Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Microbiology

Mosquito/microbiota interactions: from complex relationships to biotechnological perspectives

Irene Ricci, Claudia Damiani, Aida Capone, Chenoa DeFreece, Paolo Rossi and Guido Favia

To date around 3500 different species of mosquito have been described, several tens of which are vectors of pathogens of remarkable interest in public health. Mosquitoes are present all around the world showing a great ability to adapt to very different types of habitats where they play relevant ecological roles. It is very likely that components of the mosquito microbiota have given the mosquito a great capacity to adapt to different environments. Current advances in understanding the mosquito–microbiota relationships may have a great impact in a better understanding of some traits of mosquito biology and in the development of innovative mosquito-borne disease-control strategies aimed to reduce mosquito vectorial capacity and/or inhibiting pathogen transmission.

Address
Scuola di Bioscienze e Biotecnologie, Università degli Studi di Camerino, 62032 Camerino, Italy

Corresponding author: Favia, Guido (guido.favia@unicam.it)

Current Opinion in Microbiology 2012, **15**:278–284

This review comes from a themed issue on **Ecology and industrial microbiology**

Edited by **Bruno Lemaitre**, **Jeremy Herren** and **Eric Johnson**

For a complete overview see the Issue and the Editorial

Available online 30th March 2012

1369-5274/$ – see front matter,

http://dx.doi.org/10.1016/j.mib.2012.03.004

Introduction

Considering their biodiversity and abundance, insects can be considered among the most successful animals on Earth [1]. Mosquitoes significantly contribute to insect biodiversity and biomass, representing around 3500 described species, a few hundreds of which pose serious medical and economical risk (burdens?) [2]. In fact, among other infectious diseases, mosquitoes can transmit malaria, Yellow fever, Dengue fever, West Nile and Chikungunya. As a consequence more than half of the global human population is at risk of exposure to infections transmitted by mosquitoes; and hundreds of millions of human infections are recorded every year [3].

Mosquitoes have been recorded in almost every continent, being adapted to a variety of different habitats where they play important functional ecological roles. Larval stages represent one of the main components of the biomass in water pools worldwide.

As well as for many insect species, the symbiotic relationships between mosquitoes and several microorganisms most probably have important implications in mosquitoes' evolutionary success, including their widespread distribution. Furthermore, the resident microbiota of mosquito vectors may inhibit the development of pathogens they transmit [4•]. In this context several microbes may offer opportunities to successfully manipulate the vector competence of mosquitoes to reduce their abilities to transmit human pathogens. This latter aspect has especially led to an intensification of studies focused on the microbiota of diverse mosquito species in the last decades.

Although studies on the relationship between bacteria and mosquitoes date back to the mid 20s, beginning when Hertig and Wolbach [5] described the presence of bacteria, named *Wolbachia pipentis*, within the reproductive organs of the mosquito *Culex pipiens* [6], only recently has the study of symbiosis in mosquitoes found strong interest resulting in a significant number of publications in the field [7].

The symbiotic relationships that mosquitoes have established with different types of microorganisms have probably played a key role in the evolutionary success of insects by symbiotic microorganisms influencing various biological functions and integrating those under the control of the host genome, providing an improved adaptability to their environment.

Furthermore, there is growing interest of symbionts in mosquito disease vectors since their manipulation may offer novel control methods that are uniformly defined as Symbiotic Control. Here we review aspects of microbiota/mosquito interactions and their use in controlling mosquito-borne diseases.

Wolbachia and mosquitoes: an old story with very innovative perspectives

Bacteria of the genus *Wolbachia* represent a group of maternally inherited intracellular bacteria firstly described in *C. pipiens* [5], but more recently recorded in a remarkably high number of insect species.

In the mosquito, *Wolbachia* has been detected in several genera including *Aedes*, *Culex*, *Coquillettidia*, and *Mansonia*,

but interestingly never recorded in species of the *Anopheles* genus [7–10]. In this context it is worth acknowledging that the genus *Anopheles* comprises some 300 different species, around 60 of which are important in malaria transmission worldwide [11]. Moreover, *Wolbachia* has been never detected in natural population of the species *Aedes aegypti*, a main vector of dengue and yellow fever [12].

The maternal transmission route of *Wolbachia* occurs through the egg cytoplasm and causes several reproductive disorders in the insect host including cytoplasmic incompatibility [13], parthenogenesis [14], feminization [15] and, male killing [16]. Through cytoplasmic incompatibility, the bacteria are able to spread through populations, thus *Wolbachia* has been proposed as a gene drive system for mosquito genetic replacement, for the reduction of population size, and for interfering with population age structure to reduce disease transmission [17]. Recently, several studies have shown the potential of *Wolbachia* symbionts to control mosquito borne diseases. In particular, these studies have shown that the introduction of some strains of *Wolbachia* in *Ae. aegypti* causes a life shortening of the mosquito [18•] as well as an upregulation of the mosquito immune response that render the mosquito refractory to dengue infection and parasites [19•]. It has been shown that the strain of *Wolbachia* that halves adult lifespan in *Ae. aegypti* is also able to inhibit the ability of a range of pathogens infecting this mosquito species [20]. This study suggests that *Wolbachia*-mediated pathogen interference if coupled with the life-shortening strategy can provide a synergistic approach for the control of mosquito-transmitted diseases.

Similarly, in *An. gambiae* a *Wolbachia*-induced infection may inhibit *Plasmodium* infection [21]. It is worth noting that not all strains exert a protective phenotype on their host as shown by a comparative study aimed to assess the effect of two *Wolbachia* strains, wAlbB (isolated from *Ae. albopticus*) and wMelPop (from *Drosophila melanogaster*), on the vector competence of *An. gambiae*, the main African malaria vector [22]. The wAlbB strain significantly increases *Plasmodium* oocyst levels in the mosquito midgut, while wMelPop moderately inhibits oocyst levels. Furthermore, the wAlbB strain is avirulent to mosquitoes, while the wMelPop strain is virulent. These different effects on vector competence indicate that different *Wolbachia* strains differ in their interactions with both host and the capacity to interfere with parasites.

Very recently, it has been shown that a *Wolbachia*-infected strain of *Ae. aegypti* resistant to dengue infection is able to rapidly replace the natural, susceptible population [23••]. This latter study represents extraordinary evidence of the feasibility of *Wolbachia*-mediated population-replacement strategy to (be applied in nature to) control mosquito borne diseases, thus representing *the beginning of a new era* in this field [24].

Bacteria (beyond *Wolbachia*) and mosquitoes: from basic biology to the management of mosquito borne diseases

Aside from *Wolbachia*, the description of the microbiota associated with mosquitoes is mostly related to bacteria. For some years, it has been known that different mosquito species carry common bacteria of different genera, among these *Enterobacter*, *Escherichia*, *Klebsiella*, *Serratia*, *Pseudomonas*, and *Staphylococcus* [25–28]. These studies described genetically well-characterized gut or enteric bacteria inhabiting the midgut of mosquitoes, indicating that the mosquito midgut is a site of complex interactions between the mosquito, the malaria parasite and, the resident bacterial flora.

Symbionts of hematophagous insects may provide B vitamins to insect hosts [29] and the microbial structure of mosquito gut is strongly affected by the adults' diet. In particular, blood meal increases the density of bacteria, which reaches a peak around 48 h after feeding [30]. This bloom includes enteric bacteria, although the increase of the total bacterial load is accomplished by a reduction of the overall community diversity. Enteric bacteria show a remarkable genetic redox capacity of coping with oxidative and nitrosative stresses related to the catabolism of blood meal suggesting a beneficial role in maintaining gut redox homeostasis [31]. Furthermore, the presence of some bacterial species in the midgut of vector mosquitoes can impact not only digestion, but also other physiological traits of the insect like fecundity, and is required for the completion of the embryonic development [32].

Gut bacteria and mosquito immune system

The gut microbiome has a strong influence on host immunity too, and some bacteria can directly interfere with the mosquito vectorial capacity, as shown in studies analysing the impact of *Serratia* and *Enterobacter* species in the midgut of *Anopheles* mosquitoes on *Plasmodium* development [33]. Similarly, in *Ae. aegypti*, the regulation of viral resistance occurs through expression of specific genes and/or by the presence of natural gut microbiota [34,35]. The increase of microbiota density triggers the host innate immune responses to control the bacterial load, as the density can reach 10^7 colony forming units/milliliter before returning to pre-blood meal level 3–5 days later [36,37].

In fact, it is now a well-accepted notion that the naturally acquired microbial flora can modulate the mosquito's vectorial capacity by inhibiting *Plasmodium* and other human pathogens development. Through a functional genomic approach the molecular interplay between the bacteria and the development of *P. falciparum* in *An. gambiae* has been investigated [38]. Whole transcription

profiling of septic and aseptic mosquitoes identified a significant subset of immune genes upregulated by the mosquito microbiota. This subset includes several anti-*Plasmodium* factors such as cecropins, defensins and gambicin. Aseptic mosquitoes showed an increased susceptibility to *Plasmodium* infection while mosquitoes fed with bacteria and *P. falciparum* gametocytes showed low infection levels. This study suggests that the anti-*Plasmodium* effect induced by the indigenous bacteria is mediated by the mosquitoes' antimicrobial immune responses, probably occurring through the microbiota modulation of immune genes, some with anti-*Plasmodium* activity.

The theory of pattern-recognition by Charles Janeway suggests that the host innate immune system relies on molecular pattern cues such as lipopolysaccharides, peptidoglycan, teichoic acids of Gram-negative and Gram-positive bacteria, viral double-stranded RNA from viruses, and mannans present in yeast cell walls for mounting a response [39]. Recognitions of microbial molecules triggers various immune responses: (1) killing of microbes by hemocytes (insect blood cells) that engulf (phagocytosis) or surround (encapsulation) the pathogen, (2) activation of a serine protease cascade to activate melanization (humoral melanotic encapsulation), or (3) production of antimicrobial peptides and other effector genes [40]. The ability to mount an adapted immune response relies on the existence of various insect pattern recognition receptors (PRRs) capable of recognizing different microorganisms-associated molecular patterns (MAMPs) [41,40]. Phagocytosis involves the engulfment of bacteria and other small pathogen particles by cells. The humoral response with the production of antimicrobial peptides such as two identified defensins and cecropins found in *An. gambiae*, operates against bacteria. The expression of Defensin that encodes peptides with activities against Gram-positive bacteria is enhanced in the midgut of *An. gambiae* during *Plasmodium* infection [39]. Recently, an increasing number of immune genes triggered by native microbiota and affecting the mosquito vector competence have been described in mosquitoes. One example is the transmembrane peptidoglycan (PGN) Recognition Protein LC (PGRP-LC). This is a pattern-recognition receptor activating the Imd signaling pathway, particularly well described in *D. melanogaster*, which is activated upon recognition of peptidoglycan from bacteria. Infection of Gram-positive bacterium *Staphylococcus aureus* in *An. gambiae* activates PGRP-LC signal pathway resulting in the transcription of antimicrobial peptides. The PGRP-LC signaling also controls the density of mosquito symbiotic bacteria populations within the gut and their proliferation after a blood-meal. This defensive response also modulates mosquito resistance to *Plasmodium* [42].

Current research indicates the similarities between the immune response to symbiotic microorganisms and the parallel responses with the presence of *Plasmodium* parasites. In fact, bacterial symbionts in the midgut may also provide the opportunity to express *in situ* anti-pathogen molecules for mosquito-borne disease control purposes [43]. Mosquitoes co-fed with live or heat-inactivated bacteria with *P. falciparum*, resulted in a decrease of oocyst prevalence after 8 days of incubation [36,39]. In another experiment, mosquitoes with reduced bacterial load owing to antibiotic ingestion, displayed a higher prevalence of *P. falciparum* [36,44].

Recent identification of new mosquito bacterial symbionts with potential in vector borne diseases control

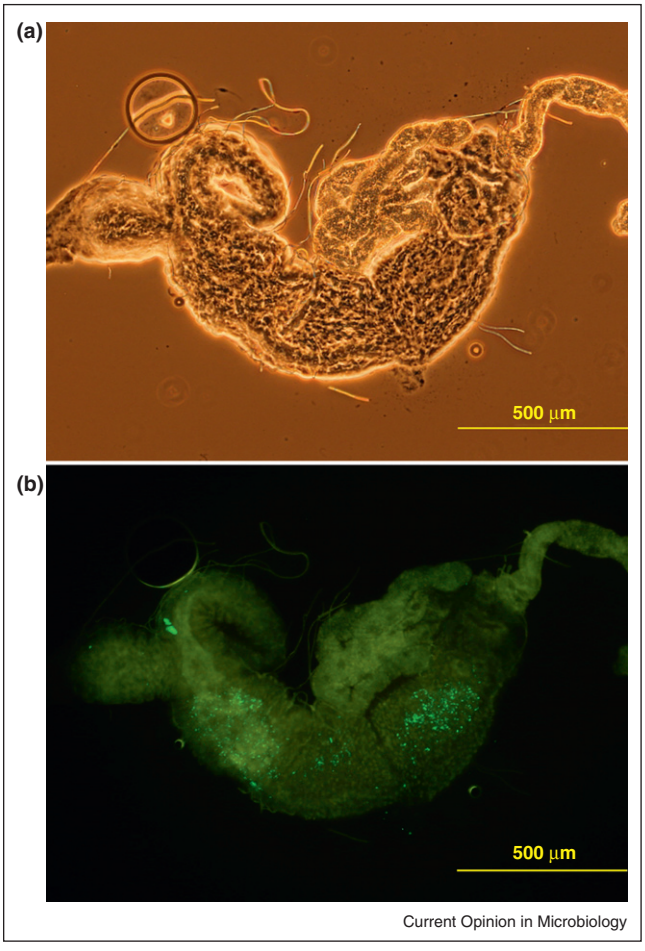
Over the past five years, the search for symbionts has been characterized by an extraordinary acceleration, which led to the identification of new bacterial species associated with midgut mosquitoes. This is the case of the Gram-positive bacterium named *Janibacter anophelis* and the Gram-negative named *Thorsellia anopheles*, both found associated with the African malaria vector *An. arabiensis* [45]. Two recently described species associated with the midgut of *An. gambiae*, are *Pantoea stewartii* and *Elizabethkingia meningoseptica* [46]. A new isolate from *An. gambiae* has also been described and named *Elizabethkingia anophelis* [47].

We recently identified a Gram-negative, α-proteobacteria belonging to the genus *Asaia* in some mosquito species including several malaria vectors, such as *An. gambiae*, *An. stephensi*, *An. maculipennis* [48,49,50] and the dengue virus vector, *Ae. aegypti* [51]. *Asaia* is the most prevalent bacteria in both natural and lab mosquito populations and its prevalence often reaches 100% [50]. Furthermore, *Asaia* was found in high numbers in the midgut, salivary glands, and reproductive organs [48]. Thus, *Asaia* could be used to express anti-*Plasmodium* molecules and may exert an additional inhibitory effect against pathogens in the salivary glands where the *Plasmodium* parasites must conclude their lifecycle within the mosquito host. Its presence in the reproductive organs permits a vertical transmission route that occurs both maternally and paternally [48,52], thus providing the basis for the introduction of engineered bacteria into mosquito populations in the field.

Experiments performed with engineered bacteria expressing fluorescent proteins has provided a proof of principle to the feasibility of using *Asaia* to express anti-parasite effectors to control malaria and others mosquito-borne diseases (Figure 1) [48,50].

Owing to its potential in vector control, this bacterium has been the focus of many studies, yet little is known about its impact on the biology of the host. Recently, we described a specific role of *Asaia* in mosquito larval development, as the presence of *Asaia* reduced the time

Figure 1



Colonization of a female gut of *Ae. aegypti* by *Gfp-Asaia*. Phase contrast **(a)** and fluorescence microscope image **(b)**.

required from the larvae to develop in pupae [53], which may provide a benefit to mosquito fitness by minimizing the action of predators. Interestingly, two commensal bacteria of Drosophila, *Acetobacter pomorum* and *Lactobacillus plantarum* promote the growth of its insect host upon nutrient scarcity [54,55].

Yeast symbionts and mosquitoes: a new field of investigation

Most, if not all studies about mosquito symbiotic relationships, have focused on bacteria. In the last few years, attention has also been focused on other symbiotic organisms like yeast. Yeasts have often been isolated from a wide variety of insect species and frequently detected in the insect gut along with other organs and tissues.

Recently, the occurrence of *Candida* and *Pichia* yeasts has been recorded in *Aedes* mosquitoes [30,56,57]. A study confirmed the presence of *Candida* sp. and *Pichia* sp., in mosquito, two fungal species that are found in association with other insects. In addition, two additional species, *Hanseniospora uvarum* and *Wickerhamomyces anomalus*, were also detected [58•]. *H. uvarum* is found in many insects while *W. anomalus* has a more specific range of host. *W. anomalus* was detected at all the developmental stages of both malaria (*An. stephensi*, *An. gambiae*) and dengue mosquito vectors (*Ae. aegypti*, *Ae. albopictus*) where it localizes in the midgut and reproductive organs [58•] possibly reflecting specific functions. The gut and gonads may provide nutrients for yeasts, thus representing optimal niches for symbionts. Furthermore, it is possible that *W. anomalus* also exerts a protective role against pathogens in mosquito as already proposed for other hosts [59,60].

The finding that *W. anomalus* associates with some mosquito vectors of several human parasites led to the proposition to use *W. anomalus* to control mosquito-borne diseases [61]. The potential to use this yeast to express anti-pathogen effector molecules is a notion that should be further explored. Additionally, this yeast has already received a Qualified Presumption of Safety status (*European Food Safety Authority*) with benefits and acceptability of novel microorganisms in food.

Moreover, some strains of *W. anomalus* produce killer toxins with antimicrobial activities against several human pathogens [62,63], including arthropod-transmitted protozoan parasites such as *Leishmania* spp. [64]. Preliminary observations indicate that the yeast strains isolated from a mosquito also produce killer toxins (I Ricci *et al.*, unpublished), thus suggesting the possible use of *W. anomalus* in the control of mosquito-borne diseases.

Conclusions

The field of mosquito–microbe interactions is rapidly expanding, providing an increasing amount of information on the contribution of microbes to mosquito nutrition, development, and defenses.

In fact, many reports clearly pinpoint the involvement of several bacterial strains in blood meal digestion. For example, oral administration of antibiotics to *Ae. aegypti* mosquitoes affected red blood cell lysis, delaying protein digestion and depriving the mosquito of essential nutrients [65]. In addition, antibiotic treated mosquitoes also showed reduction in the production of viable eggs suggesting an effect of bacteria on oocyte maturation. These results indicate the synergistic action of the mosquito gut and its midgut bacteria in blood meal digestion.

Furthermore, commensal bacteria also interfere with parasites either directly or indirectly within the mosquito vector. Indirect effect is mediated by the mosquitoes' antimicrobial immune responses, probably through the activation of basal immunity [40].

The study of mosquito–microbiota interactions has raised more interest in the possibility of genetically transforming mosquito symbionts to express anti-parasite effector molecules to develop effective disease-control strategies. One example of this strategy involves the symbiont bacterium *Rhodococcus rhodnii*, which is naturally resident in the gut lumen of the triatomine vector *Rhodnius prolixus* (Hemiptera: Reduviidae) contributing to vector nutrition. These symbionts have been transformed with some anti-parasite effector genes. Interestingly, laboratory para-transgenic populations of triatomine unable to transmit the diseases have been generated for some years [66].

An approach aimed at introducing the genetically modified bacterial symbionts into natural populations of Chagas disease vectors has already been developed by the coprophagic behaviour [66]. A number of relevant interactions between symbionts and mosquito have already been described and quite a few symbionts have been identified as potentially effective for *Symbiotic Control Strategies* to combat mosquito-borne diseases. In this context *Asaia* and *Pantoea* bacteria are potentially very useful.

Recently, *Pantoea agglomerans*, another bacterial symbiont of *Anopheles* mosquitoes has been engineered to express and secrete anti-*Plasmodium* effector proteins, such as pelB or hlyA, from related species [67]. These strains are now under evaluation for anti-*Plasmodium* activity in infected mosquitoes.

Furthermore, several microorganisms and microbial products, which do not derive from symbionts relationships, may also be applied for mosquito control. This is the case of some isolates of *Bacillus thuringiensis* and *Serratia* species that exhibit lethal activity against early fourth instar larvae of *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus* [68]. The applications of non-symbiotic microorganisms to fight mosquito-borne diseases are not restricted to bacteria only. Recently, applications based on genetically modified organisms to express anti-pathogens effectors within the mosquito body, have been developed using densonucleosis viruses [69] and the ascomycetes *Metarhizium anisopliae* [70••]. While further studies will be required to better exploit mosquito-symbiotic relationships for the purpose of reducing disease transmission, the evidence provided so far suggests the feasibility of Symbiotic Control applications to reduce the dissemination of mosquito-transmitted pathogenic microbes.

Acknowledgements

We thank Clare Whitmell for the revision of English language. This work was supported by Firb-Ideas (grant RBID082MLZ) and Prin 2009 (grant 2009L27YC8_003), both from the Italian Ministry of Education, University and Research (MIUR) and by the EU-FP7 Capacities-Infrastructure 2008 (grant 228421) to G.F.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Feldhaar H, Gross R: **Immune reactions of insects on bacterial pathogens and mutualists**. *Microbes Infect* 2008, **10**:1082-1088.

2. Fang J: **Ecology: a world without mosquitoes**. *Nature* 2010, **466**:432-434.

3. Tolle MA: **Mosquito-borne diseases**. *Curr Probl Pediatr Adolesc Health Care* 2009, **4**:97-140.

4. • Cirimotich CM, Dong Y, Clayton AM, Sandiford SL, Souza-Neto JA, Mulenga M, Dimopoulos G: **Natural microbe-mediated refractoriness to *Plasmodium* infection in *Anopheles gambiae***. *Nature* 2011, **13**:855-858.
This manuscript shows that bacterial flora in *Anopheles gambiae* mediates an anti-*Plasmodium* effect through the generation of reactive oxygen species.

5. Hertig M, Wolbach SB: **Studies on *Rickettsia*-like microorganisms in insects**. *J Med Res* 1924, **44**:329-374.

6. Werren JH: ***Wolbachia* run amok**. *Proc Natl Acad Sci USA* 1997, **94**:11154-11155.

7. Ricci I, Cancrini G, Gabrielli S, D'Amelio S, Favia G: **Searching for *Wolbachia* (Rickettsiales: Rickettsiaceae) in mosquitoes (Diptera: Culicidae): large polymerase chain reaction survey and new identifications**. *J Med Entomol* 2002, **39**:562-567.

8. Kittayapong P, Baisley KJ, Baimai V, O'Neill SL: **Distribution and diversity of *Wolbachia* infections in Southeast Asian mosquitoes (Diptera: Culicidae)**. *J Med Entomol* 2000, **37**:340-345.

9. Dean JL, Dobson SL: **Characterization of *Wolbachia* infections and interspecific crosses of *Aedes* (Stegomyia) polynesiensis and *Ae.* (Stegomyia) riversi (Diptera: Culicidae)**. *J Med Entomol* 2004, **41**:894-900.

10. Tsai KH, Huang CG, Wu WJ, Chuang CK, Lin CC, Chen WJ: **Parallel infection of Japanese encephalitis virus and *Wolbachia* within cells of mosquito salivary glands**. *J Med Entomol* 2006, **43**:752-756.

11. The malERA Consultative Group on Vector Control: **A research agenda for malaria eradication: vector control**. *PLoS Med* 2011, **8**:e1000401.

12. Service MW: **Importance of ecology in *Aedes aegypti* control**. *Southeast Asian J Trop Med Public Health* 1992, **23**:681-690.

13. Yen JH, Barr AR: **New hypothesis of the cause of cytoplasmic incompatibility in *Culex pipiens L***. *Nature* 1971, **232**:657-658.

14. Stouthamer R, Breeuwer JA, Hurst GD: ***Wolbachia pipientis*: microbial manipulator of arthropod reproduction**. *Annu Rev Microbiol* 1999, **53**:71-102.

15. Rousset F, Bouchon D, Pintureau B, Juchault P, Solignac M: ***Wolbachia* endosymbionts responsible for various alterations of sexuality in arthropods**. *Proc Biol Sci* 1992, **250**:91-98.

16. Hurst GD, Johnson AP, Schulenburg JH, Fuyama Y: **Male-killing *Wolbachia* in *Drosophila*: a temperature-sensitive trait with a threshold bacterial density**. *Genetics* 2000, **156**:699-709.

17. Sinkins SP: ***Wolbachia* and cytoplasmic incompatibility in mosquitoes**. *Insect Biochem Mol Biol* 2004, **34**:723-729.

18. • McMeniman CJ, Lane RV, Cass BN, Fong AW, Sidhu M, Wang YF, O'Neill SL: **Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti***. *Science* 2009, **323**:141-144.
The successful transfer of a life-shortening strain of the inherited bacterial symbiont, *Wolbachia*, into the major mosquito vector of dengue, *Aedes aegypti*, halved adult life span under laboratory conditions, thus suggesting that targeting mosquito age with inherited *Wolbachia* infections may be a viable strategy to reduce the transmission of pathogens such as dengue viruses.

19. Kambris Z, Cook PE, Phuc HK, Sinkins SP: **Immune activation by life-shortening *Wolbachia* and reduced filarial competence in mosquitoes**. *Science* 2009, **326**:134-136.
• The *Wolbachia* strain named wMelPop induces upregulation of the mosquito's innate immune system and its presence inhibits the development of filarial nematodes in the mosquito. wMelPop could be used in the global effort to eliminate lymphatic filariasis and possibly for the control of other mosquito-borne parasites where immune pre-activation inhibits their development.

20. Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu G, Pyke AT, Hedges LM, Rocha BC, Hall-Mendelin S, Day A, Riegler M *et al.*: **A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, *Chikungunya*, and *Plasmodium***. *Cell Dec* 2009, **139**:1268-1278.

21. Kambris Z, Blagborough AM, Pinto SB, Blagrove MS, Godfray HC, Sinden RE, Sinkins SP: ***Wolbachia* stimulates immune gene expression and inhibits *Plasmodium* development in *Anopheles gambiae***. *PLoS Pathog* 2010, **6**:e1001143.

22. Hughes GL, Vega-Rodriguez J, Xue P, Rasgon JL: ***Wolbachia* strain wAlbB enhances infection by the rodent malaria parasite *Plasmodium berghei* in *Anopheles gambiae* mosquitoes**. *Appl Environ Microbiol* 2011, **78**:1491-1495.

23. Hoffmann AA, Montgomery BL, Popovici J, Iturbe-Ormaetxe I, Johnson PH, Muzzi F, Greenfield M, Durkan M, Leong YS, Dong Y *et al.*: **Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission**. *Nature* 2011, **474**:454-457.
•• The *Wolbachia* strain named wMel infection, when introduced into the dengue vector *Aedes aegypti* from *Drosophila melanogaster*, successfully invade two natural *A. aegypti* populations in Australia, reaching near-fixation in a few months following releases of wMel-infected *A. aegypti* adults. This study shows that *Wolbachia*-based strategies can be a feasible approach to dengue suppression with potential for area-wide implementation.

24. Rasgon JL: **Dengue fever: mosquitoes attacked from within**. *Nature* 2011, **476**:407-408.

25. Pidiyar V, Kaznowski A, Narayan NB, Patole M, Shouche YS: ***Aeromonas culicicola* sp. nov., from the midgut of *Culex quinquefasciatus***. *Int J Syst Evol Microbiol* 2002, **52**:1723-1728.

26. Pidiyar VJ, Jangid K, Patole MS, Shouche YS: **Studies on cultured and uncultured microbiota of wild *Culex quinquefasciatus* mosquito midgut based on 16s ribosomal RNA gene analysis**. *Am J Trop Med Hyg* 2004, **70**:597-603.

27. Touré AM, Mackey AJ, Wang ZX, Beier JC: **Bactericidal effects of sugar-fed antibiotics on resident midgut bacteria of newly emerged anopheline mosquitoes (Diptera: Culicidae)**. *J Med Entomol* 2000, **37**:246-249.

28. Demaio J, Pumpuni CB, Kent M, Beier JC: **The midgut bacterial flora of wild *Aedes triseriatus*, *Culex pipiens*, and *Psorophora columbiae* mosquitoes**. *Am J Trop Med Hyg* 1996, **54**:219-223.

29. Douglas AE, Smith DC: **Are endosymbioses mutualistic?** *Trends Ecol Evol* 1989, **4**:350-352.

30. Gusmão DS, Santos AV, Marini DC, Bacci M Jr, Berbert-Molina MA, Lemos FJ: **Culture-dependent and culture-independent characterization of microorganisms associated with *Aedes aegypti* (Diptera: Culicidae) (L.) and dynamics of bacterial colonization in the midgut**. *Acta Trop* 2010, **115**:275-281.

31. Wang Y, Gilbreath TM 3rd, Kukutla P, Yan G, Xu J: **Dynamic gut microbiome across life history of the malaria mosquito *Anopheles gambiae* in Kenya**. *PLoS ONE* 2011, **6**:e24767.

32. Fouda MA, Hassan MI, Al-Daly AG, Hammad KM: **Effect of midgut bacteria of *Culex pipiens* L. on digestion and reproduction**. *J Egypt Soc Parasitol* 2001, **31**:767-780.

33. Gonzalez-Ceron L, Santillan F, Rodriguez MH, Mendez D, Hernandez-Avila JE: **Bacteria in midguts of field-collected *Anopheles albimanus* block *Plasmodium vivax* sporogonic development**. *J Med Entomol* 2003, **40**:371-374.

34. Xi Z, Ramirez JL, Dimopoulos G: **The *Aedes aegypti* toll pathway controls dengue virus infection**. *PLoS Pathog* 2008, **4**:e1000098.

35. Weiss B, Aksoy S: **Microbiome influences on insect host vector competence**. *Trends Parasitol* 2011, **27**:514-522.

36. Cirimotich CM, Dong Y, Garver LS, Sim S, Dimopoulos G: **Mosquito immune defenses against *Plasmodium* infection**. *Dev Comp Immunol* 2010, **34**:387-395.

37. Pumpuni CB, Demaio J, Kent M, Davis JR, Beier JC: **Bacterial population dynamics in three anopheline species: the impact on *Plasmodium* sporogonic development**. *Am J Trop Med Hyg* 1996, **54**:214-218.

38. Dong Y, Manfredini F, Dimopoulos G: **Implication of the mosquito midgut microbiota in the defense against malaria parasites**. *PLoS Pathog* 2009, **5**:e1000423.

39. Marquardt WC, Kondratieff BC: *Biology of Disease Vectors*. edn 2. Elsevier Academic Press; 2005.

40. Dimopoulos G: **Insect immunity and its implication in mosquito-malaria interactions**. *Cell Microbiol* 2003, **5**:3-14.

41. Gross TL, Myles KM, Adelman ZN: **Identification and characterization of heat shock 70 genes in *Aedes aegypti* (Diptera: Culicidae)**. *J Med Entomol* 2009, **46**:496-504.

42. Meister S, Agianian B, Turlure F, Relógio A, Morlais I, Kafatos FC, Christophides GK: ***Anopheles gambiae* PGRPLC-mediated defense against bacteria modulates infections with malaria parasites**. *PLoS Pathog* 2009, **8**:e1000542.

43. Riehle MA, Jacobs-Lorena M: **Using bacteria to express and display anti-parasite molecules in mosquitoes: current and future strategies**. *Insect Biochem Mol Biol* 2005, **35**:699-707.

44. Beier MS, Pumpuni CB, Beier JC, Davis JR: **Effects of para-aminobenzoic acid, insulin, and gentamicin on *Plasmodium falciparum* development in anopheline mosquitoes (Diptera: Culicidae)**. *J Med Entomol* 1994, **31**:561-565.

45. Kämpfer P, Terenius O, Lindh JM, Faye I: ***Janibacter anophelis* sp. nov., isolated from the midgut of *Anopheles arabiensis***. *Int J Syst Evol Microbiol* 2006, **56**:389-392.

46. Lindh JM, Borg-Karlson AK, Faye I: **Trans stadial and horizontal transfer of bacteria within a colony of *Anopheles gambiae* (Diptera: Culicidae) and oviposition response to bacteria-containing water**. *Acta Trop* 2008, **107**:242-250.

47. Kämpfer P, Matthews H, Glaeser SP, Martin K, Lodders N, Faye I: ***Elizabethkingia anophelis* sp. nov., isolated from the midgut of the mosquito *Anopheles gambiae***. *Int J Syst Evol Microbiol* 2011, **61**:2670-2675.

48. Favia G, Ricci I, Damiani C, Raddadi N, Crotti E, Marzorati M, Rizzi A, Urso R, Brusetti L, Borin S *et al.*: **Bacteria of the genus *Asaia* stably associate with *Anopheles stephensi*, an Asian malarial mosquito vector**. *Proc Natl Acad Sci USA* 2007, **104**:9047-9051.

49. Favia G, Ricci I, Marzorati M, Negri I, Alma A, Sacchi L, Bandi C, Daffonchio D: **Bacteria of the genus *Asaia*: a potential paratransgenic weapon against malaria**. *Adv Exp Med Biol* 2008, **627**:49-59.

50. Damiani C, Ricci I, Crotti E, Rossi P, Rizzi A, Scuppa P, Capone A, Ulissi U, Epis S, Genchi M *et al.*: **Mosquito-bacteria symbiosis: the case of *Anopheles gambiae* and *Asaia***. *Microb Ecol* 2010, **60**:644-654.

51. Crotti E, Damiani C, Pajoro M, Gonella E, Rizzi A, Ricci I, Negri I, Scuppa P, Rossi P, Ballarini P *et al.*: ***Asaia*, a versatile acetic acid bacterial symbiont, capable of cross-colonizing insects of phylogenetically distant genera and orders**. *Environ Microbiol* 2009, **11**:3252-3264.

52. Damiani C, Ricci I, Crotti E, Rossi P, Rizzi A, Scuppa P, Esposito F, Bandi C, Daffonchio D, Favia G: **Paternal transmission of symbiotic bacteria in malaria vectors**. *Curr Biol* 2008, **18**:1087-1088.

53. Chouaia B, Rossi P, Epis S, Mosca M, Ricci I, Damiani C, Ulissi U, Crotti E, Daffonchio D, Bandi C, Favia G: **Delayed larval development in *Anopheles* mosquitoes deprived of *Asaia* bacterial symbionts**. *BMC Microbiol* 2012, **12** Suppl 1:S2 [Epub ahead of print].

54. Shin SC, Kim SH, You H, Kim B, Kim AC, Lee KA, Yoon JH, Ryu JH, Lee WJ: ***Drosophila*** **microbiome modulates host developmental and metabolic homeostasis via insulin signaling**. *Science* 2011, **334**:670-674.

55. Storelli G, Defaye A, Erkosar B, Hols P, Royet J, Leulier F: ***Lactobacillus plantarum*** **promotes** ***Drosophila*** **systemic growth by modulating hormonal signals through TOR-dependent nutrient sensing**. *Cell Metab* 2011, **14**:403-414.

56. Gusmão DS, Santos AV, Marini DC, Russo Ede S, Peixoto AM, Bacci Júnior M, Berbert-Molina MA, Lemos FJ: **First isolation of microorganisms from the gut diverticulum of** ***Aedes aegypti*** **(Diptera: Culicidae): new perspectives for an insect-bacteria association**. *Mem Inst Oswaldo Cruz* 2007, **102**:919-924.

57. Ignatova EA, Nagornaia SS, Povazhnaia TN, Ianishevskaia GS: **The yeast flora of blood-sucking mosquitoes**. *Mikrobiol Z* 1996, **58**:12-15.

58. (a).
• Ricci I, Mosca M, Valzano M, Damiani C, Scuppa P, Rossi P, Crotti E, Cappelli A, Ulissi U, Capone A *et al.*: **Different mosquito species host** ***Wickerhamomyces anomalus*** **(*Pichia anomala*): perspectives on vector-borne diseases symbiotic control**. *Antonie Van Leeuwenhoek* 2011, **99**:43-50;
(b).
Ricci I, Damiani C, Scuppa P, Mosca M, Crotti E, Rossi P, Rizzi A, Capone A, Gonella E, Ballarini P *et al.*: **The yeast** ***Wickerhamomyces anomalus*** **(*Pichia anomala*) inhabits the midgut and reproductive system of the Asian malaria vector** ***Anopheles stephensi***. *Environ Microbiol* 2011, **13**:911-921.
First description of a yeast symbiont inhabiting midgut and reproductive organs of malaria vectors.

59. Wang X, Chi Z, Yue L, Li J, Li M, Wu L: **A marine killer yeast against the pathogenic yeast strain in crab (*Portunus trituberculatus*) and an optimization of the toxin production**. *Microbiol Res* 2007, **62**:77-85.

60. Wang L, Yue L, Chi Z, Wang X: **Marine killer yeasts active against a yeast strain pathogenic to crab** ***Portunus trituberculatus***. *Dis Aquat Organ* 2008, **80**:211-218.

61. Fredlund E, Druvefors U, Boysen ME, Lingsten KJ, Schnürer J: **Physiological characteristics of the biocontrol yeast** ***Pichia anomala*** **J121**. *FEMS Yeast Res* 2002, **2**:395-402.

62. Séguy N, Polonelli L, Dei-Cas E, Cailliez JC: **Effect of a killer toxin of** ***Pichia anomala*** **to** ***Pneumocystis*****. Perspectives in the control of pneumocystosis**. *FEMS Immunol Med Microbiol* 1998, **22**:145-149.

63. Magliani W, Conti S, Arseni S, Frazzi R, Salati A, Polonelli L: **Killer anti-idiotypes in the control of fungal infections**. *Curr Opin Investig Drugs* 2001, **2**:477-479.

64. Savoia D, Avanzini C, Conti S, Magliani V, Frazzi R, Polonelli L: **In vitro leishmanicidal activity of a monoclonal antibody mimicking a yeast killer toxin**. *J Eukaryot Microbiol* 2002, **49**:319-323.

65. Gaio Ade O, Gusmão DS, Santos AV, Berbert-Molina MA, Pimenta PF, Lemos FJ: **Contribution of midgut bacteria to blood digestion and egg production in** ***Aedes aegypti*** **(Diptera: Culicidae) (L.)**. *Parasit Vectors* 2011, **4**:105.

66. Beard CB, Dotson EM, Pennington PM, Eichler S, Cordon-Rosales C, Durvasula RV: **Bacterial symbiosis and paratransgenic control of vector-borne Chagas disease**. *Int J Parasitol* 2001, **31**:621-627.

67. Bisi DC, Lampe DJ: **Secretion of anti-*Plasmodium* effector proteins from a natural** ***Pantoea agglomerans*** **isolate by using PelB and HlyA secretion signals**. *Appl Environ Microbiol* 2011, **77**:4669-4675.

68. Patil CD, Patil SV, Salunke BK, Salunkhe RB: **Insecticidal potency of bacterial species** ***Bacillus thuringiensis*** **SV2 and** ***Serratia nematodiphila*** **SV6 against larvae of mosquito species** ***Aedes aegypti*****,** ***Anopheles stephensi*****, and** ***Culex quinquefasciatus***. Parasitol Res 2011, in press. [Epub ahead of print]

69. Ren X, Hoiczyk E, Rasgon JL: **Viral paratransgenesis in the malaria vector** ***Anopheles gambiae***. *PLoS Pathog* 2008, **4**:e1000135.

70. Fang W, Vega-Rodríguez J, Ghosh AK, Jacobs-Lorena M, Kang A,
•• St Leger RJ: **Development of transgenic fungi that kill human malaria parasites in mosquitoes**. *Science* 2011, **331**:1074-1077.
By using recombinant strains of the Ascomycete, *Metarhizium anisopliae*, expressing molecules that target sporozoite stage of the malarial parasite, the authors showed reduced sporozoite counts. *Metarhizium*-mediated inhibition of *Plasmodium* development could be a powerful weapon for combating malaria.