

Microbial symbiosis and the control of vector-borne pathogens in tsetse flies, human lice, and triatomine bugs

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Symbiosis is a widespread biological phenomenon, and is particularly common in arthropods. Bloodsucking insects are among the organisms that rely on beneficial bacterial symbionts to complement their unbalanced diet. This review is focused on describing symbiosis, and possible strategies for the symbiont-based control of insects and insect-borne diseases, in three bloodsucking insects of medical importance: the flies of the genus *Glossina*, the lice of the genus *Pediculus*, and triatomine bugs of the subfamily *Triatominae*. *Glossina* flies are vector of *Trypanosoma brucei*, the causative agent of sleeping sickness and other pathologies. They are also associated with two distinct bacterial symbionts, the primary symbiont *Wigglesworthia* spp., and the secondary, culturable symbiont *Sodalis glossinidius*. The primary symbiont of human lice, *Riesia pediculicola*, has been shown to be fundamental for the host, due to its capacity to synthesize B-group vitamins. An antisymbiotic approach, with antibiotic treatment targeted on the lice symbionts, could represent an alternative strategy to control these ectoparasites. In the case of triatominae bugs, the genetic modification of their symbiotic *Rhodococcus* bacteria, for production of anti-*Trypanosoma* molecules, is an example of paratransgenesis, i.e. the use of symbiotic microorganism engineered in order to reduce the vector competence of the insect host.

Keywords: *Glossina*, *Pediculus*, Triatominae, *Trypanosoma*, *Rhodococcus*, *Wigglesworthia*, *Sodalis*, *Riesia*, Symbiotic control, Paratransgenesis

Introduction

The interactions between arthropods and bacteria encompass the entire range of possible symbiotic associations, from strict parasitism to obligate mutualism. Several insect species that hold a role of medical importance, either directly or as pathogen vectors, present microbial associations that strongly affect their biology. Some of these medically important species have been shown to be engaged in mutualistic relationships with bacterial symbionts, which give their hosts distinct selective advantages. A recent review highlighted the importance of the study of symbiosis in mosquitoes, for developing novel strategies for the control mosquito-borne diseases.¹ The present review is focused on symbiosis in other bloodsucking insects of medical importance: tsetse flies (genus *Glossina*), human lice (genus *Pediculus*), and triatomine bugs. Mutualistic bacteria are frequently observed in hematophagous insects, due to the lack of balance in the insect diet and the subsequent need of metabolic integration. The contribution of microbial symbionts to the insect

physiology is of particular importance where hematophagy is the sole form of feeding during all of the phases of the life cycle, as in the case of the head and body lice of humans. Diptera, on the other hand, having a larval stage, in general have the possibility to exploit very different ecological niches during their development. However, an uncommon situation in this insect order is that of *Glossina* spp., the vectors of sleeping sickness. Indeed, in *Glossina* spp. the larvae develop within the maternal uterus where they are fed with the secretions of the 'milk gland'. In other words, the larva indirectly relies on the same, unbalanced, trophical niche of the mother (based on blood), and this could be expected to create a condition favorable to the development of a strict symbiotic association with mutualistic symbionts.

The symbionts of *Glossina* flies, *Sodalis*, *Wigglesworthia*, and *Wolbachia*

The flies of the genus *Glossina* (Diptera: Glossinidae) are the vectors of Human African Trypanosomiasis (HAT), also known as sleeping sickness. This disease is caused by two subspecies of the protozoan *Trypanosoma brucei*. Most of the cases of sleeping sickness in Africa (95%) are caused by *T. b. gambiense* (West African sleeping sickness), predominant in

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Central and Western Africa, while *T. b. rhodesiense* (East African sleeping sickness) is primarily localized in Eastern and Southeastern Africa and is responsible for a limited percentage of cases.^{2–4} Human African Trypanosomiasis is estimated to affect tens of thousands of people each year; however, the application of integrated control measures is reducing the annual incidence of the disease, as indicated by the reduction of the cases recorded by WHO, from about 37 000 to about 17 500 in the period 1998–2004, to less than 10 000 in 2009 and to about 7 000 in 2010.⁴ It must however be emphasized that recorded cases are only a fraction of the actual cases, and the estimates on the actual incidence and prevalence of HAT are to be interpreted with caution. There are indeed several difficulties in calculating the actual number of cases of this neglected tropical disease. The diagnosis of HAT can be difficult,^{5,6} also considering that the disease mainly occurs in rural areas where health facilities are limited or even absent. It has been reported that, across the African continent, up to 50 000 HAT cases might remain undetected.^{2,7} For example, it has been estimated that in Uganda, in 2005, only about 10% of the deaths caused by HAT had actually been attributed to this diseases, while the total numbers remained undetermined.⁸ Cases of HAT have also been described in Europe and in the USA, mainly in travelers returned from Africa. During the 2000–2010 period, 94 cases have been reported in non-endemic countries (43% in Europe and 23% in the USA).² In addition, African trypanosomes (i.e. *T. b. brucei*) also cause a disease of great veterinary and economic importance,⁹ the Nagana, or Animal African Trypanosomiasis (AAT).

The typical progression of a *T. b. rhodesiense* infection is rapid; most patients develop fever, headache, muscle and joint aches, enlarged lymph nodes, and rash after the infecting *Glossina* bite. After a few weeks of infection, the protozoa invades the central nervous system causing mental deterioration and other neurological symptoms. For these reasons the *T. b. rhodesiense* HAT is classified as acute, while the HAT caused by *T. b. gambiense* is classified as chronic. Indeed, infection by *T. b. gambiense* progresses more slowly, as in the first phase the infected person may just develop intermittent fever, headache, muscle and joint ache. After 1–2 years, the pathology progresses, involving the central nervous system, with personality changes, daytime sleepiness, night-time sleep disturbance, and progressive confusion, a syndrome that threatens the life of the patients.³ Tsetse flies bite during daylight hours and both sexes, necessitating obligate blood meals, can transmit trypanosomes. In the East African savannah the *Glossina* flies live in the rural areas, in the woodlands and thickets. In central and West Africa, these Diptera live in the forests and near rivers.

Glossina flies harbor two distinct bacterial symbiont populations: the primary (and thus necessary for the host survival) symbionts of the genus *Wigglesworthia* and the secondary symbiont *Sodalis glossinidius*. Both bacteria are vertically transmitted to the developing progeny via the secretions of the mother's milk.¹⁰ *Wigglesworthia* bacteria live within specialized epithelial cells (bacteriocytes) that form an organ (bacteriome) located in the anterior midgut.¹¹ Each bacteriome contains about 10⁸ *Wigglesworthia* cells that populate the cytoplasm of the bacteriocytes.¹² The unique function of this symbiont can be understood by considering the peculiar form of reproduction of tsetse flies. *Glossina* flies are viviparous, meaning that the single fertilized egg develops to the third larval instar within the maternal uterus. At the end of larval development, the female lays a mature larva that turns into a pupa. The intrauterine larval development is made possible by the availability of vitamins/cofactors produced by the primary symbiont, *Wigglesworthia*, that are fed to the larva through the secretions of the 'milk gland'. It has been shown that the absence of *Wigglesworthia* symbionts in *Glossina* flies increases the numbers of trypanosome parasites and influences the vectorial competence.¹⁰ A mechanism that explains the *Glossina*–*Wigglesworthia* interaction has recently been proposed. According to this model, the symbiont induces the transcription of the host gene PGRP-LB, which, in turn, exhibits bactericidal and trypanocidal activity, potentially mediating the immune response of tsetse to infections.¹³

The genomes of two species of this symbiotic bacterial genus, *W. glossinidia* and *W. morsitans*, have been sequenced, and they present many interesting features, most of which are common to both species.^{14,15} Both genomes have a size around 700 Kb, with a very strong adenine-thymine percentage content, as high as 78% in *W. glossinidia*. High percentage of AT nucleotides in the genome is a characteristic of obligate endosymbionts with reduced genomes (for a recent analysis of the genomes of endosymbionts see Ref. 16). It has been suggested that part of the genome of the symbionts has been incorporated into that of *Glossina* during the course of the host–symbiont co-evolution.¹⁷ Despite its small size, the genome of *Wigglesworthia* contains over 60 genes necessary to synthesize a remarkable variety of vitamins, essential to supplement the qualitatively deficient diet of the host. Another indirect evidence of the obligatory mutualistic relationship between *Glossina* and *Wigglesworthia* can be obtained by producing aposymbiotic strains (i.e. devoid of symbionts) by treating the insect with antibiotics. Individuals deprived of their primary symbionts show a delay in development and an almost total reproductive impairment.¹⁸ This situation can be partially

rescued by providing aposymbiotic flies with a blood meal supplemented with vitamins of the B group.¹⁹

The secondary symbionts of *Glossina* are bacteria of the species *Sodalis glossinidius*. *Sodalis* and related bacteria were found in different insect hosts: tsetse flies,²⁰ aphids,²¹ weevils,²² hippoboscids,²³ ants,²⁴ stinkbugs,²⁵ and beetles.²⁶ In *Glossina* these symbionts colonize different organs and tissues, the midgut, the muscles, the fat body, the hemolymph, the milk gland, and the salivary glands.¹¹ Additionally these bacteria use a type III secretion system to invade the larval tissues.²⁷ Recently, it has been reported that the density of *S. glossinidius* in *Glossina palpalis gambiensis* is stable in pupae and increases significantly in adults, particularly in males. With an opposite trend, the density of *Wigglesworthia* increases in pupae and is stable in adults. These results show that tsetse fly colonization by both symbionts is permanent throughout the host life span.²⁸

Unlike *Wigglesworthia*, the symbiotic relationship of *S. glossinidius* with *Glossina* has been dated to be a recent evolution. The genome was found to be about 4.5 mb, similar in size to those of related free-living enteric microbes such as *Escherichia coli*. The characteristics of this symbiont genome, such as a high number of pseudogenes, were proposed to possibly indicate an ongoing transition from free-life to intracellular symbiosis.²⁹ Observing the biosynthetic capabilities of *Sodalis* and *Wigglesworthia*, it is interesting to note that one of the distinctions between the genomes of these bacteria is in thiamine (vitamin B1) biosynthesis and transport. While *Wigglesworthia* possesses thiamine biosynthetic capabilities, *Sodalis* is incapable of its production but its genome contains a putative thiamine ABC transport system, likely used to salvage exogenous thiamine. This supply of vitamin B1 by *Wigglesworthia* is important for the integration of the blood diet of tsetse flies, deficient in B-complex vitamins. The mechanisms of the symbiotic relationship of *S. glossinidius* with *Glossina* are less clear, possibly ranging from commensalism to a limited, facultative mutualistic symbiosis. In fact, the selective elimination of this symbiont does not produce any significant reduction in the development and reproductive capacity of the aposymbiotic flies. It has been suggested that *S. glossinidius* is implicated in the capacity of tsetse fly to transmit trypanosome,³⁰ and the symbiont has indeed been shown to be involved in the host vector competence.³¹ Moreover, *S. glossinidius* appears to be resistant to antimicrobial peptides: the recombinant attacin from *Glossina* (an inducible immune peptide, active against tripanosomes and different Gram-negative bacteria) is not effective on *S. glossinidius*.³²

The presence of *S. glossinidius* is thus not essential to the host (unlike *Wigglesworthia*), and this symbiont

is linked with its host by a rather weak symbiotic 'connection', as indicated by the fact that it can be cultured *in vitro*. This is an important characteristic, that could help to achieve the genetic transformation of the symbiont, and its use in paratransgenesis applications, i.e. the use of symbionts genetically modified for the expression molecules capable of reducing the vector capacity of the insect host.³² Genetic manipulation of *S. glossinidius* was applied to generate recombinant strains expressing the green fluorescent protein (GFP) marker. These labeled bacteria, injected in the hemolymph of gravid females, were acquired successfully by intrauterine larvae and thus transmitted to the following generation, in which bacteria continued to express the GFP marker, even after maturation into adults.^{33,34} These first studies were followed by a second phase of research on paratransgenesis, consisting in the manipulation of the symbiont for the expression of products able to interfere negatively with the development of trypanosomes responsible for sleeping sickness and Nagana.³⁵ In this recent work, a single domain anti-*Trypanosoma* antibody (Nanobody®), Nb_An33, directed toward distinct regions of the variant-specific surface glycoprotein of trypanosomes, was successfully expressed and secreted by *S. glossinidius*.

The development of effective paratransgenesis-based methods to reduce vector capacity would possibly represent an important step for a further implementation of integrated control strategies. However, the application of paratransgenesis implies the release of genetically modified microorganisms (directly, or indirectly through the release of insects harboring the modified microbes). It is not certain whether the release of genetically engineered microbes will ever be allowed, even in areas endemic for a vector-borne disease that could be controlled by this strategy, considering the public perception of the risks associated with the use in the environment of transgenic organisms. Under this respect, it is interesting that various *Glossina* species also harbor *Wolbachia*,^{36,37} a bacterial symbiont that hold the potential of leading to symbiont-based control methods of vector-borne diseases, without the need of genetic manipulation of the symbiont itself. Indeed, as shown by Scott L. O'Neill and coworkers^{38,39} and by other research groups,^{40,41} non-manipulated *Wolbachia* bacteria have the capability to reduce the vector capacity of different insects, through different mechanisms, as the shortening of the life of the insect itself (as determined by the wMelPop strain of *Wolbachia*) and the activation of the insect immune response. These effects, associated with the capacity of *Wolbachia* to spread into the host population through cytoplasmic incompatibility, or CI,³⁸ makes this bacterium very promising for the control of different vector-borne

diseases, with an approach that does not foresee the release of genetically modified organisms and that perfectly falls into the area of biological control. Considering tsetse flies, a recent study showed that *Wolbachia* in *G. morsitans* induces a strong CI and shortens the life of the insects,⁴² two characteristics that are very important toward the development of these bacteria into tools for the biological control of trypanosome transmission. In fact, *Wolbachia*-induced CI is currently under consideration as a novel tool for the control of insect pests and disease vectors.^{43,44}

***Reisia*, symbiont of lice**

The suborder Anoplura includes about 500 species of insects, all obligate bloodsucking parasites, scattered on a wide range of vertebrate hosts. The two most important members of the Anoplura are the closely related *Pediculus humanus capitis* and *P. h. humanus*, respectively the head and the body lice. The members of the first subspecies live on the scalp and lay their eggs 'cemented' on the hair, whereas members of the second feed on the body and lay their eggs on clothing.⁴⁵ Body louse is an important disease vector,^{46,47} while head louse has generally been regarded as of minor importance.^{48–51} Diseases vectored by body lice include epidemic typhus, trench fever, and relapsing fever, caused respectively by *Rickettsia prowazekii*, *Bartonella quintana*, and *Borrelia recurrentis*. At the moment the taxonomic status of these two insect parasites is uncertain. Biological traits, including morphological characters and behavioral and ecological differences,^{52,53} as well as isoenzyme markers (e.g. Ref. 54), have been used to investigate the taxonomy of human lice, with results supporting the existence of the two subspecies. Most authors agree in believing them to be two closely related subspecies that diverged around 100 000 years ago, possibly when humans started intensive usage of clothes. However, two recent studies, based on a wide sampling in a high number of geographical areas, disagree on this conclusion.^{55,56} These studies indicate that body and head lice could be just two genetically indistinct ecotypes of the same species. They highlight that it is possible to observe genetic differentiations between different louse populations over different geographical areas, while all the individuals collected in the same area, even if sampled both from the head and the body, are genetically uniform. Furthermore, these authors show that genetic differentiations between different geographical areas may lead to the evolution of different alleles of resistance to insecticides among different lice populations.

Head and body lice are thus certainly closely related in many aspects, including in the primary bacterial symbionts they harbor. In fact, during their common evolutionary journey, the ectoparasites of

the genus *Pediculus* have interacted with various species of microorganisms, establishing commensal, parasitic, and mutualistic relations, the tightest of which being that with *Riesia pediculicola*. The symbiont is associated with a disk-shaped structure (mycetoma), at the level of the midgut of the lice, formed by an aggregate of bacteriocytes, whose first observation can be traced back to a study by Robert Hooke performed 350 years ago.⁵⁷ An in-depth study on endosymbiont localization during the entire life cycle of lice highlights very complex interactions between the host and the symbiont. This symbiosis is characterized by the sequential development of three different mycetomal stages, that completes before the hatch of the first nymphal instar.

A fourth and final stage is the maternal mycetoma, which is formed in the second and third instar stages, and only in female individuals, because it is directly involved in the vertical transmission to the developing oocytes.⁵⁸ The complexity of the host–endosymbiont interaction is moreover confirmed by the alternation of both endocellular and extracellular phases of the symbionts during the host life cycle.⁵⁸ *R. pediculicola* is a member of the family Enterobacteriaceae that forms a well defined phyletic line within the Gamma-proteobacteria.⁵⁹ *Riesia* maintains a relationship of mutual symbiosis with the *Pediculus* host as it provides vitamins that cannot be obtained through the hematophagous diet.⁵⁸ This capability is reflected in the genome of *Riesia*, which, albeit encoding less than 600 proteins, includes several genes for the synthesis of B-group vitamins, essential to supplement the diet of the host. The obligate mutualistic symbiosis has been further proved by microsurgical removal of the mycetoma in females of *Pediculus*, which deprived of their symbionts die after a few days and produce deformed eggs.⁶⁰ These dramatic effects can be mitigated in part by integrating the blood meal with pantothenic acid, nicotinic acid, and beta biotin. A detailed study showed that the symbiont could provide the host with up to seven vitamins (B1, B2, B3, B5, B6, B7, B9), but it supplements the diet differently in the two sexes.⁶¹

The control of lice in humans is usually performed with insecticide treatments and through the mechanical removal of eggs (nits). In the future, a new therapeutic approach, that identifies as target the symbiotic bacterium rather than the louse, could be considered. This strategy, known as antisymbiotic therapy, already proposed and tested for filarial nematodes and their symbiont *Wolbachia*, has the advantage of targeting the bacteria, which are sensitive to the action of antibiotics.⁶² Since *Riesia* is an obligatory symbiont, essential to the metabolism of the host, the deleterious action exerted by the antibiotic treatment should reflect on the host. Single-case reports

show promising results for this strategy: an example is the case of an antibiotic-treated girl of 12 years: the antibiotic treatment, performed to combat a respiratory infection, resulted as a side effect in the death of head lice.⁶³ It is however obvious that the importance of single-case observations must not be overestimated, and that *ad hoc* studies in this area are required.

Rhodococcus, symbiont of Triatominae bugs

The subfamily Triatominae of the family Reduviidae (Rhynchota) includes the bugs of the genera *Triatoma*, *Rhodnius*, and *Panstrongylus*, which are the main vectors of the protozoan *Trypanosoma cruzi*, the causative agent of Chagas disease.⁶⁴ The triatomine are fundamental in the life cycle of the parasite, both for their vector capability through their blood-feeding habits, and for the interaction they establish with the *Trypanosoma*, at the physiological and at the biochemical level.^{65–67} Triatomine usually consume their blood meal at night, while the host is sleeping and, if they feed on infected hosts, they are likely to acquire *T. cruzi*, allowing it to complete its life-cycle and to be released with the feces. Furthermore triatomine bugs tend to defecate during the blood meal, contaminating host's skin and hair. Moreover, they can acquire trypanosomes through consumption of infected feces expelled by other bugs, or through cannibalism. The life cycle can be easily completed as the mammalian infection happens through Triatominae's fecal droplets containing trypanosomes. Thanks to their flagellar motility, trypanosomes are able to penetrate into the mammalian host by exploiting the microlesions caused by bug's bite or by self scratching, or to penetrate through the mucous membranes.

Chagas disease is endemic in the rural/poor zones of Central and South America, where an estimated 8–11 million people are infected by *T. cruzi*, and over 90 million are at risk of contracting the infection.⁶⁸ Chagas is a chronic disease with a highly debilitating course, that leads to the development of alterations to the cardiovascular (e.g. dilated cardiomyopathy), digestive (e.g. mega-esophagus and mega-colon), and nervous systems. It thus causes severe disability and a significant shortening of the life expectancy of patients. Moreover, *T. cruzi* has a low host specificity, and thus can be found in several animal reservoirs (dog, opossum, armadillo, etc.), a characteristics that makes eradication virtually impossible.⁶⁹ For these reasons, adequate control of Chagas disease can only be achieved by designing an integrated approach that includes actions to contain the insect populations as well as their vector capacity.

The gut microbial community of Triatominae has been studied for over 50 years.⁷⁰ Of particular interest are the bacteria of the genus *Rhodococcus*

(e.g. *R. rhodnii*, *R. corynebacteroides*, *R. triatomae*), which are believed to give an important contribution to the insect metabolism (i.e. through the synthesis of group B vitamins, deficient in the blood, or by being directly digested by the bugs in order to provide the missing nutrients).^{71,72} The first nymphal stage of triatominae is characterized by a transient lack of gut-associated symbionts (aposymbiotic), which are later acquired by coprophagy. Coprophagia therefore allows insects to acquire both the symbionts released by other bugs, and, when present, the trypanosomes. The host–symbiont relationship between triatomine and *Rhodococcus* is particularly attractive for the development of a strategy for control of trypanosomiasis based on paratransgenesis. In fact, *Rhodococcus* meets the necessary characteristics for a successful paratransgenic control strategy, as defined by Beard and colleagues.⁷¹ *Rhodococcus* bacteria can indeed be easily cultured and genetically modified to stably express proteins, an approach that has been shown to effectively harm the pathogen. Furthermore, the symbionts and trypanosomes colocalize in the gut of the insect vector, and the natural way of acquisition of symbionts through ingestion of fecal material has allowed to envisage a strategy for the administration of modified bacteria to the insects.

The first step toward the application of this strategy has been to show that it is possible to reduce symbiont populations naturally present in the intestines of wild-type bugs through the inclusion of antibiotics in the diet and, subsequently, to promote colonization by genetically manipulated (GM) antibiotic-resistant bacteria.⁷¹ The following step was thus to feed the bugs with bacteria modified to produce anti-trypanosome factors (e.g. cecropin A).⁷³ This allowed to obtain the colonization of the insects with bacteria that are 'toxic' for the protozoan, significantly reducing the vector capacity of the bugs. The studies on this particular host–GM symbiont system continued over the years, not only determining in laboratory conditions the effectiveness of various anti-trypanosome molecules (apidaecin, magainin II, melittin), but also through the expression of active single-chain antibodies for potential transmission-blocking activity against *T. cruzi*.^{72,74} One of the most important goals of these studies was the development of a synthetic 'anti-trypanosome diet' named Cruziguard®, to be used for field experiments.⁷⁵ This synthetic substance, added with GM *R. rhodnii*, was dispensed in pellets that simulate feces of *R. prolixus*, thereby stimulating coprophagy. Trial experiments in field conditions were conducted by spreading Cruziguard® in cages which simulate the building materials used in huts in Central America regions endemic for the disease.⁷² At the same time, in order to evaluate potential transgene dispersion

linked to the Horizontal Gene Transfer phenomenon (HGT), appropriate experiments on risk assessment were conducted. The first lines of evidence obtained from mathematical models in laboratory experiments show an HGT frequency of less than 1.14×10^{-16} per 100 000 bacterial generations.⁷⁶ Of course, it will be necessary to confirm these preliminary data with experimental trials in field conditions. A possible cause of concern when designing a paratransgenesis approach is the conservation and spread of the exogenous gene in the symbiont population. The gene could be costly in terms of fitness, and thus likely to be lost. This issue has been discussed in different publications (e.g. Ref. 77) and has been a focal point in multiple experimental studies. For example in the approach described here, it has been shown that the transformed *Rhodococcus* stably maintain the transgene, even in the absence of antibiotic driven selective pressure.^{73,74} This issue requires further investigation and application of theoretical models, also considering the potential benefit to the insect vector that could derive from being infected by GM bacteria, that are protective toward pathogens detrimental not only to vertebrate hosts, but also to the insect vector itself. To date, it is not yet possible to launch large-scale campaigns for the control of Chagas disease based on this strategy, but the published results are certainly encouraging, making the symbiotic triatomine–*Rhodococcus* model one of the most advanced systems toward the application of paratransgenesis-based control methods.

Conclusions

Investigations on the microbial community associated with insects revealed that the biology of these animals can be profoundly influenced by the microorganisms they harbor. Such microbe–host interactions range from parasitism to a variety of beneficial associations.⁷⁸ In this range of effects that the microbial community can exert on its host, two examples relevant to the area of medical entomology are the resistance to insecticides⁷⁹ and the association between the parasitic phenotype of an insect and its microbiota.⁸⁰ Focusing investigations on invertebrate animals as complex symbiotic systems, scientists in different research areas have achieved concrete progresses and applications, e.g. for the control of human, animal, and plant diseases. For example, the discovery of *Wolbachia* in filarial nematodes led to the development of the anti-symbiotic therapy to cure filarial diseases.^{62,68} These studies showed that, targeting the symbionts rather than the nematode, these diseases can be cured with a safe elimination of the adults worms, a results that is not possible using traditional anti-parasitic drugs.⁸¹ Other studies on *Wolbachia* revealed that this bacterium can stimulate

the resistance of mosquitoes to pathogens, reducing their vector capacity.^{40,82} In addition, components of the microbiota have been shown to protect against pathogens and environmental factors.⁸³ The molecular dialog between the host and the microbiota is emerging as a fundamental aspect of insect biology.^{84,85} It is now clear that the composition of the microbiota affects the phenotype of its host, e.g. immune response, resistance to environmental conditions, overall health. In summary, investigations on the role of the microbiota in invertebrate biology are allowing to develop novel strategies to control pest and parasitic arthropods, and to reduce their vector competence.

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