interactions between tsetse and trypanosomes with implications for the control of trypanosomiasis. *Adv. Parasitol.* 53, 1
61 Hedges, L.M. *et al.* (2008) *Wolbachia* and virus protection in insects. *Science* 322, 702
62 Teixeira, L. *et al.* (2008) The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. *PLoS Biol.* 6, e2
63 Osborne, S.E. *et al.* (2009) Variation in antiviral protection mediated by different Wolbachia strains in *Drosophila simulans*. *PLoS Pathogens* 5, e1000656
64 Kambris, Z. *et al.* (2009) Immune activation by life-shortening *Wolbachia* and reduced filarial competence in mosquitoes. *Science* 326, 134
65 Bian, G. *et al.* (2010) The endosymbiotic bacterium *Wolbachia* induces resistance to dengue virus in *Aedes aegypti*. *PLoS Pathog.* 6, e1000833
66 Moreira, L.A. *et al.* (2009) A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and *Plasmodium*. *Cell* 139, 1268
67 Glaser, R.L. and Meola, M.A. (2010) The native *Wolbachia* endosymbionts of *Drosophila melanogaster* and *Culex quinquefasciatus* increase host resistance to West Nile virus infection. *PloS ONE* 5, e11977
68 Kambris, Z. *et al.* (2010) *Wolbachia* stimulates immune gene expression and inhibits *Plasmodium* development in *Anopheles gambiae*. *PLoS Pathog.* 6, e1001143
69 Hughes, G.L. *et al.* (2011) *Wolbachia* infections in *Anopheles gambiae* cells: transcriptomic characterization of a novel host-symbiont interaction. *PLoS Pathog.* 7, e1001296
70 Enserink, M. (2010) Infectious diseases. Australia to test 'mosquito vaccine' against human disease. *Science* 330, 1460
71 Aksoy, S. (2000) Tsetse–a haven for microorganisms. *Parasitol. Today* 16, 114
72 Rio, R.V. and Aksoy, S. (2005) Interactions among multiple genomes: tsetse, its symbionts and trypanosomes. *Insect Biochem. Mol. Biol.* 35, 691–698

73 Lindh, J.M. and Lehane, M.J. (2011) The tsetse fly *Glossina fuscipes fuscipes* (Diptera: Glossina) harbours a surprising diversity of bacteria other than symbionts. *Antonie Van Leeuwenhoek* 99, 711–720
74 Geiger, A. *et al.* (2010) *Serratia glossinae* sp. nov., isolated from the midgut of the tsetse fly *Glossina palpalis gambiensis*. *Int. J. Syst. Evol. Microbiol.* 60, 1261
75 Geiger, A. *et al.* (2009) First isolation of *Enterobacter*, *Enterococcus*, and *Acinetobacter* spp. as inhabitants of the tsetse fly (*Glossina palpalis palpalis*) midgut. *Infect. Genet. Evol.* 9, 1364
76 Aksoy, S. *et al.* (1995) Mycetome endosymbionts of tsetse flies constitute a distinct lineage related to Enterobacteriaceae. *Insect Mol. Biol.* 4, 15
77 Chen, X. *et al.* (1999) Concordant evolution of a symbiont with its host insect species: molecular phylogeny of genus *Glossina* and its bacteriome-associated endosymbiont, *Wigglesworthia glossinidia*. *J. Mol. Evol.* 48, 49
78 Akman, L. and Aksoy, S. (2001) A novel application of gene arrays: *Escherichia coli* array provides insight into the biology of the obligate endosymbiont of tsetse flies. *Proc. Natl. Acad. Sci. U.S.A.* 98, 7546
79 Weiss, B.L. *et al.* (2006) Inter-specific transfer of bacterial endosymbionts between tsetse species: infection establishment and effect on host fitness. *Appl. Environ. Microbiol.* 72, 7013
80 Kaiwa, N. *et al.* (2010) Primary gut symbiont and secondary, *Sodalis*-allied symbiont of the Scutellerid stinkbug *Cantao ocellatus*. *Appl. Environ. Microbiol.* 76, 3486
81 Novakova, E. and Hypsa, V. (2007) A new *Sodalis* lineage from bloodsucking fly *Craterina melbae* (Diptera, Hippoboscoidea) originated independently of the tsetse flies symbiont *Sodalis glossinidius*. *FEMS Microbiol. Lett.* 269, 131
82 Heddi, A. *et al.* (1998) Molecular characterization of the principal symbiotic bacteria of the weevil *Sitophilus oryzae*:apeculiar G+C contents of an endocytobiotic DNA. *J. Mol. Evol.* 47, 52
83 Fukatsu, T. *et al.* (2007) Bacterial endosymbiont of the slender pigeon louse, *Columbicola columbae*, allied to endosymbionts of grain weevils and tsetse flies. *Appl. Environ. Microbiol.* 73, 6660
84 Cheng, Q. and Aksoy, S. (1999) Tissue tropism, transmission and expression of foreign genes in vivo in midgut symbionts of tsetse flies. *Insect Mol. Biol.* 8, 125
85 Toh, H. *et al.* (2006) Massive genome erosion and functional adaptations provide insights into the symbiotic lifestyle of *Sodalis glossinidius* in the tsetse host. *Genome Res.* 16, 149
86 Belda, E. *et al.* (2010) Mobile genetic element proliferation and gene inactivation impact over the genome structure and metabolic capabilities of *Sodalis glossinidius*, the secondary endosymbiont of tsetse flies. *BMC Genomics* 11, 449
87 Dale, C. and Moran, N.A. (2006) Molecular interactions between bacterial symbionts and their hosts. *Cell* 126, 453
88 Dale, C. *et al.* (2002) Type III secretion systems and the evolution of mutualistic endosymbiosis. *Proc. Natl. Acad. Sci. U.S.A.* 99, 12397
89 Dale, C. *et al.* (2001) The insect endosymbiont *Sodalis glossinidius* utilizes a type III secretion system for cell invasion. *Proc. Natl. Acad. Sci. U.S.A.* 98, 1883
90 Dale, C. and Welburn, S.C. (2001) The endosymbionts of tsetse flies: manipulating host-parasite interactions. *Int. J. Parasitol.* 31, 628
91 Welburn, S.C. and Maudlin, I. (1999) Tsetse-trypanosome interactions: rites of passage. *Parasitol. Today (Personal ed)* 15, 399
92 Weiss, B.L. *et al.* (2008) An insect symbiosis is influenced by bacterium-specific polymorphisms in outer-membrane protein A. *Proc. Natl. Acad. Sci. U.S.A.* 105, 15088
93 Geiger, A. *et al.* (2007) Vector competence of *Glossina palpalis gambiensis* for *Trypanosoma brucei* s.l. and genetic diversity of the symbiont *Sodalis glossinidius*. *Mol. Biol. Evol.* 24, 102
94 Farikou, O. *et al.* (2010) Tripartite interactions between tsetse flies, *Sodalis glossinidius* and trypanosomes–an epidemiological approach in two historical human African trypanosomiasis foci in Cameroon. *Infect Genet. Evol.* 10, 115
95 Geiger, A. *et al.* (2006) Vector competence of *Glossina palpalis gambiensis* for *Trypanosoma brucei* s.l. and genetic diversity of the symbiont *Sodalis glossinidius*. *Mol. Biol. Evol.* 24, 102–109
96 O'Neill, S.L. *et al.* (1993) Phylogenetically distant symbiotic microorganisms reside in *Glossina* midgut and ovary tissues. *Med. Vet. Entomol.* 7, 377
97 Cheng, Q. *et al.* (2000) Tissue distribution and prevalence of *Wolbachia* infections in tsetse flies, *Glossina* spp. *Med. Vet. Entomol.* 14, 44
98 Haines, L.R. *et al.* (2003) Cationic antimicrobial peptide killing of African trypanosomes and *Sodalis glossinidius*, a bacterial symbiont of the insect vector of sleeping sickness. *Vector Borne Zoonotic Dis.* 3, 175
99 Haines, L.R. *et al.* (2009) Killing of trypanosomatid parasites by a modified bovine host defense peptide, BMAP-18. *PLoS Neglected Trop. Dis.* 3, e373
100 Durvasala, R. *et al.* (2003) *Rhodnius prolixus* and its symbiont, *Rhodococcus rhodnii*: a model for paratransgenic control of disease transmission. In *Insect Symbiosis* (Bourtzis, K. and Thomas, A.M., eds), pp. 83–96, CRC Press
101 Durvasula, R.V. *et al.* (2008) Genetic transformation of a Corynebacterial symbiont from the Chagas disease vector *Triatoma infestans*. *Exp. Parasitol.* 119, 94
102 Sundaram, R.K. *et al.* (2008) Expression of a functional single-chain antibody via *Corynebacterium pseudodiphtheriticum*. *Eur. J. Clin. Microbiol. Infect. Dis.* 27, 617
103 Durvasula, R. *et al.* (1999) Expression of a functional antibody fragment in the gut of *Rhodnius prolixus* via transgenic bacterial symbiont *Rhodococcus rhodnii*. *Med. Vet. Entomol.* 13, 115
104 Favia, G. *et al.* (2008) Bacteria of the genus *Asaia*: a potential paratransgenic weapon against malaria. *Adv. Exp. Med. Biol.* 627, 49