



# Microbial symbiosys in mosquito vectors

Guido Favia  
School of Biosciences and Veterinary Medicine  
[guido.favia@unicam.it](mailto:guido.favia@unicam.it)



# Syllabus

- ✓ **Microbiota:** the microorganisms permanently resident in a particular site / habitat
- ✓ **Microbiome:** are the collective genomes of microorganisms residing in an environmental niche
- ✓ **Hologenome:** The hologenome theory is a postulate that states that the object of natural genomic selection is not a single organism, but the organism and its microbial communities

# The concept of hologenome



- ✓ **Hologenome:** This hypothesis proposes that a dynamic relationship exists between organisms (hosts) and their symbiotic microbial communities. By changing its composition, this "**holobiont**" can adapt to changing environmental conditions much more rapidly than with genetic mutation and selection alone.

# Insect symbiosis



REVIEW ARTICLE

## **The gut microbiota of insects – diversity in structure and function**

Philipp Engel & Nancy A. Moran

Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT, USA

Review



Cell  
PRESS

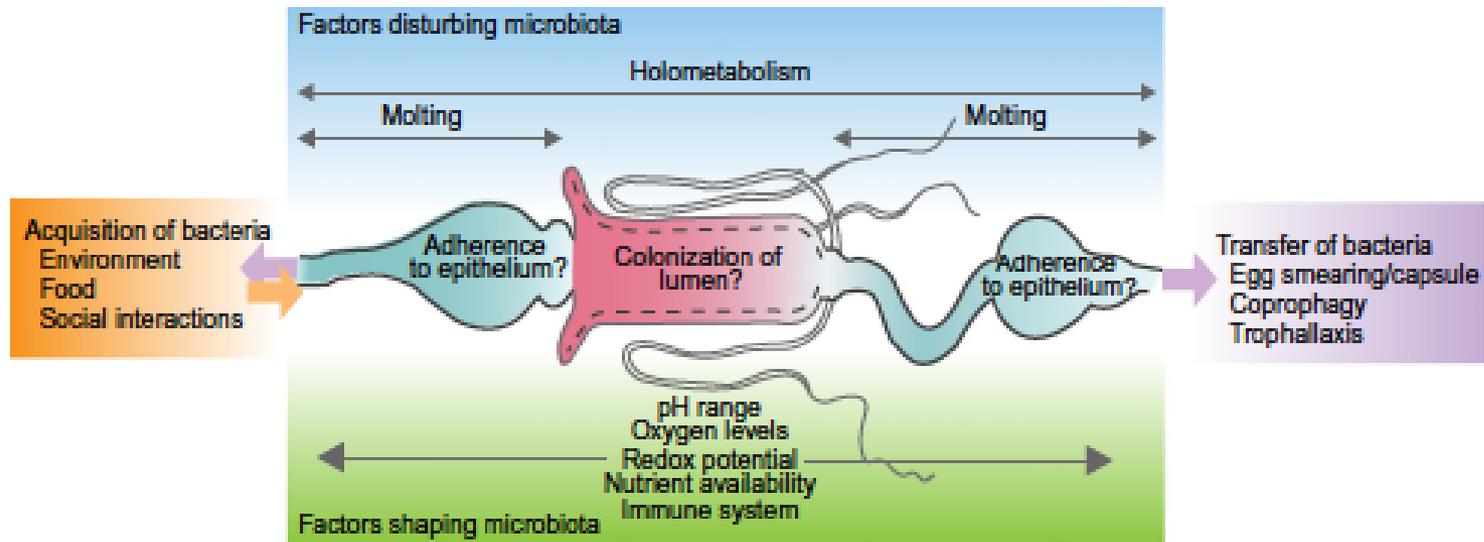
*Feature Review*

## **Microbiome influences on insect host vector competence**

Brian Weiss and Serap Aksoy

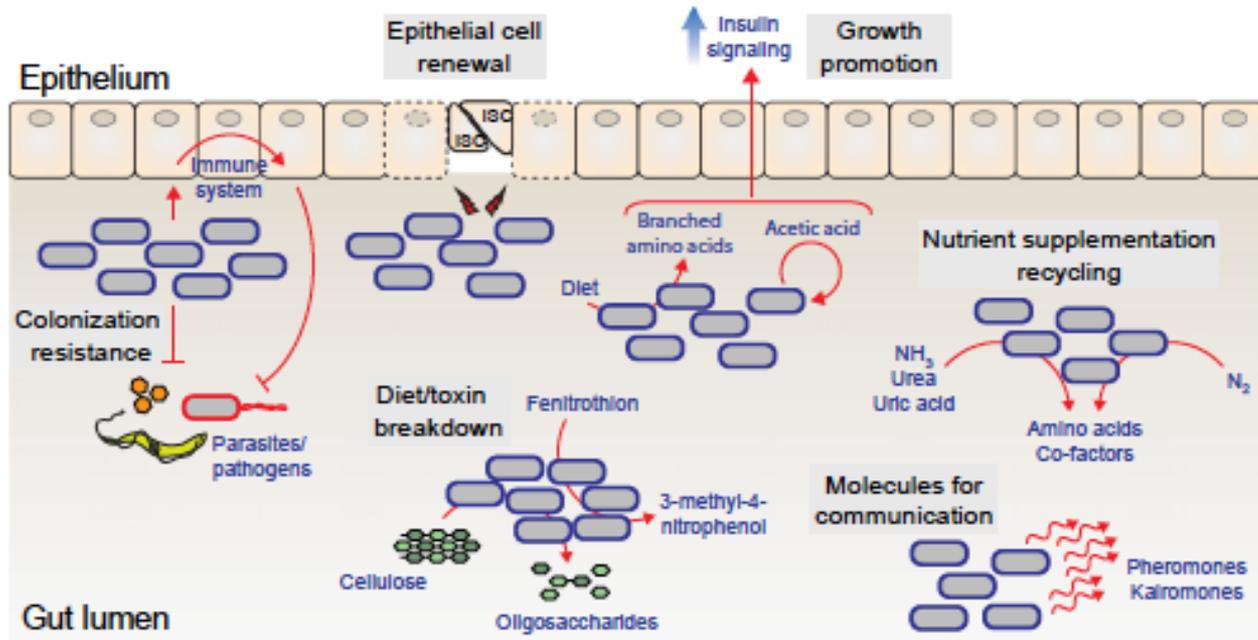
Department of Epidemiology and Public Health, Yale School of Public Health, New Haven, CT 06520, USA

# Insect symbiosis



**Fig. 2.** Factors influencing composition of the gut microbiota of insects include insect development, physiochemical conditions in different gut compartments, available sources for bacteria acquisition, and capability to transfer bacteria to progeny.

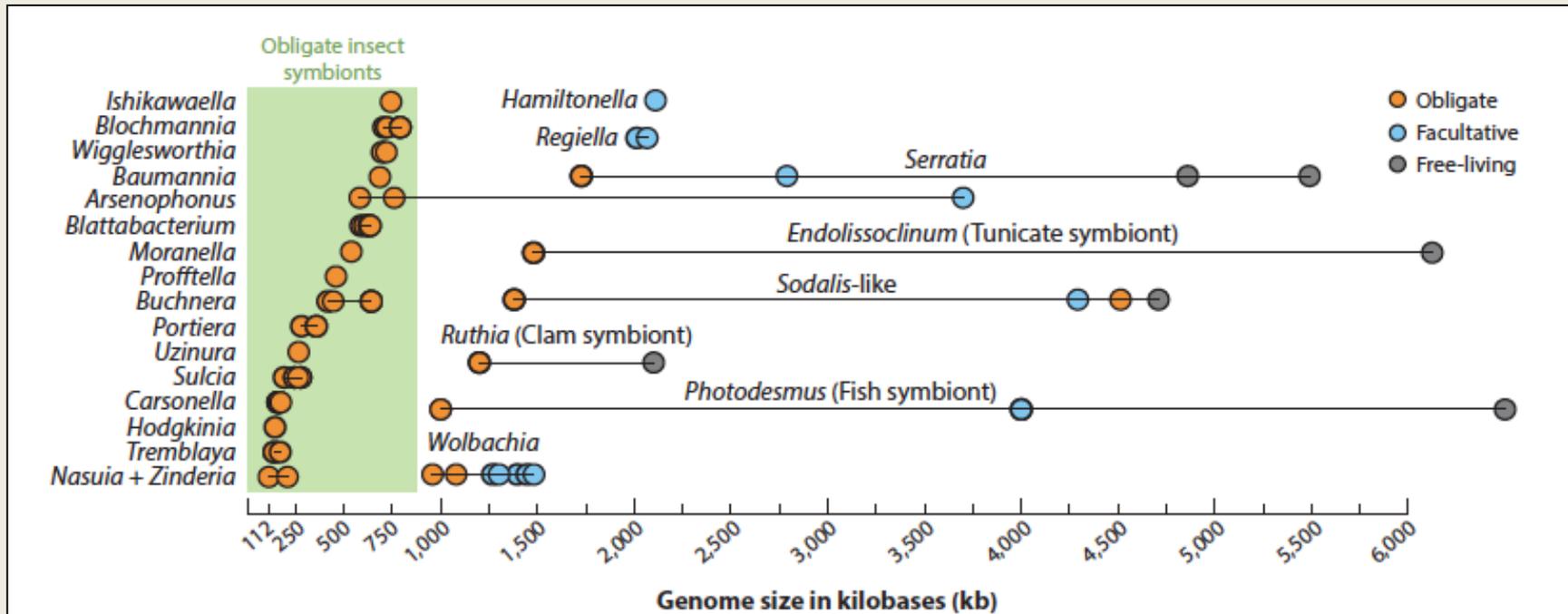
# Insect symbiosis



**Fig. 4.** Known functions of bacteria in insect guts. Colonization resistance against pathogens or parasites has been described for the bumble bee, *Bombus terrestris*, the desert locust, *Schistocerca gregaria*, and various mosquito species (Pumpuni *et al.*, 1993; Gonzalez-Ceron *et al.*, 2003; Dillon *et al.*, 2005; Cirimotich *et al.*, 2011a; Koch & Schmid-Hempel, 2011b). In *Drosophila melanogaster*, the commensal gut microbiota has been shown to be involved in intestinal cell renewal and promotion of systemic growth (Buchon *et al.*, 2009a, b; Shin *et al.*, 2011; Storelli *et al.*, 2011). A prime example for diet breakdown is the degradation of cellulose by the characteristic gut microbiota in the hindgut of termites (Warnecke *et al.*, 2007). Gut bacteria have also been shown to degrade toxins ingested with the diet (Ping *et al.*, 2007; Kikuchi *et al.*, 2012). The insecticide fenitrothion is hydrolyzed into 3-methyl-4-nitrophenol by the *Burkholderia* gut symbiont of the stinkbug *Riptortus pedestris*. Nutrient supplementation, such as the synthesis of vitamins and essential amino acids or the fixation of nitrogen, has been shown for gut symbionts of blood-feeding kissing bugs, stinkbugs, and termites, respectively (Eichler & Schaub, 2002; Hongoh *et al.*, 2008b; Nikoh *et al.*, 2011). Certain gut bacteria of termites can also recycle nitrogenous waste products excreted by the host by converting them into high-value nutrients (Hongoh *et al.*, 2008b). Similar functions might also be carried out by gut bacteria of ants and cockroaches (Russell *et al.*, 2009a; Sabree *et al.*, 2009). In a number of insects, gut bacteria produce molecules involved in intraspecific and interspecific communication, such as pheromones and kairomones (Dillon *et al.*, 2002; Sharon *et al.*, 2010; Leroy *et al.*, 2011).



# “The tiniest tiny genomes”



**Figure 3**

Genome size ranges in closely related clusters of symbiotic bacteria and relatives. Obligate symbionts are shown in orange, facultative symbionts in blue, and free-living bacteria in gray. Obligate symbionts show the tiniest genomes; those in the green box are obligate insect symbionts and include the tiniest genomes known in cellular organisms. Symbionts in younger associations with hosts are sometimes only slightly reduced in genome size.

# Aphids and Buchnera

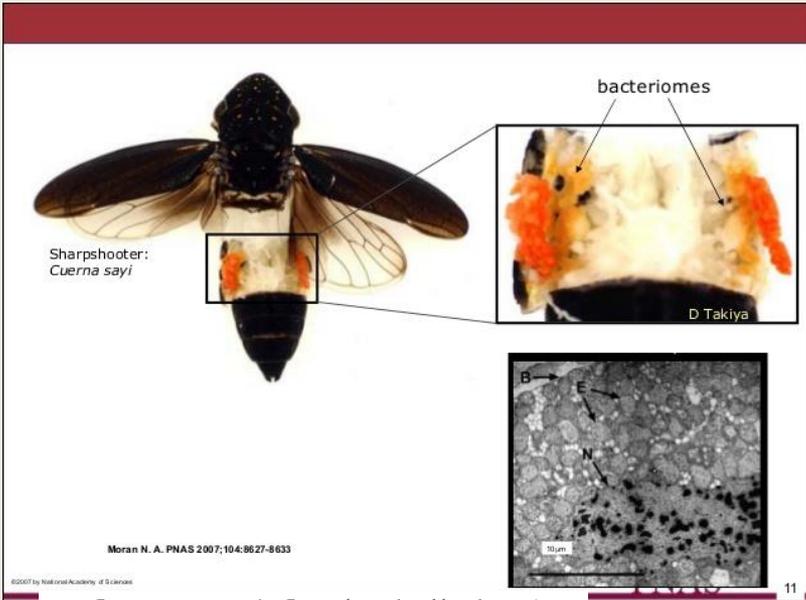


In the body of aphids, bilobed bacteriomes are present, containing 60-80 bacteriocytes.

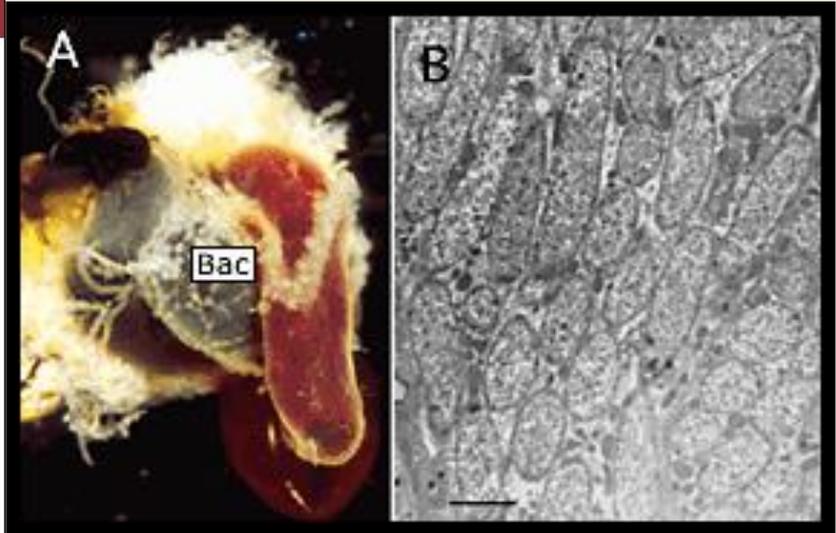
Inside the bacteriocyte there are vesicles that contain the primary Gram negative symbiont *Buchnera aphidicola*, belonging to the phylum gamma-Proteobacteria (extreme reduction of the genome: 425-500 kb).

Vertical transmission of *Buchnera* occurs through transfer into the egg (in the case of sexual reproduction) or into the embryo (in the case of reproduction by parthenogenesis).

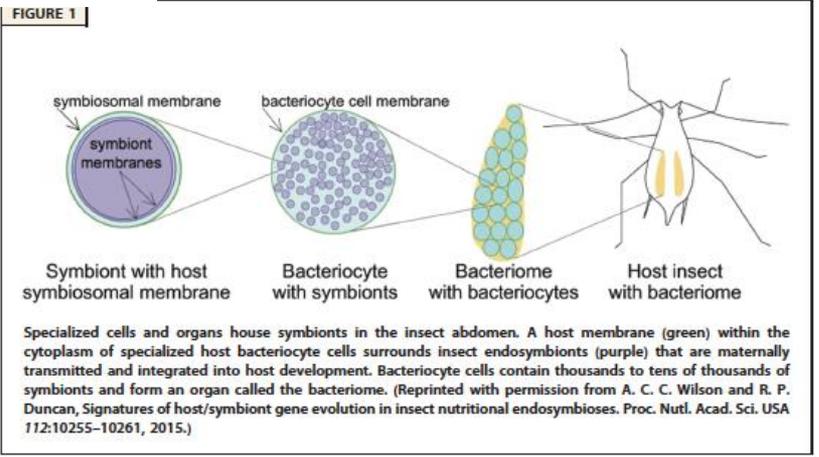
# Other examples



*Cuerna (Cicladellidae)*



- Glossina*



# Potential effects of the symbiont in the biology of the host

1. Ecology of insect nutrition
2. Defense against pathogens
3. Adaptation to different environmental conditions
4. Influence on plant-insect interactions
5. Impact on population dynamics
6. Resistance to insecticides
7. .... many others ... ..

# What kind of interactions?

## Influence of microbial symbionts on insect pheromones

Tobias Engl  and Martin Kaltenpoth \*

mate localization and mate choice as well as other social interactions in insects. A growing body of literature indicates that microbial symbionts can modulate their hosts' chemical profiles, mate choice decisions and social behavior. This modulation can occur by the direct biosynthesis of pheromone components or the provisioning of precursors, or through general changes in the metabolite pool of the host and its resource allocation into pheromone production. Here we review and discuss the contexts in which

resource allocation into pheromone production. Here we review and discuss the contexts in which microbial modulation of intraspecific communication in insects occurs and emphasize cases in which microbes are known to affect the involved chemistry. The described examples for a symbiotic influence on mate attraction and mate choice, aggregation, nestmate and kin recognition highlight the context-dependent costs and benefits of these symbiotic interactions and the potential for conflict and manipulation among the interacting partners. However, despite the increasing number of studies reporting on symbiont-mediated effects on insect chemical communication, experimentally validated connections between the presence of specific symbionts, changes in the host's chemistry, and behavioral effects thereof, remain limited to very few systems, highlighting the need for increased collaborative efforts between symbiosis researchers and chemical ecologists to gain more comprehensive insights into the influence of microbial symbionts on insect pheromones.

**Table 1** Overview of systems for which a symbiotic influence on insect chemical profiles, mate choice or social behavior has been demonstrated or suggested. For completeness, two non-insect arthropod systems are also included

Host order	Host species	Symbiont	Chemistry	Behavior	References
Blattodea	<i>Blattella germanica</i>	Gut microbiota	Carboxylic acids	Aggregation	124
Blattodea	<i>Reticulitermes speratus</i>	Microbial community		Nestmate aggression	110
Blattodea	<i>Zootermopsis nevadensis</i>	Gut microbiota	Precursors for methyl-branched CHCs		60
Coleoptera	<i>Costelytra zealandica</i>	<i>Morganella morgani</i>	Conversion of tyrosine to phenole (1)	Attraction of males	57 and 58
Coleoptera	<i>Ips typographus</i>	Grosmannia penicillata and G. europhioides	2-Methyl-3-buten-2-ol (15)	Aggregation	136
Coleoptera	<i>Oryzaephilus surinamensis</i>	Bacteroidetes bacteria	CHCs under desiccation stress		37
Diptera	<i>Bactrocera dorsalis</i>	Gut microbiota, <i>Klebsiella oxytoca</i>		Attraction of males	68
Diptera	<i>Ceratitis capitata</i>	Gut microbiota incl. <i>Klebsiella</i> sp., <i>Enterobacter</i> sp.		Mating latency of males	63 and 70
Diptera	<i>Drosophila melanogaster</i>	Gut microbiota, <i>Lactobacillus plantarum</i>	CHCs (6,7)	Sexual isolation through assortative mating of males in certain lab populations	69–84
Diptera	<i>Drosophila melanogaster</i>	<i>Wolbachia</i>		Sexual isolation through assortative mating of males	81, 85–87
Diptera	<i>Drosophila melanogaster</i>	Pathogens: <i>Pseudomonas entomophila</i> , <i>Serratia marcescens</i> , <i>Erwinia carotovora</i>	Methyl laurate, methyl myristate, methyl palmitate	Aggregation	16
Diptera	<i>Drosophila melanogaster</i> and <i>D. simulans</i>	<i>Wolbachia</i>		<i>Wolbachia</i> infected female pupae influence testes development of male pupae	88 and 89
Diptera	<i>Drosophila melanogaster</i> and <i>D. simulans</i>	<i>Wolbachia</i>		Mating rate & promiscuity	87
Diptera	<i>Drosophila paulistorum</i>	<i>Wolbachia</i>	CHCs and male anti-aphrodisiacs (8–10)	Sexual isolation through assortative mating of males	91–95
Diptera	<i>Glossina morsitans</i>	<i>Wigglesworthia glossinidia</i>	CHCs, incl. female contact pheromone (11)	Prefrential mating	104
Diptera	<i>Musca domestica</i>	Bacteria		Oviposition choice	125
Hymenoptera	<i>Pogonomyrmex barbatus</i>	Microbial community		Nestmate aggression	111
Orthoptera	<i>Schistocerca gregaria</i>	Gut microbiota incl. <i>Pantoea agglomerans</i> , <i>Klebsiella pneumoniae</i> , <i>Enterobacter cloacae</i>	Synthesis of phenol (1) and guaiacol (12)	Aggregation	120–123
Isopoda (Malacostraca)	<i>Armadillidium vulgare</i>	<i>Wolbachia</i>		Preferential mating with true <i>versus</i> feminized neo-females	97
Trombidiformes (Arachnida)	<i>Tetranychus urticae</i>	<i>Wolbachia</i>		Preferential mating with uninfected males	96

# Commensal bacteria play a role in mating preference of *Drosophila melanogaster*

Gil Sharon<sup>a</sup>, Daniel Segal<sup>a</sup>, John M. Ringo<sup>b</sup>, Abraham Hefetz<sup>c</sup>, Ilana Zilber-Rosenberg<sup>d</sup>, and Eugene Rosenberg<sup>a,1</sup>

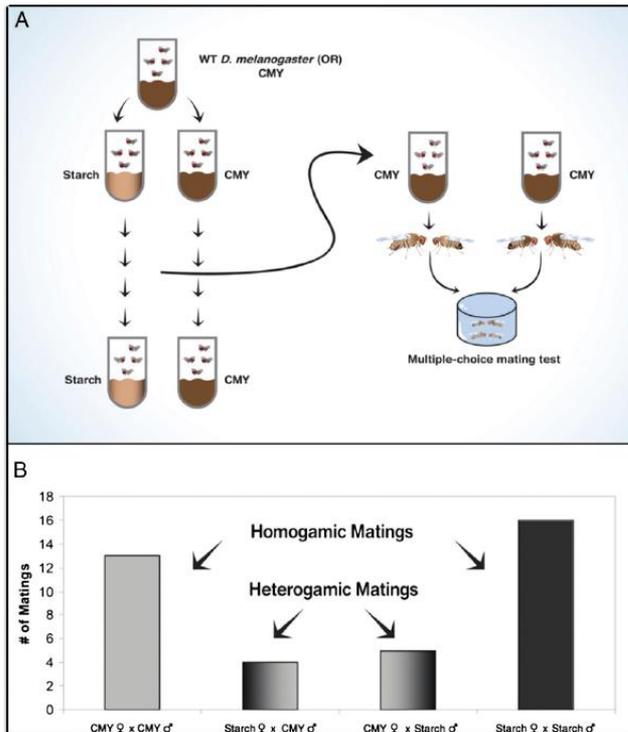


Fig. 1. (A) Schematic representation of the experimental procedure. A population of flies was divided, serially transferred in two different media, and then examined for mating preference. After rearing the flies for a number of generations on starch or CMY media, each population was grown separately for one generation on CMY medium and then tested for mating preference. The multiple-choice mating tests were performed in 24-well plastic plates; each well contained four flies: one male and one female starch-reared and one male and one female CMY-reared. The multiple-choice mating tests were performed in 24-well plastic plates; each well contained four flies: one male and one female starch-reared and one male and one female CMY-reared. (B) Mating preference tests of *D. melanogaster* after growing 11 generations on starch or CMY medium. Matings were recorded every 4 min for 1 h.

How can a bacterially induced mating preference, as described here, contribute to speciation and evolution in nature?

One possibility is that, in the natural world, multiple environmental factors act synergistically to differentiate the microbiota and strengthen the homogamic mating preference.

For example, it is reasonable to assume that fly populations living on different nutrients will be, at least to some extent, geographically separated.

The combination of partial geographic separation and diet-induced mating preference would reduce interbreeding of the populations. Slower changes in the host genome could further enhance the mating preference.

The stronger the mating preference, the greater the chance that two populations will become sexually isolated.

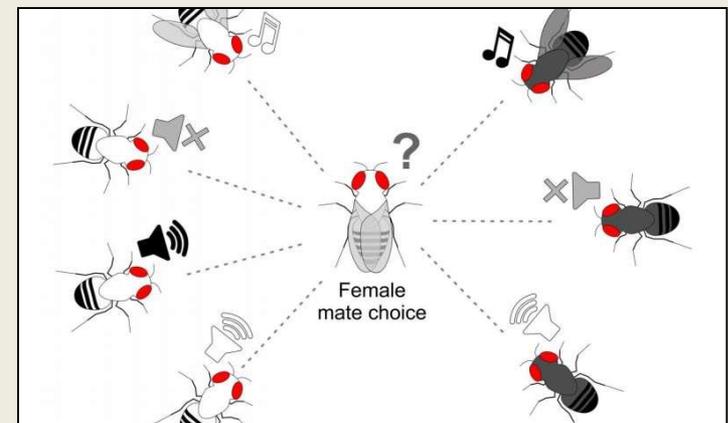
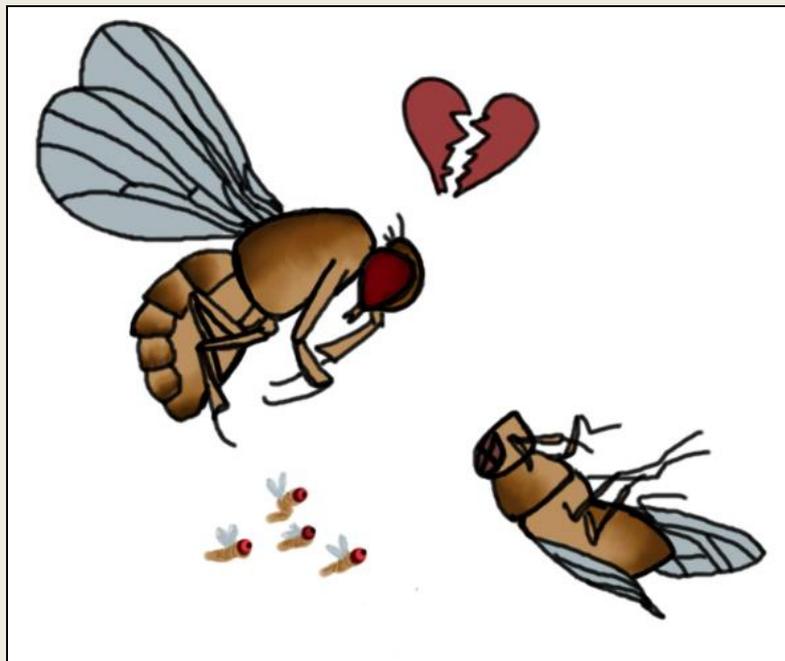
RESEARCH

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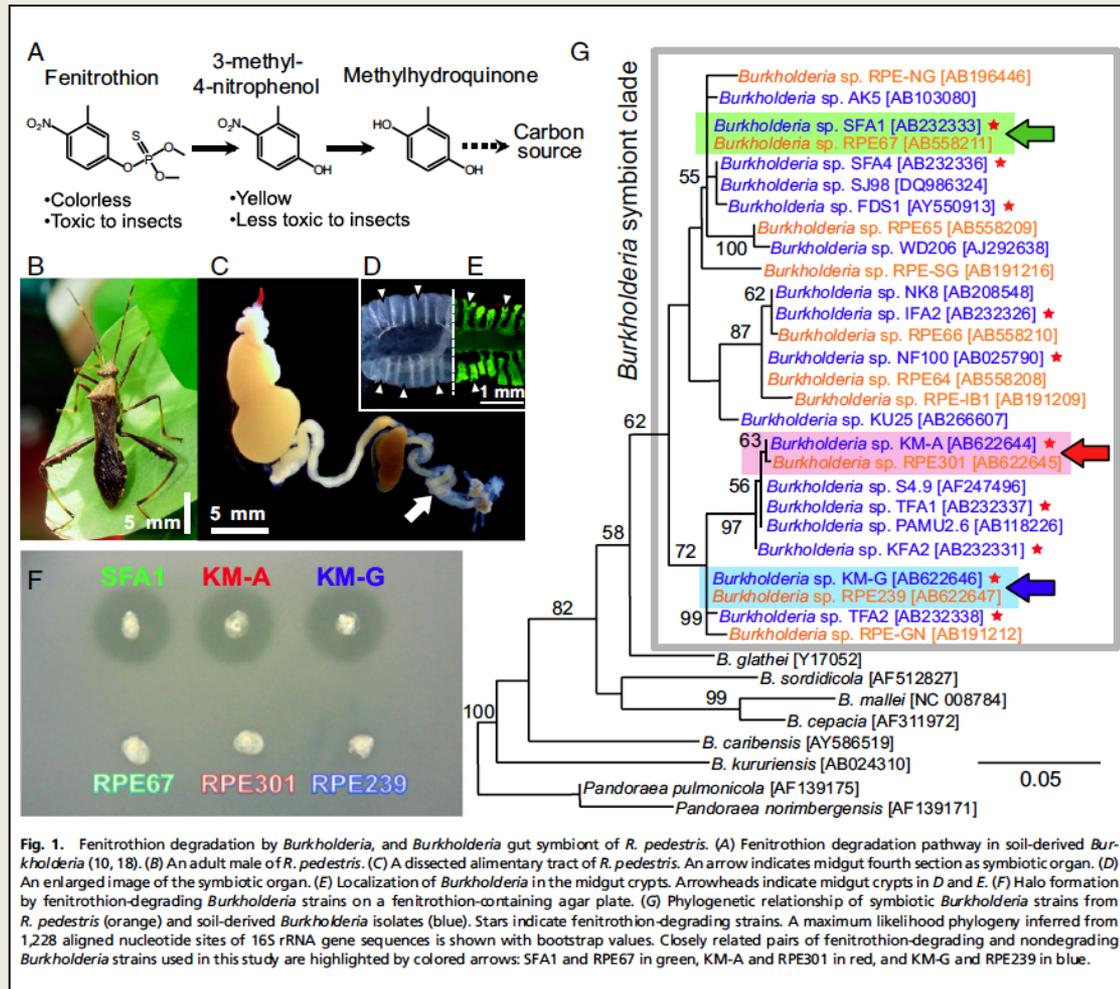
# Effect of antibiotic treatment and gamma-irradiation on cuticular hydrocarbon profiles and mate choice in tsetse flies (*Glossina m. morsitans*)

Tobias Engl<sup>1,2\*</sup>, Veronika Michalkova<sup>3,4,5</sup>, Brian L. Weiss<sup>3</sup>, Güler D. Uzel<sup>6,7</sup>, Peter Takac<sup>4</sup>, Wolfgang J. Miller<sup>8</sup>, Adly M. M. Abd-Alla<sup>6</sup>, Serap Aksoy<sup>3</sup> and Martin Kaltenpoth<sup>1,2\*</sup>

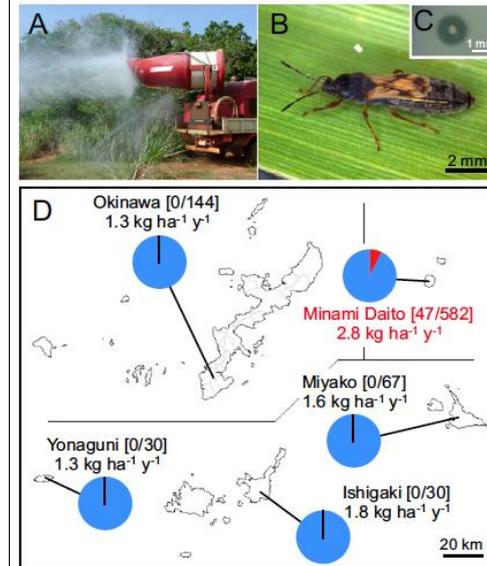


# Symbiont-mediated insecticide resistance

Yoshitomo Kikuchi<sup>a,b,1</sup>, Masahito Hayatsu<sup>c</sup>, Takahiro Hosokawa<sup>d</sup>, Atsushi Nagayama<sup>e</sup>, Kanako Tago<sup>c</sup>, and Takema Fukatsu<sup>d</sup>



**Fig. 1.** Fenitrothion degradation by *Burkholderia*, and *Burkholderia* gut symbiont of *R. pedestris*. (A) Fenitrothion degradation pathway in soil-derived *Burkholderia* (10, 18). (B) An adult male of *R. pedestris*. (C) A dissected alimentary tract of *R. pedestris*. An arrow indicates midgut fourth section as symbiotic organ. (D) An enlarged image of the symbiotic organ. (E) Localization of *Burkholderia* in the midgut crypts. Arrowheads indicate midgut crypts in D and E. (F) Halo formation by fenitrothion-degrading *Burkholderia* strains on a fenitrothion-containing agar plate. (G) Phylogenetic relationship of symbiotic *Burkholderia* strains from *R. pedestris* (orange) and soil-derived *Burkholderia* isolates (blue). Stars indicate fenitrothion-degrading strains. A maximum likelihood phylogeny inferred from 1,228 aligned nucleotide sites of 16S rRNA gene sequences is shown with bootstrap values. Closely related pairs of fenitrothion-degrading and nondegrading *Burkholderia* strains used in this study are highlighted by colored arrows: SFA1 and RPE67 in green, KM-A and RPE301 in red, and KM-G and RPE239 in blue.



**Fig. 5.** Discovery of fenitrothion-degrading *Burkholderia* infection in *C. saccharivorus* at Minami-Daito Island, Japan. (A) Fenitrothion spraying in a sugarcane field at Minami-Daito Island. (B) An adult female of *C. saccharivorus*. (C) Degradation of fenitrothion by a *Burkholderia* strain (MDT2) isolated from *C. saccharivorus*. (D) Infection frequencies with fenitrothion-degrading (red) and nondegrading (blue) *Burkholderia* symbionts in *C. saccharivorus* populations in subtropical islands of Japan. The number of infected insects per number of all insects examined is shown in brackets. Mean annual use of fenitrothion in each of the islands is also shown [the data was from the Japan Agricultural Cooperatives, Okinawa office (JA Okinawa), and the Annual Report of Sugarcane in Okinawa Prefecture (Okinawa Prefectural Government)].



# The symbionts "know" to protect the host

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Review

**The Insect Microbiome Modulates Vector Competence for Arboviruses**

Natapong Jupatanakul<sup>1</sup>, Shuzhen Sim<sup>2</sup> and George Dimopoulos<sup>1,\*</sup>

<sup>1</sup> W. Harry Feinstone Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, 615 N. Wolfe Street, Baltimore, MD 21205, USA; E-Mail: njupata1@jhu.edu

<sup>2</sup> Genome Institute of Singapore, 60 Biopolis Street, #02-01 Genome, Singapore 138672, Singapore; E-Mail: shuzhens@eis.a-star.edu.sg

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**Protection against a fungal pathogen conferred by the aphid facultative endosymbionts *Rickettsia* and *Spiroplasma* is expressed in multiple host genotypes and species and is not influenced by co-infection with another symbiont**

P. ŁUKASIK<sup>\*1</sup>, H. GUO<sup>\*†</sup>, M. VAN ASCH<sup>\*</sup>, J. FERRARI<sup>\*‡</sup> & H. C. J. GODFRAY<sup>\*</sup>

<sup>\*</sup>Department of Zoology, University of Oxford, Oxford, UK  
<sup>†</sup>Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, Nanjing, China  
<sup>‡</sup>Department of Biology, University of York, York, UK

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PLOS PATHOGENS

**Symbionts Commonly Provide Broad Spectrum Resistance to Viruses in Insects: A Comparative Analysis of *Wolbachia* Strains**

Julien Martinez<sup>1\*</sup>, Ben Longdon<sup>1</sup>, Simone Bauer<sup>1</sup>, Yuk-Sang Chan<sup>1</sup>, Wolfgang J. Miller<sup>2</sup>, Kostas Bourtzis<sup>3,4,5</sup>, Luis Teixeira<sup>6</sup>, Francis M. Jiggins<sup>1</sup>

<sup>1</sup>Department of Genetics, University of Cambridge, Cambridge, United Kingdom, <sup>2</sup>Laboratories of Genome Dynamics, Department Cell and Developmental Biology, Center of Anatomy and Cell Biology, Medical University of Vienna, Vienna, Austria, <sup>3</sup>Department of Environmental and Natural Resources Management, University of Patras, Agrinio, Greece, <sup>4</sup>Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece, <sup>5</sup>Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria, <sup>6</sup>Instituto Gulbenkian de Ciência, Oeiras, Portugal

**Abstract**

In the last decade, bacterial symbionts have been shown to play an important role in protecting hosts against pathogens. *Wolbachia*, a widespread symbiont in arthropods, can protect *Drosophila* and mosquito species against viral infections. We have investigated antiviral protection in 19 *Wolbachia* strains originating from 16 *Drosophila* species after transfer into the same genotype of *Drosophila simulans*. We found that approximately half of the strains protected against two RNA viruses. Given that 40% of terrestrial arthropod species are estimated to harbour *Wolbachia*, as many as a fifth of all arthropods species may benefit from *Wolbachia*-mediated protection. The level of protection against two distantly related RNA viruses – DCV and FHV – was strongly genetically correlated, which suggests that there is a single mechanism of protection with broad specificity. Furthermore, *Wolbachia* is making flies resistant to viruses, as increases in survival can be largely explained by reductions in viral titer. Variation in the level of antiviral protection provided by different *Wolbachia* strains is strongly genetically correlated to the density of the bacteria strains in host tissues. We found no support for two previously proposed mechanisms of *Wolbachia*-mediated protection — activation of the immune system and upregulation of the methyltransferase *Dnmt2*. The large variation in *Wolbachia*'s antiviral properties highlights the need to carefully select *Wolbachia* strains introduced into mosquito populations to prevent the transmission of arboviruses.

But... insects **recycle** endosymbionts when the benefit no longer occurs

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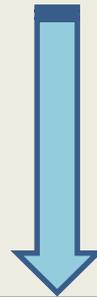
**Insects Recycle Endosymbionts when the Benefit Is Over**

Report

# Symbiotic Control

Current advances in understanding the relationships between microbiota and vectors could have a major impact on:

- i) a better understanding of some traits of mosquito biology;
- ii) the development of innovative control strategies for mosquito-borne diseases aimed at reducing the vector capacity of mosquitoes and / or inhibiting the transmission of the pathogen.



**PARATRANSGENESIS:** genetic modification of the symbiont to express "anti-pathogenic" molecules within the insect to stop transmission.

# SC of insect pests/vectors



Adult *Rhodnius prolixus*, a kissing bug.  
WHO/TDR/Stammers

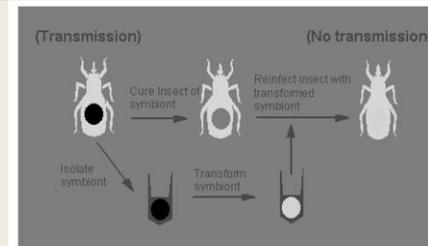
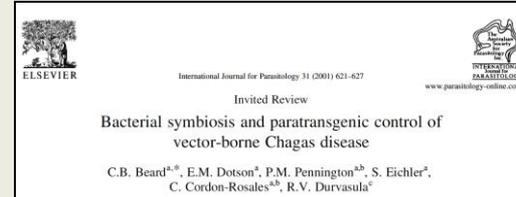


Fig. 5. Generation of *T. cruzi*-refractory *R. prolixus* via genetically modified symbionts.



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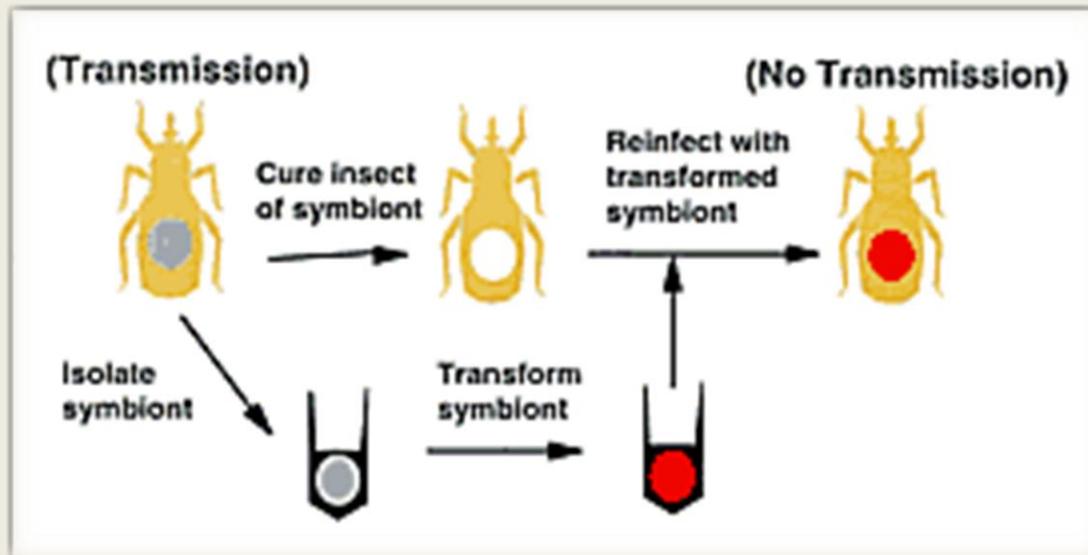
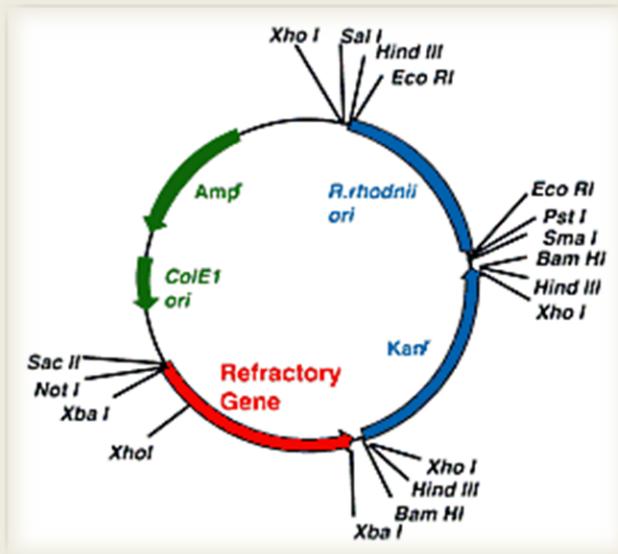
Delivery of a Genetically Marked *Alcaligenes* sp. to the Glassy-winged Sharpshooter for Use in a Paratransgenic Control Strategy

Blake Bextine,<sup>1</sup> Carol Lauzon,<sup>2</sup> Sarah Potter,<sup>2</sup> David Lampe,<sup>3</sup> Thomas A. Miller<sup>1</sup>

Modification of the extracellular bacterial symbiont of the Chagas disease vector *Rhodnius prolixus* (Beard et al. 1992, 1993, 2000). The symbiont is transmitted to the progeny by contaminating egg shells or of food with infected feces and genetically modified symbionts can be transmitted to hosts lacking symbionts (Richard 1993, Beard et al. 2000).

A paratransgenic approach to interrupt transmission of *Xylella fastidiosa* (the bacterial pathogen causing Pierce's disease of grape), by insect vectors has been recently set-up.

# SC of Chagas Diseases



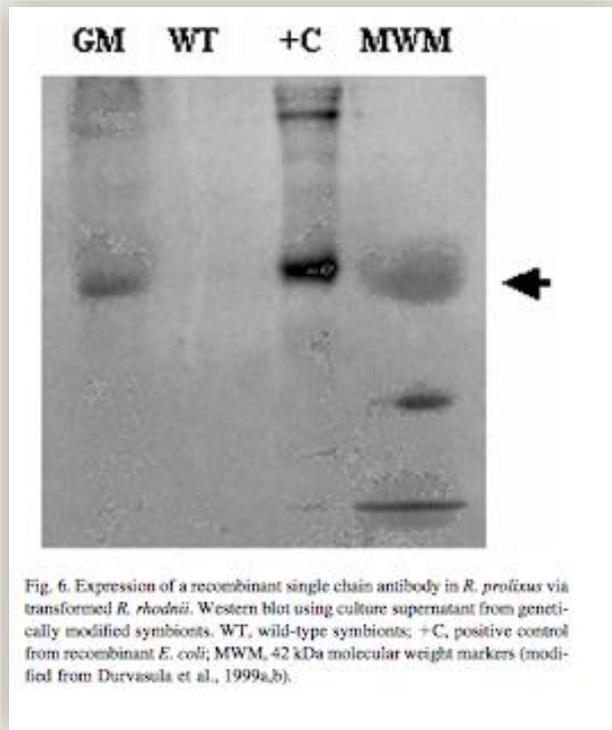
Beard et al, 1998. EID; 4: 581-591.

*Synopses*

## Bacterial Symbiosis in Arthropods and the Control of Disease Transmission

Charles B. Beard,\* Ravi V. Durvasula,† and Frank F. Richards†  
\*Centers for Disease Control and Prevention, Atlanta, Georgia, USA; and  
†Yale University School of Medicine, New Haven, Connecticut, USA

# SC of Chagas Disease



- Genetically modified bacterial formulations are applied to new homes or to insecticide-treated homes.
- Insects infest or reinfest homes.
- Triatomine nymphs ingest modified bacteria.
- Genetically modified symbionts are amplified and dispersed by newly infected insects.

Fig. 7. A theoretical strategy for controlling Chagas disease transmission using genetically modified symbionts.

# Symbionts and mosquitoes: new opportunities for MBD control?

Mosquitoes are potential vectors of many pathogens responsible for diseases of great health importance:

Malaria,

Dengue,

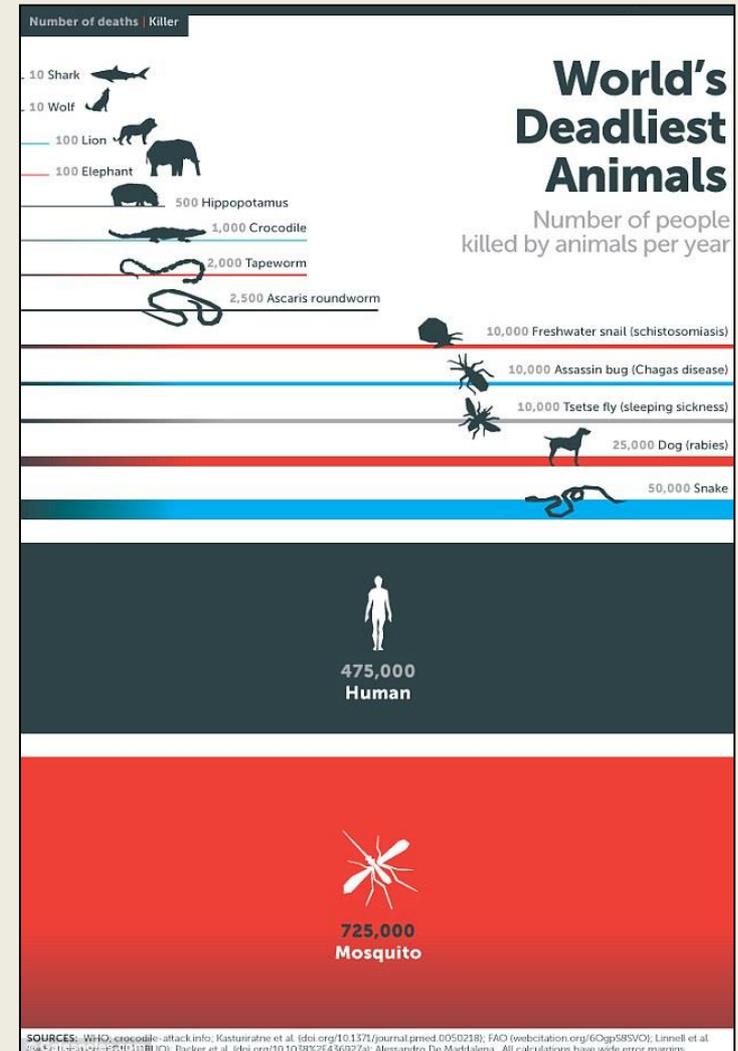
Yellow Fever,

West Nile Virus,

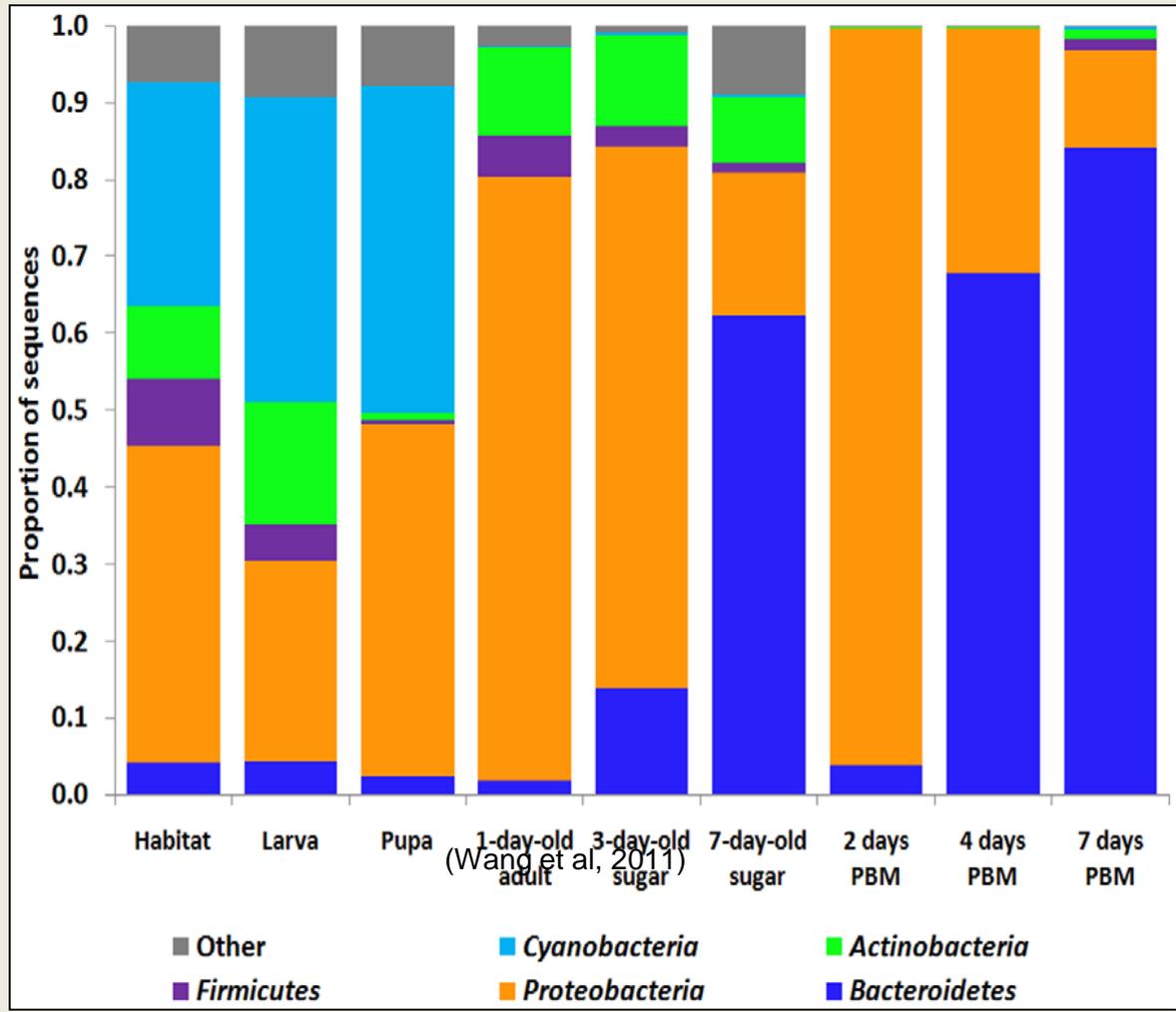
Chikungunya,

Filariasis,

and many others!



# Microbiota fluctuations in mosquito



**Larval-pupal stage:**  
*Cyanobacteria*

**Adult (sugar):**  
*Proteobacteria*  
(*Enterobacteriaceae*)

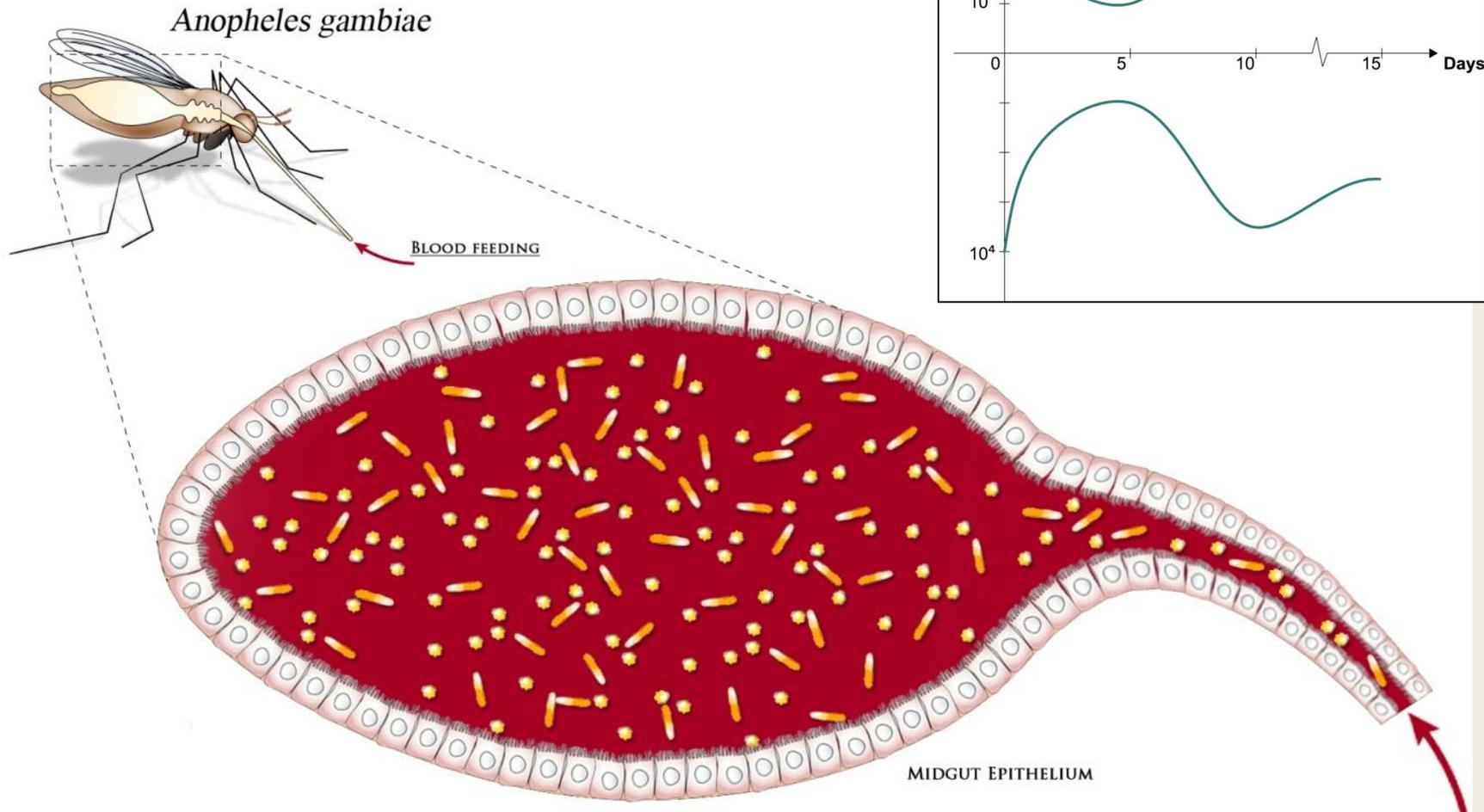
**Adult (7-day-old) :**  
*Bacteroides*  
(*Flavobacteriaceae*)

**Adults (PBM):**  
*Proteobacteria*  
(*Enterobacteriaceae*,  
*Aeromonadaceae* and  
*Pseudomonadaceae*)

**Adults (7 days PBM):**  
*Bacteroides*

# Effects on *Plasmodium* transmission

Colonization of Gram-negative bacteria species has been associated with an **inhibitory activity** on the sporogonic development of the parasite



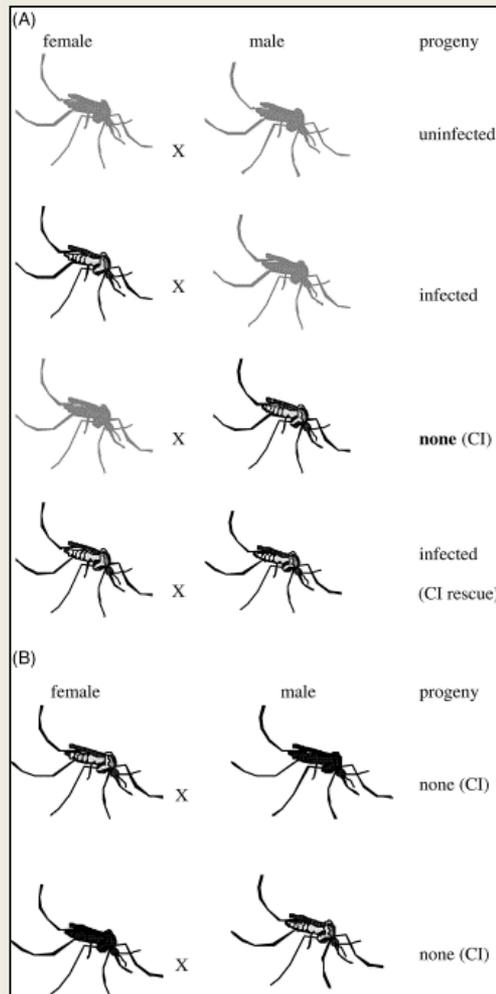
*Wolbachia* as a tool for  
controlling mosquito-  
borne diseases



# *Wolbachia* in insects

- *Wolbachia* is an  $\alpha$ -proteobacterium, first identified in the ovaries of *Culex* mosquitoes in 1924 and is probably the best known endosymbiotic microbe in the biosphere. It is thought to infect up to 76% of the millions of insect species on Earth

The success of these intracellular bacteria has been attributed to their ability to induce a range of reproductive distortions in their hosts to increase the reproductive success of infected females, thus improving maternal transmission of *Wolbachia*.



These traits include transforming genotypic males into phenotypic females, modifying male sperm so that females cannot produce offspring unless they mate with a male infected with the same *Wolbachia* strain, or inducing parthenogenetic reproduction of females.

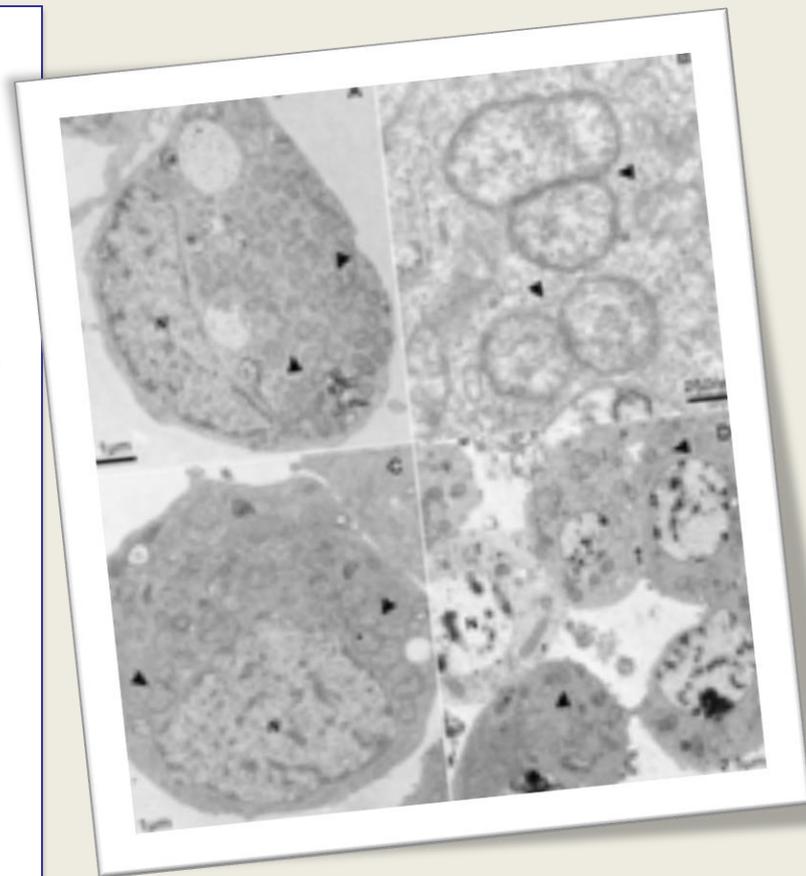
*Wolbachia* can also increase host fitness by influencing nutrition and development and providing resistance to pathogens

# *Wolbachia* in mosquitoes

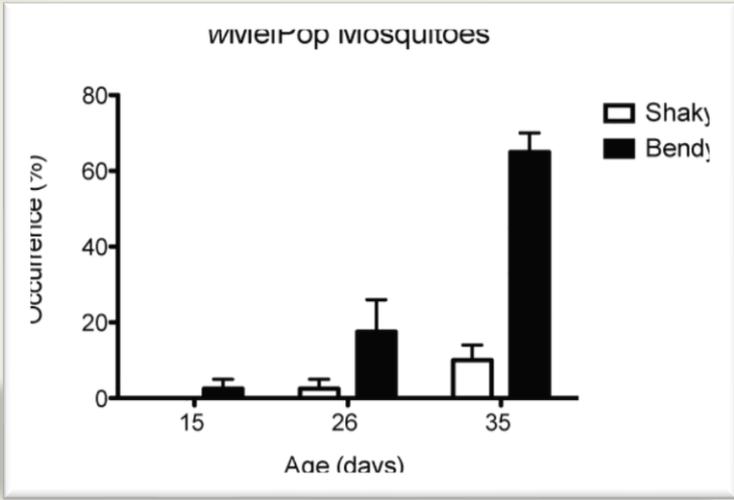
- Many species of mosquitoes are home to *Wolbachia* (eg *Ae. albopictus*)
- Rarely detected in the whole genus *Anopheles* \* and in *Ae. aegypti*
- We can "force" *Wolbachia* to infect Anopheline and *Ae. aegypti*

# *Wolbachia* in Mosquitoes

- ✓ The approaches using *Wolbachia* for the control of the mosquito-borne diseases mentioned above rely on the successful stabilization of *Wolbachia* infections, usually by purified *Wolbachia* microinjection from infected insect hosts.



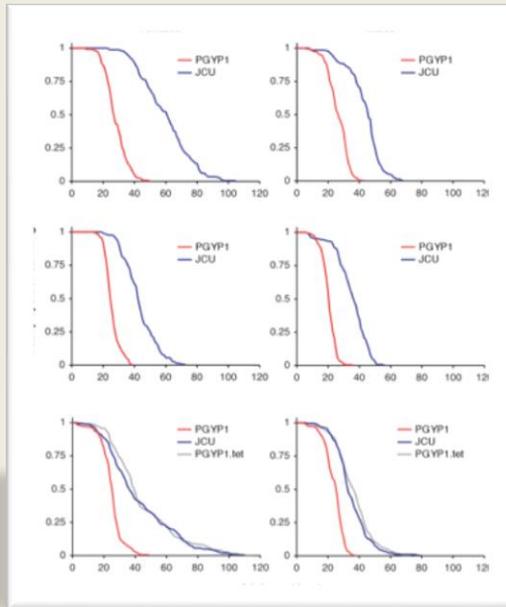
# How to use Wolbachia to control MBD's ?



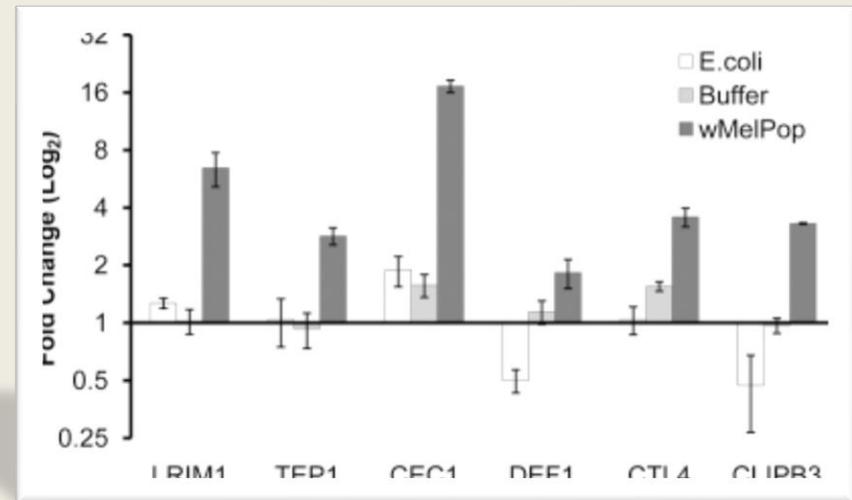
# A “drunk” mosquito!



# Life shortening and priming the immune system



- Most pathogens require a relatively long development period in their vector before they can be transmitted to a new human host; therefore, only the "older" insects have epidemiological importance.
- Wolbachia can shorten the life span of the mosquito by reducing / abolishing the competence of the vector



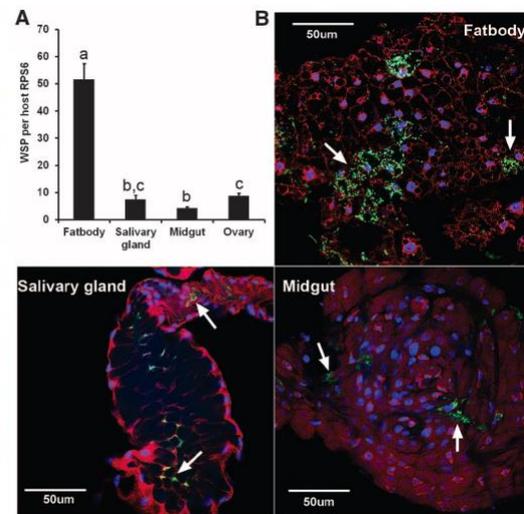
- The expression of six immune genes were analyzed by qRT-PCR: leucine-rich repeat immune protein, *LRIM1*; thioester-containing protein, *TEP1*; cecropin, *CEC1*; defensin, *DEF1*; C-type lectin, *CTL4*; and clip-domain serine protease, *CLIPB3*.
- Adult *An. gambiae* females were injected with *E. coli*, *wMelPop* or the buffer alone, 2–3 days post-eclosion, and RNA was extracted from these adults eight days after injection. Expression was normalized to non-injected adult females of the same age from the same colony.

# *Wolbachia* Invades *Anopheles stephensi* Populations and Induces Refractoriness to *Plasmodium* Infection

Guowu Bian,<sup>1,2</sup> Deepak Joshi,<sup>1</sup> Yuemei Dong,<sup>3</sup> Peng Lu,<sup>1</sup> Guoli Zhou,<sup>1</sup> Xiaoling Pan,<sup>1</sup> Yao Xu,<sup>1</sup> George Dimopoulos,<sup>3</sup> Zhiyong Xi<sup>1,4\*</sup>

*Wolbachia* is a maternally transmitted symbiotic bacterium of insects that has been proposed as a potential agent for the control of insect-transmitted diseases. One of the major limitations preventing the development of *Wolbachia* for malaria control has been the inability to establish inherited infections of *Wolbachia* in anopheline mosquitoes. Here, we report the establishment of a stable *Wolbachia* infection in an important malaria vector, *Anopheles stephensi*. In *A. stephensi*, *Wolbachia* strain *wAlbB* displays both perfect maternal transmission and the ability to induce high levels of cytoplasmic incompatibility. Seeding of naturally uninfected *A. stephensi* populations with infected females repeatedly resulted in *Wolbachia* invasion of laboratory mosquito populations. Furthermore, *wAlbB* conferred resistance in the mosquito to the human malaria parasite *Plasmodium falciparum*.

**Fig. 3. *Wolbachia wAlbB* distribution in somatic tissues of LB1 mosquitoes (G<sub>27</sub>).** (A) The genome copy of *Wolbachia* surface protein (WSP) was measured by real-time PCR and normalized by *A. stephensi* ribosomal protein S6 (RPS6). Different letters above each column signify distinct statistical groups ( $P < 0.05$  for comparison between a, b, and c; Student's *t* test). Error bars indicate SEM of at least 10 biological replicates. (B) *Wolbachia wAlbB* distribution in fat body, midgut, and salivary gland of an LB1 mosquito, assayed by FISH as described in Fig. 1A. White arrows indicate *Wolbachia*.

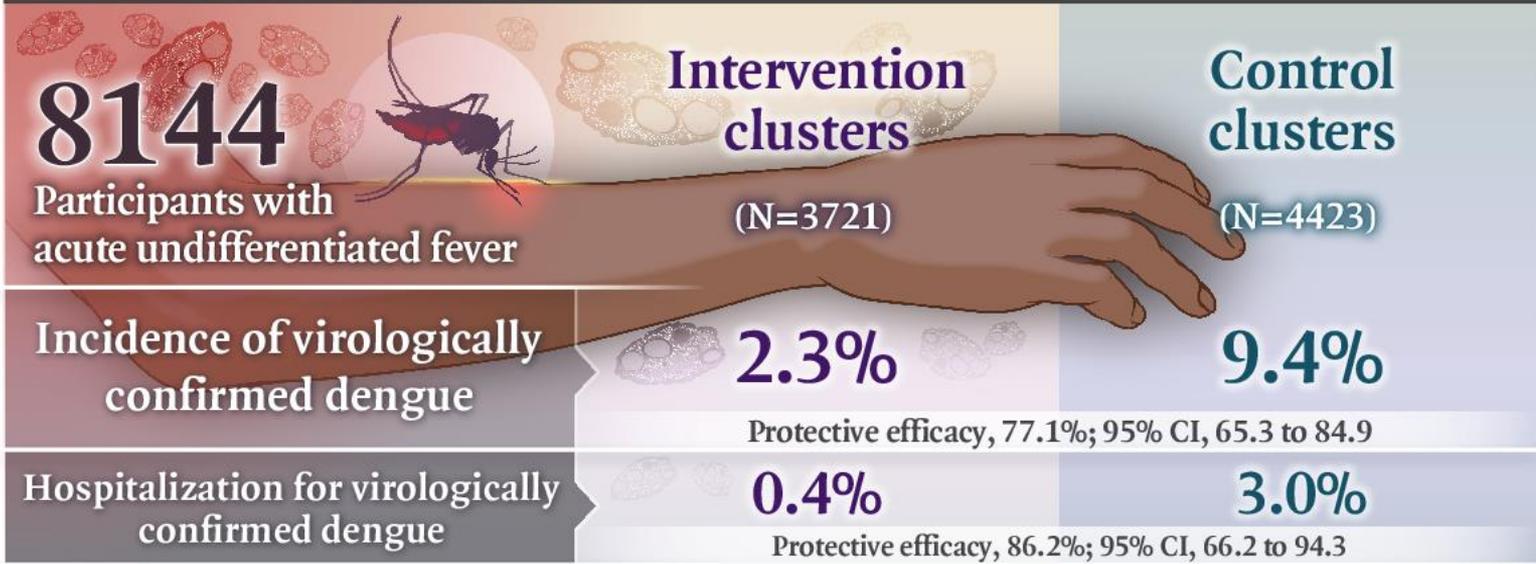


# Wolbachia to eliminate Dengue ([www.eliminatedengue.com](http://www.eliminatedengue.com))

The NEW ENGLAND JOURNAL of MEDICINE

## Wolbachia-Infected Mosquito Deployments for Dengue Control

CLUSTER-RANDOMIZED TRIAL



Protective efficacy similar against the four dengue virus serotypes

Introduction of wolbachia-infected *A. aegypti* was effective in reducing the incidence of symptomatic dengue and hospitalizations.

A. Utarini et al. 10.1056/NEJMoa2030243

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populations to suppress dengue transmission

A. A. Hoffmann<sup>1</sup>, B. L. Montgomery<sup>2</sup>, J. Popovici<sup>2,3</sup>, I. Iturbe-Ormaetxe<sup>2,3</sup>, P. H. Johnson<sup>4</sup>, F. Muzzi<sup>2</sup>, M. Greenfield<sup>2</sup>, M. Durkan<sup>2</sup>, Y. S. Leong<sup>2</sup>, Y. Dong<sup>2,3</sup>, H. Cook<sup>2</sup>, J. Axford<sup>1</sup>, A. G. Callahan<sup>1</sup>, N. Kenny<sup>2,3</sup>, C. Omodei<sup>4</sup>, E. A. McGraw<sup>2,3</sup>, P. A. Ryan<sup>2,3,5</sup>, S. A. Ritchie<sup>4</sup>, M. Turelli<sup>6</sup> & S. L. O'Neill<sup>2,3</sup>

invades caged *Aedes aegypti* populations

T. Walker<sup>1\*</sup>, P. H. Johnson<sup>1\*</sup>, L. A. Moreira<sup>1†</sup>, I. Iturbe-Ormaetxe<sup>1</sup>, F. D. Frentiu<sup>1</sup>, C. J. McMeniman<sup>1†</sup>, Y. S. Leong<sup>1</sup>, Y. Dong<sup>1</sup>, J. Axford<sup>2</sup>, P. Kriesner<sup>2</sup>, A. L. Lloyd<sup>4,5</sup>, S. A. Ritchie<sup>3</sup>, S. L. O'Neill<sup>1,6</sup> & A. A. Hoffmann<sup>2</sup>

# Anopheles and symbionts

For many years we have started an investigation aimed at:

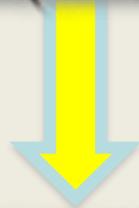
- i) Identifying and characterizing mosquito symbionts "suitable" for the SC;
- ii) Use them in the paratransgenic control of vector-borne disease.

# Symbiosis in *Anopheles*

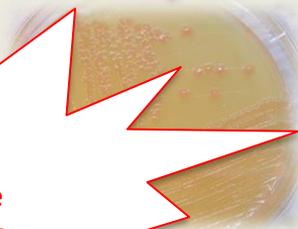


- This enormous scientific effort was not accompanied by in-depth studies aimed at characterizing the bacterial components associated with the mosquito, therefore, until a few years ago, only little information was available (Lindh et al 2005).

# Malaria paratransgenesis



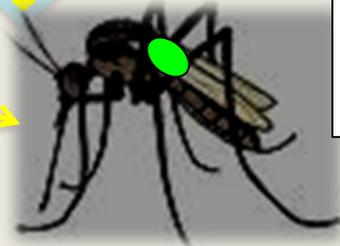
Symbiont identification and isolation



Symbiont transformation



Reintroduction of modified symbiont



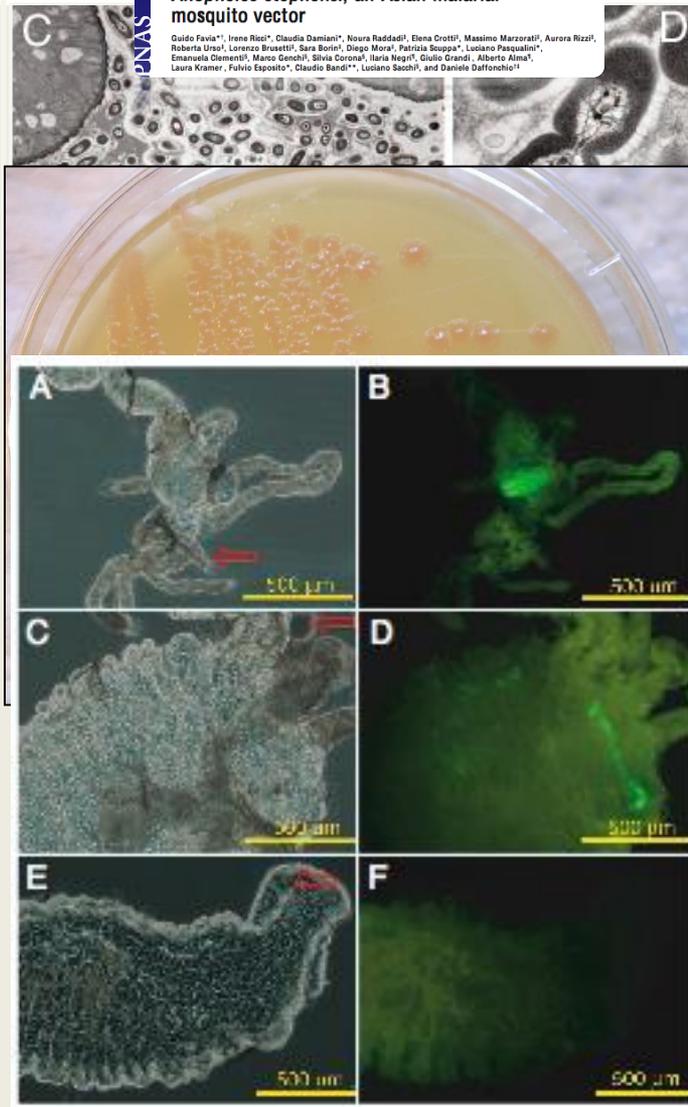
Block of the transmission



# The Gram- a-proteobacterium *Asaia*: a dominant symbiont of mosquito vectors

Bacteria of the genus *Asaia* stably associate with *Anopheles stephensi*, an Asian malarial mosquito vector

Guido Favio<sup>1\*</sup>, Irene Ricci<sup>1\*</sup>, Claudia Damiani<sup>1\*</sup>, Noura Raddadi<sup>1</sup>, Elena Drotti<sup>1</sup>, Massimo Marzorati<sup>1</sup>, Aurora Rizzi<sup>1</sup>, Roberta Urso<sup>1</sup>, Lorenzo Brusetti<sup>1</sup>, Sara Borini<sup>1</sup>, Diego Mora<sup>1</sup>, Patrizia Scoppa<sup>1</sup>, Luciano Pasqualini<sup>1</sup>, Emanuela Diemanti<sup>1</sup>, Marco Genchi<sup>1</sup>, Silvia Coronati<sup>1</sup>, Ilaria Negri<sup>1</sup>, Giulio Grandi<sup>1</sup>, Alberto Alma<sup>1</sup>, Laura Kramer<sup>1</sup>, Fulvio Esposito<sup>1</sup>, Claudio Bandi<sup>1\*</sup>, Luciano Sacchi<sup>1</sup>, and Daniele Daffonchio<sup>1</sup>



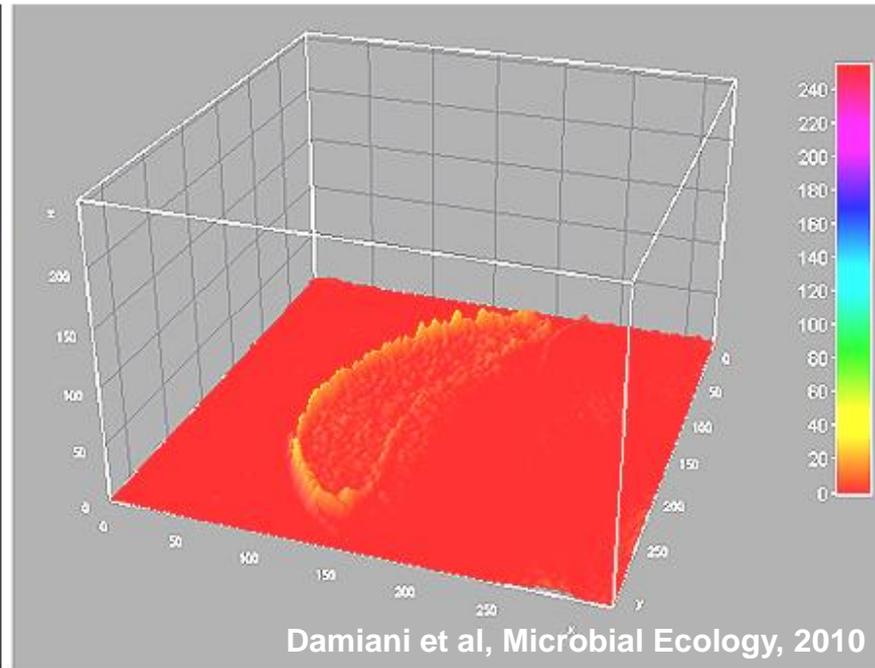
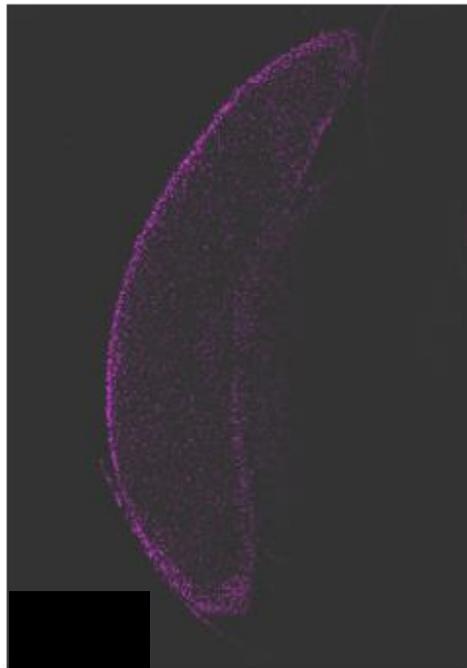
√ Localizes properly: in the **midgut** and **salivary glands** thus overlapping Plasmodium localization, and in the **gonads**.

√ It's **cultivable** and **transformable** with exogenous DNA.

√ Modified strains of *Asaia* are able to efficiently **colonize** midgut, salivary glands and gonad

√ Transmission occurs through horizontal (co-feeding, mating) and vertical routes (maternal transmission). In particular vertical routes offers the chance to introduce engineered bacteria into mosquito populations in the field!

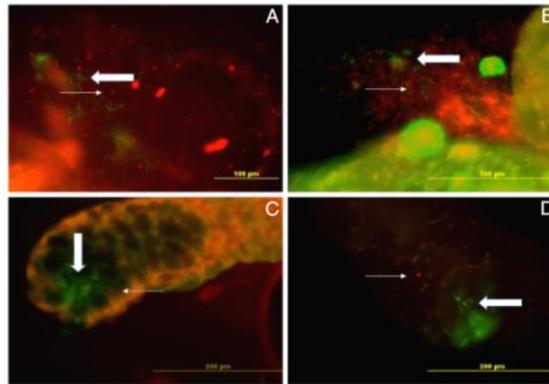
# We have provided evidence that the transfer of *Asaia* from parents to offspring is likely mediated by a mechanisms of egg smearing



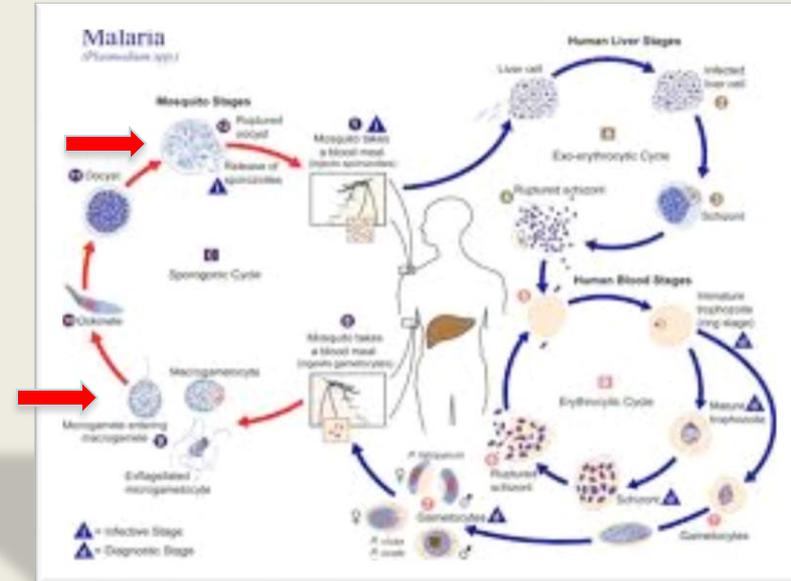
Our demonstration of vertical transmission of *Asaia* from female mosquito to progeny is important because it suggests means to spread recombinant bacteria in field populations. A female mosquito typically lays 50~80 eggs (half of which develop into female adults).

This amplification of the number of mosquitoes carrying *Asaia* should result in rapid spread of the bacterium into the local population. If mosquitoes not carrying *Asaia* lay eggs in the same breeding site, the speed of the spread would be enhanced

# Asaia: potentially a “suitable” candidate for paratransgenesis!



**Figure 7** Co-localization of DsRed-*Asaia* and *P. berghei* PbGFP<sub>Cox2</sub> sporozoites in the proximity of mature oocysts and within the salivary glands of *An. stephensi*. Microscopic fluorescence analysis was carried out on the 17th day after infection and at the 21st day after bacterial administration. The presence of red fluorescent *Asaia* (thin arrow) in the proximity of mature oocysts and GFP-tagged sporozoites (thick arrow) (A and B) as well as the co-localisation of the two microorganisms in the salivary gland lobes (C and D) can be detected.



Capone *et al. Parasites & Vectors* 2013, **6**:182  
<http://www.parasitesandvectors.com/content/6/1/182>



RESEARCH

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## Interactions between *Asaia*, *Plasmodium* and *Anopheles*: new insights into mosquito symbiosis and implications in Malaria Symbiotic Control

Aida Capone<sup>1†</sup>, Irene Ricci<sup>1†</sup>, Claudia Damiani<sup>1</sup>, Michela Mosca<sup>1</sup>, Paolo Rossi<sup>1</sup>, Patrizia Scuppa<sup>1</sup>, Elena Crotti<sup>2</sup>, Sara Epis<sup>1,3</sup>, Mauro Angeletti<sup>1</sup>, Matteo Valzano<sup>1</sup>, Luciano Sacchi<sup>4</sup>, Claudio Bandi<sup>3</sup>, Daniele Daffonchio<sup>2</sup>, Mauro Mandrioli<sup>5</sup> and Guido Favia<sup>1\*</sup>

# Anti-*Plasmodium* effector molecules

**Parasite killing:** around 15 i.e. **Scorpin**, Shiva, Gambicin;

**Interaction with parasite:** i.e. single chain antibodies;

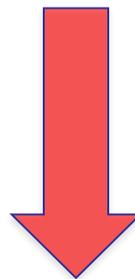
**Interaction with mosquito midgut or salivary gland epithelia:** i.e. SM1

**Manipulation of mosquito immune system:** i.e. AKT, Rel2

Fighting malaria with engineered symbiotic bacteria from vector mosquitoes.

# Possible applications in malaria control

*Asaia* has been transformed for expressing the scorpion as effector molecule



Interference with malaria transmission has been already proved!

# *Asaia*-PM mosquitoes: release in confined field conditions

- By the use of facilities hosted in PERUGIA we are now monitoring the spread of the bacteria in the “recipient populations” and in subsequent generations to acquire the basic parameters for the development of mathematical models.



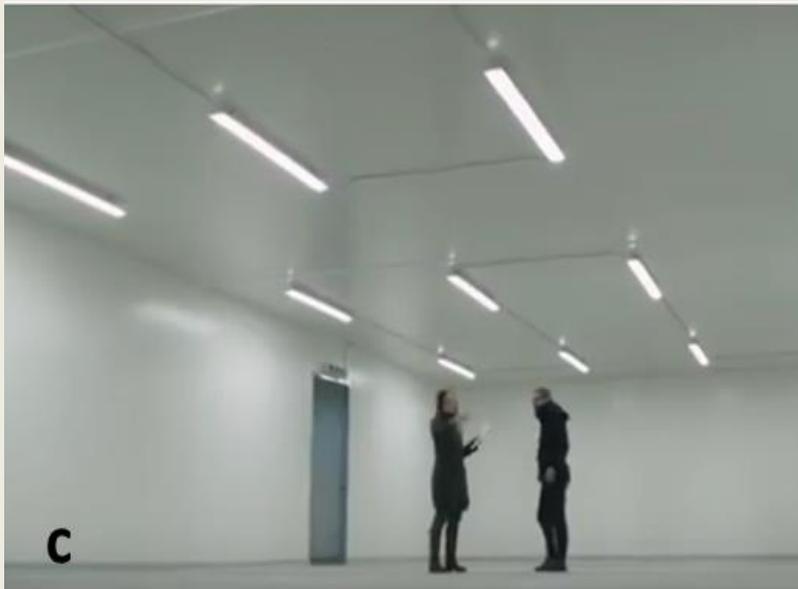
# *Asaia*-PM mosquitoes: release in confined field conditions



- 1) Release of paratransgenic males in cages hosting a w.t. population
- 2) Release of paratransgenic bacteria in feeding stations within the cages to monitor the spread of the bacteria in a SAD population

At defined time intervals, aliquots of mosquitoes will be analyzed to verify the horizontal spread of the bacteria, as well as the vertical transmission through analysis of next generations

# SEMI-FIELD CAGES in Muccia (8 kms from Camerino)



# *Asaia*-PM mosquitoes: release in confined field conditions

Mancini et al. *Parasites & Vectors* (2016) 9:140  
DOI 10.1186/s13071-016-1427-3

Parasites & Vectors

RESEARCH

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## Paratransgenesis to control malaria vectors: a semi-field pilot study

Maria Vittoria Mancini<sup>1†</sup>, Roberta Spaccapelo<sup>2†</sup>, Claudia Damiani<sup>1</sup>, Anastasia Accoti<sup>1</sup>, Mario Tallarita<sup>2</sup>, Elisabetta Petraglia<sup>1</sup>, Paolo Rossi<sup>1</sup>, Alessia Cappelli<sup>1</sup>, Aida Capone<sup>1</sup>, Giulia Peruzzi<sup>2</sup>, Matteo Valzano<sup>1</sup>, Matteo Picciolini<sup>2</sup>, Abdoulaye Diabaté<sup>3</sup>, Luca Facchinelli<sup>2</sup>, Irene Ricci<sup>1</sup> and Guido Favia<sup>1\*</sup>

### Abstract

**Background:** Malaria still remains a serious health burden in developing countries, causing more than 1 million deaths annually. Given the lack of an effective vaccine against its major etiological agent, *Plasmodium falciparum*, and the growing resistance of this parasite to the currently available drugs repertoire and of *Anopheles* mosquitoes to insecticides, the development of innovative control measures is an imperative to reduce malaria transmission. Paratransgenesis, the modification of symbiotic organisms to deliver anti-pathogen effector molecules, represents a novel strategy against *Plasmodium* development in mosquito vectors, showing the potential to reduce parasite development. However, the field application of laboratory-based evidence of paratransgenesis imposes the use of more realistic confined semi-field environments.

**Methods:** Large cages were used to evaluate the ability of bacteria of the genus *Asaia* expressing green fluorescent protein (*Asaia*<sup>GFp</sup>), to diffuse in *Anopheles stephensi* and *Anopheles gambiae* target mosquito populations. *Asaia*<sup>GFp</sup> was introduced in large cages through the release of paratransgenic males or by sugar feeding stations. Recombinant bacteria transmission was directly detected by fluorescent microscopy, and further assessed by molecular analysis.

**Results:** Here we show the first known trial in semi-field condition on paratransgenic anophelines. Modified bacteria were able to spread at high rate in different populations of *An. stephensi* and *An. gambiae*, dominant malaria vectors, exploring horizontal ways and successfully colonising mosquito midguts. Moreover, in *An. gambiae*, vertical and trans-stadial diffusion mechanisms were demonstrated.

**Conclusions:** Our results demonstrate the considerable ability of modified *Asaia* to colonise different populations of malaria vectors, including species where its association is not primary, in large environments. The data support the potential to employ transgenic *Asaia* as a tool for malaria control, disclosing promising perspective for its field application with suitable effector molecules.

**Keywords:** *Asaia*, *Anopheles*, Paratransgenesis, Large cages trials

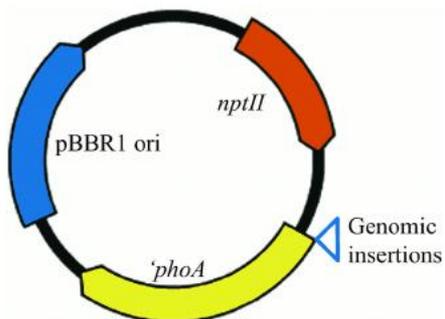
# Asaia-PM mosquitoes: proof of principle for malaria control

RESEARCH ARTICLE

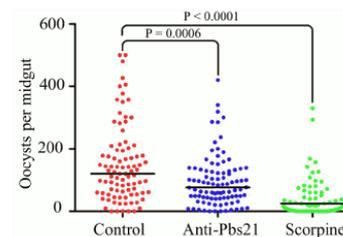
## Inhibition of *Plasmodium berghei* Development in Mosquitoes by Effector Proteins Secreted from *Asaia* sp. Bacteria Using a Novel Native Secretion Signal

Nicholas J. Bongio<sup>1</sup>, David J. Lampe<sup>1\*</sup>

Department of Biological Sciences, Duquesne University, Pittsburgh, Pennsylvania, United States of America



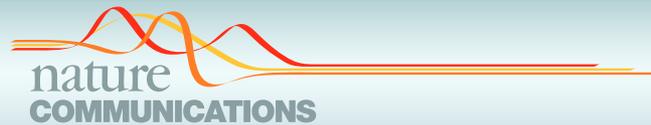
**Fig 1. pNB90 backbone for the genomic library screen.** Random size-selected genomic fragments were cloned 5' to 'phoA' to create a library that could be screened to detect sequences capable of mediating secretion of PhoA. pBBR1 ori = origin of replication; nptII = neomycin phosphotransferase II conferring kanamycin resistance; 'phoA' = *E. coli* alkaline phosphatase gene with no native signal sequence.



Sample set	Control	Anti-Pbs21	Scorpine
N	134	120	96
Range	500	420	330
Prevalence	83.6	83.3	63.5
Tbp	-	0.4	24.0
Mean	120.4	82.2	44.6
Mean % Inhibition	-	31.7%	63.0%
Median	90.5	61.5	18.0
Median % Inhibition	-	32.0%	80.1%
P-value	-	0.0006	<0.0001

**Fig 6. Disruption of *P. berghei* development by anti-Pbs21 scFv-Shiva1 immunotoxin and scorpine constructs.** Three-day old *A. stephensi* mosquitoes were fed paratransgenic strains of *Asaia* expressing either a fusion construct combining the siderophore receptor protein and PhoA (= control), or a fusion construct combining the siderophore receptor protein, an effector protein, and PhoA. These mosquitoes were then fed on an infective mouse and the parasite was allowed to develop for 14 days. The mosquitoes were then dissected, and oocysts on the midgut were counted for each individual. Each dot on the chart represents a single midgut count. The median number of oocysts for each data set is marked with a horizontal line. Inhibition = inhibition of oocyst formation relative to the control; Mean = mean oocyst number per midgut; Median = median oocyst number per midgut; N = number of mosquitoes analyzed; Prevalence = percentage of mosquitoes carrying at least one oocyst; Range = range of oocyst numbers per midgut; Tbp = transmission-blocking potential:  $100 - ((\text{prevalence of mosquitoes fed with recombinant } P. agglomerans) / \text{prevalence of control mosquitoes}) \times 100$ . The scorpine construct produced a significant inhibition of 80.1% calculated by the median oocyst number.

# *Asaia*-PM mosquitoes: proof of principle for malaria control



## ARTICLE

DOI: [10.1038/s41467-018-06580-9](https://doi.org/10.1038/s41467-018-06580-9)

OPEN

## Blood meal-induced inhibition of vector-borne disease by transgenic microbiota

Jackie L. Shane <sup>1</sup>, Christina L. Grogan <sup>1</sup>, Caroline Cwalina<sup>1</sup> & David J. Lampe <sup>1</sup>

Vector-borne diseases are a substantial portion of the global disease burden; one of the deadliest of these is malaria. Vector control strategies have been hindered by mosquito and pathogen resistances, and population alteration approaches using transgenic mosquitos still have many hurdles to overcome before they can be implemented in the field. Here we report a paratransgenic control strategy in which the microbiota of *Anopheles stephensi* was engineered to produce an antiplasmodial effector causing the mosquito to become refractory to *Plasmodium berghei*. The midgut symbiont *Asaia* was used to conditionally express the antiplasmodial protein scorpine only when a blood meal was present. These blood meal inducible *Asaia* strains significantly inhibit pathogen infection, and display improved fitness compared to strains that constitutively express the antiplasmodial effector. This strategy may allow the antiplasmodial bacterial strains to survive and be transmitted through mosquito populations, creating an easily implemented and enduring vector control strategy.

# Combining *Asaia* and *Wolbachia*



## COMMUNICATIONS BIOLOGY

ARTICLE



<https://doi.org/10.1038/s42003-020-0835-2>

OPEN

Chimeric symbionts expressing a *Wolbachia* protein stimulate mosquito immunity and inhibit filarial parasite development

Sara Epis <sup>1,2,10</sup>, Ilaria Varotto-Boccazzi <sup>1,2,10</sup>, Elena Crotti<sup>3</sup>, Claudia Damiani<sup>2,4</sup>, Laura Giovati <sup>5</sup>, Mauro Mandrioli <sup>6</sup>, Marco Biggiogera <sup>7</sup>, Paolo Gabrieli<sup>1,2</sup>, Marco Genchi<sup>8</sup>, Luciano Polonelli<sup>5</sup>, Daniele Daffonchio <sup>9</sup>, Guido Favia<sup>2,4</sup> & Claudio Bandi <sup>1,2</sup>✉

# *Asaia* circulation in human samples

One of the problems related to the use of bacteria in the paratransgenic control of insect-borne diseases is their potential infectivity and virulence for humans and animals.

This is particular crucial for bacteria that localize at the level of the salivary gland implying potential transmission by the mosquito saliva.

We have studied the “circulation” of *Asaia* within human samples.

Table I. Blood and serum samples from humans, and summary of the results of the screenings for *Asaia* spp.

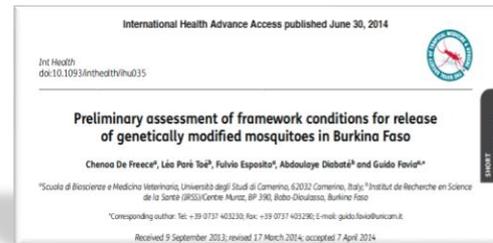
Human samples and health or disease status	Samples Origin	qPCR results on blood samples positive/total	IFA results on sera samples positive/total
Healthy blood donors	St. Orsola Malpighi University Hospital	0/338	0/221
Donors exposed to mosquito bites	Insectary at the University of Camerino	0/10	0/10
	Physicians working in rural or urban areas of Burkina Faso	0/13	0/14
Lyme disease Patients	St. Orsola Malpighi University Hospital	0/10	0/16
HIV patients	St. Orsola Malpighi University Hospital	0/90	0/0
Malaria patients	Spedali Civili di Brescia (Institute for Infectious and Tropical Diseases)	0/5	0/14
Enteric disease Patients	St. Orsola Malpighi University Hospital	0/19	0/0
	Total	0/485	0/275

# *Asaia* circulation in human samples

This data coupled with the fact that *Asaia* has been found in tropical flowers (now quite widespread like for instance the plumbago tree), in grapevine, in glutinous fermented rice and in insects **is a strong evidence that *Asaia* is most likely a non-pathogenic bacterium** (none of the known acetic acid bacteria has thus far been shown to be pathogenic).

# Ethical issues

✓ Consequently, we have performed a pilot study in Burkina Faso to explore the perspectives of Burkinabè citizens and of national and government organisations familiar with Bt cotton to gauge paratransgenesis receptiveness



✓ We aim to progress this approach in Burkina faso and extend it to other African (i.e. Mali, Ghana) and Asian countries (i.e. Laos) in endemic region for MBD to properly address all the concerns related to the release of paratransgenic mosquitoes in field.

# *Asaia* may stimulate host immune response



## ***Asaia* Activates Immune Genes in Mosquito Eliciting an Anti- *Plasmodium* Response: Implications in Malaria Control**

*Alessia Cappelli<sup>‡</sup>, Claudia Damiani<sup>‡</sup>, Maria Vittoria Mancini<sup>‡</sup>, Matteo Valzano<sup>‡</sup>,  
Paolo Rossi<sup>‡</sup>, Aurelio Serrao<sup>‡</sup>, Irene Ricci<sup>‡</sup> and Guido Favia<sup>‡\*</sup>*

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*School of Biosciences & Veterinary Medicine, University of Camerino, Camerino, Italy*

# Asaia activates immune genes in mosquito eliciting an anti-*Plasmodium* response.

frontiers in Genetics

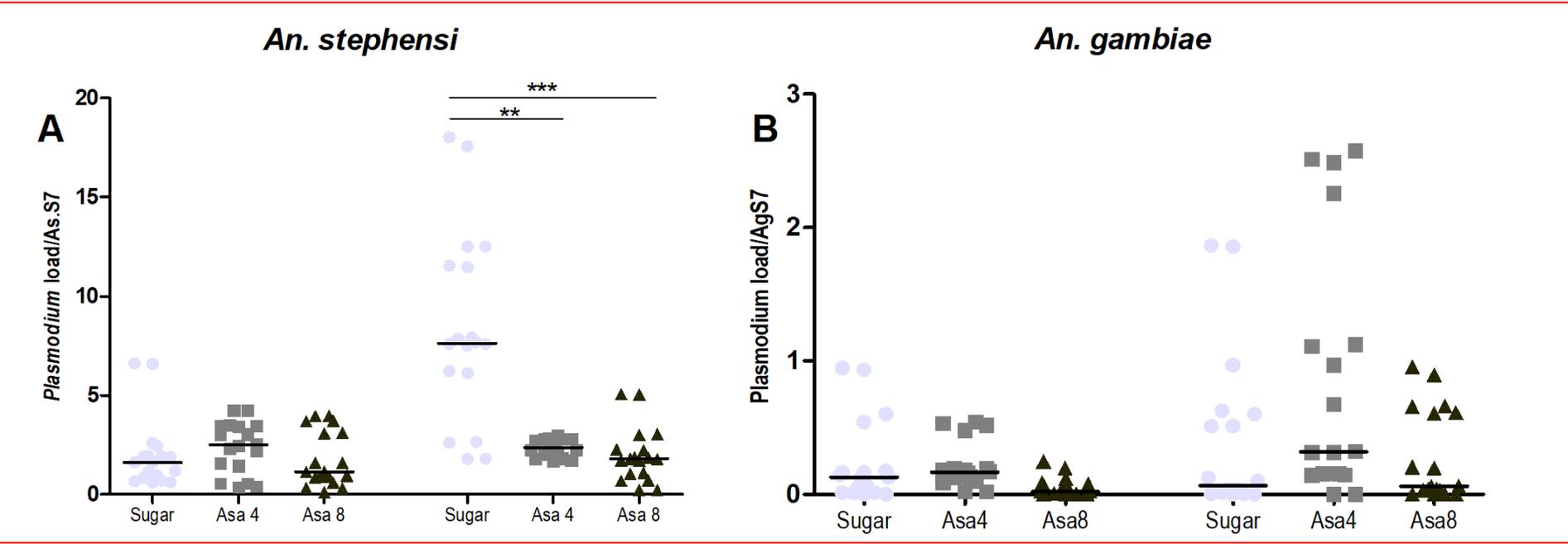
ORIGINAL RESEARCH  
published: 23 September 2019  
doi: 10.3389/fgene.2019.00836

Check for updates

## Asaia Activates Immune Genes in Mosquito Eliciting an Anti-*Plasmodium* Response: Implications in Malaria Control

Alessia Cappelli<sup>†</sup>, Claudia Damiani<sup>†</sup>, Maria Vittoria Mancini<sup>†</sup>, Matteo Valzano<sup>†</sup>, Paolo Rossi<sup>†</sup>, Aurelio Serrao<sup>†</sup>, Irene Ricci<sup>†</sup> and Guido Favia<sup>†\*</sup>

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# Activation of immune genes in leafhopper by *Asaia*



## Activation of Immune Genes in Leafhoppers by Phytoplasmas and Symbiotic Bacteria

Elena Gonella<sup>1</sup>, Mauro Mandrioli<sup>2</sup>, Rosemarie Tedeschi<sup>1</sup>, Elena Crotti<sup>3</sup>, Marianna Pontini<sup>1</sup> and Alberto Alma<sup>1\*</sup>

<sup>1</sup> Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), Università degli Studi di Torino, Grugliasco, Italy, <sup>2</sup> Dipartimento di Scienze della Vita (DSV), Università degli Studi di Modena e Reggio Emilia, Modena, Italy, <sup>3</sup> Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente (DeFENS), Università degli Studi di Milano, Milan, Italy



# *Asaia* shows sign of genome reduction

GBE

## Genome Reduction in the Mosquito Symbiont *Asaia*

Diego Peres Alonso<sup>1,†</sup>, Maria Vittoria Mancini<sup>2,4,†</sup>, Claudia Damiani<sup>2</sup>, Alessia Cappelli<sup>2</sup>, Irene Ricci<sup>2</sup>, Marcus Vinicius Niz Alvarez<sup>1</sup>, Claudio Bandi<sup>3</sup>, Paulo Eduardo Martins Ribolla<sup>1,\*</sup>, and Guido Favia<sup>2,\*</sup>

<sup>1</sup>Biotechnology Institute (IBTEC) & Biosciences Institute at Botucatu (IBB), Sao Paulo State University (UNESP), Sao Paulo, Brazil

<sup>2</sup>School of Biosciences and Veterinary Medicine, University of Camerino, Italy

<sup>3</sup>Clinical Pediatric Research Center Romeo and Enrica Invernizzi, Department of Biosciences, University of Camerino, Italy

<sup>4</sup>Present address: MRC-University of Glasgow-Centre for Virus Research, Glasgow, U.K.

**Table 2**

Number of Total Genes in Each Isolate

Isolate	Total Genes	Flagellum Complex	Mobile Elements
<i>AAeg</i>	3,464	30	25
<i>AAIb</i>	3,718	30	34
<i>ADar</i>	2,909	6	7
<i>AFun</i>	3,508	30	24
<i>AGam</i>	3,604	30	26
<i>ASte</i>	3,431	30	23

Note.—the table compares the number of total genes identified in each isolate. Specific annotated genes belonging to the flagellum machinery and mobile elements were also included.

analyses: In fact, flagella genes were differentially missing in endosymbiotic bacteria of insects, suggesting a functional divergence, including a development and specialization in the export of proteins from the bacterium to the insect (Toft and Fares 2008).

The investigation of *ADar* absence of flagella genes has been expanded by using the hanging drop method, correlating genotypic and phenotypic evidences. It confirms that the cells of *ADar* isolate do not display a motile behavior, appearing static during microscope observations, when compared with other isolates.

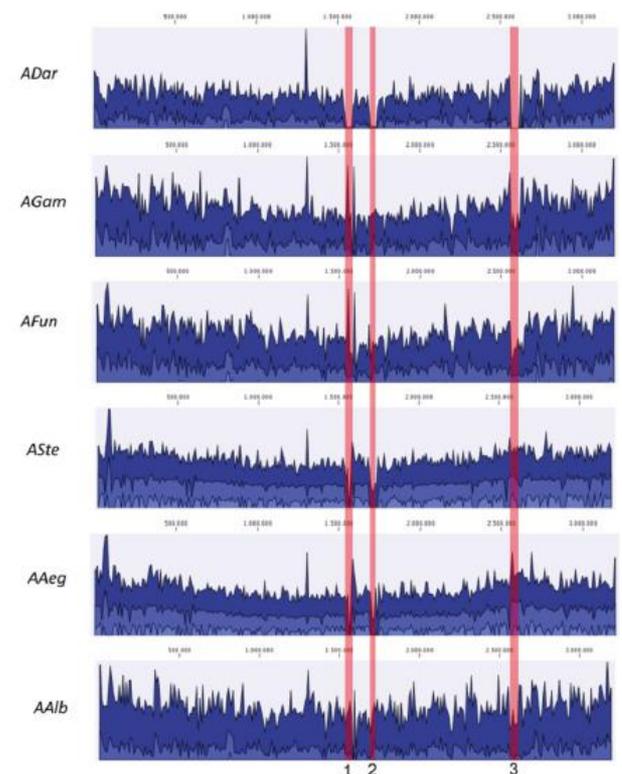


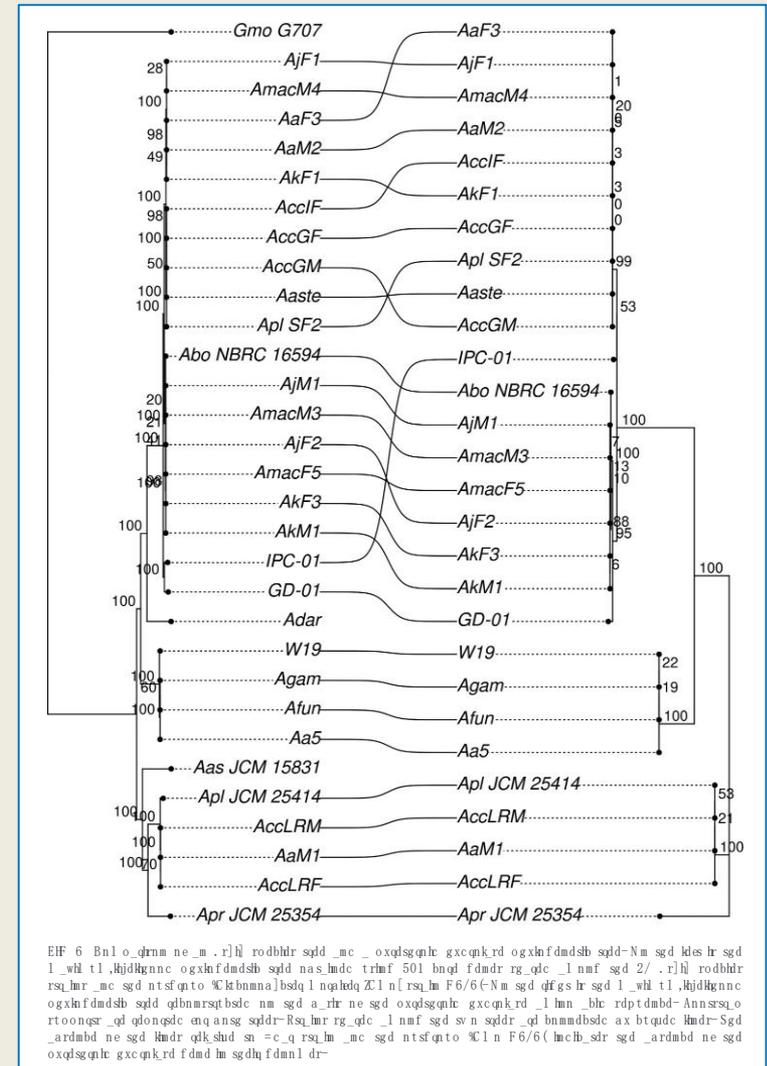
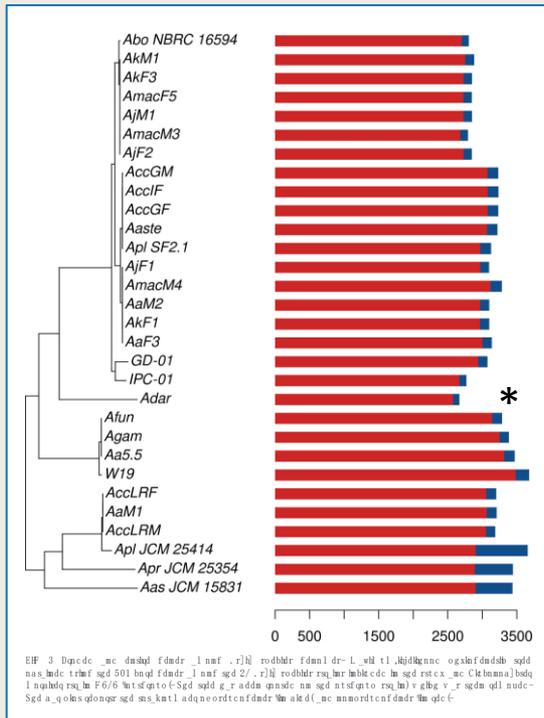
FIG. 1.—Genome alignment comparison between *Asaia* isolates. Red bars highlight gap regions in the genome of *ADar* isolate.

# *Asaia* symbionts from insects underwent convergent genome reduction, preserving an insecticide-degrading gene

RESEARCH ARTICLE

## Phylogenomics Reveals that *Asaia* Symbionts from Insects Underwent Convergent Genome Reduction, Preserving an Insecticide-Degrading Gene

Francesco Comandatore,<sup>a</sup> Claudia Damiani,<sup>b,c</sup> Alessia Cappelli,<sup>b,c</sup> Paulo Eduardo Martins Ribolla,<sup>d,e</sup> Giuliano Gasperi,<sup>f</sup> Francesco Gradoni,<sup>g</sup> Gioia Capelli,<sup>g</sup> Aurora Piazza,<sup>g</sup> Fabrizio Montarsi,<sup>g</sup> Maria Vittoria Mancini,<sup>h</sup> Paolo Rossi,<sup>b,c</sup> Irene Ricci,<sup>b,c</sup> Claudio Bandi,<sup>i</sup> Guido Favia<sup>b,c</sup>



EIF 6 Bn1 o\_dmm ne\_m .r]h] rodbhr sgd\_mc \_ oxqsgnqhc gxcqkrd ogxhfdmsdb sgd-Nm sgd kds hr sgd l\_w] t1 ,hjdHgncnnc ogxhfdmsdb sgd nas\_lmdc trmf 501 bnqf fdmrd rg\_qlc \_l nmf sgd 2/ .r]h] rodbhr rsq\_lmr \_mc sgd ntsfnto %hbnmna]bsdq l nqledq Zl n[rsq\_lm F6/6(-Nm sgd qfTgs hr sgd l\_w] t1 ,hjdHgncnnc ogxhfdmsdb sgd qbnarsqbsdc nm sgd a\_rlr ne sgd oxqsgnqhc gxcqkrd \_l hm\_n\_blc rdpfdmbd-Annsrs\_o rtoonqr \_qd qlonqdc enq ansq sgd-rsq\_lmr rg\_qlc \_l nmf sgd sv n sgd\_r \_qd bnmdbsdc ax btqdc Hndr-Sgd \_ardmbd ne sgd Hndr qtkshud sn =c\_q rsq\_lm \_mc sgd ntsfnto %l n F6/6(hmch\_sdr sgd \_ardmbd ne sgd oxqsgnqhc gxcqkrd fdmd hm sgdq fdmnl dr-

# Not only *Asaia*...

SCIENTIFIC  
REPORTS



**OPEN** Intra-specific diversity of *Serratia marcescens* in *Anopheles* mosquito midgut defines *Plasmodium* transmission capacity

SUBJECT AREAS:  
PARASITE HOST RESPONSE  
PATHOGENS  
ENTOMOLOGY  
PARASITIC INFECTION

Hironori Bando<sup>1,2</sup>, Kiyoshi Okado<sup>1,2</sup>, Wamdaogo M. Guelbeogo<sup>3</sup>, Athanase Badolo<sup>3,4</sup>, Hiroka Aonuma<sup>1,2</sup>, Bryce Nelson<sup>5</sup>, Shinya Fukumoto<sup>2</sup>, Xuenan Xuan<sup>2</sup>, N'Fale Sagnon<sup>3</sup> & Hirotaka Kanuka<sup>1,2</sup>

**Fighting malaria with engineered symbiotic bacteria from vector mosquitoes**

Sibao Wang<sup>a</sup>, Anil K. Ghosh<sup>a</sup>, Nicholas Bongio<sup>b</sup>, Kevin A. Stebbings<sup>b,1</sup>, David J. Lampe<sup>b</sup>, and Marcelo Jacobs-Lorena<sup>a,2</sup>

<sup>a</sup>Department of Molecular Microbiology and Immunology, Malaria Research Institute, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205; and <sup>b</sup>Department of Biological Sciences, Duquesne University, Pittsburgh, PA 15282

# Metagenomic analysis in three different organs / tissues of nine different mosquito species

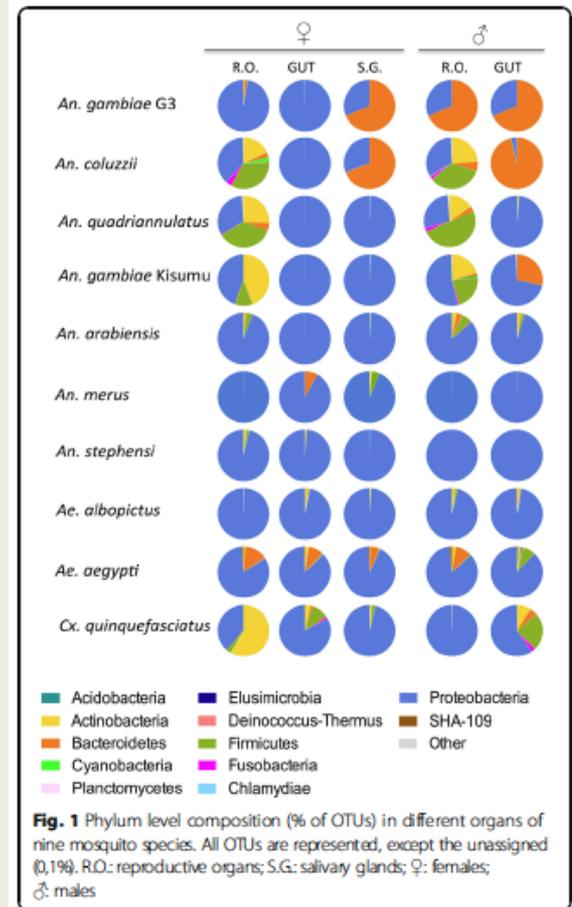
## RESEARCH ARTICLE

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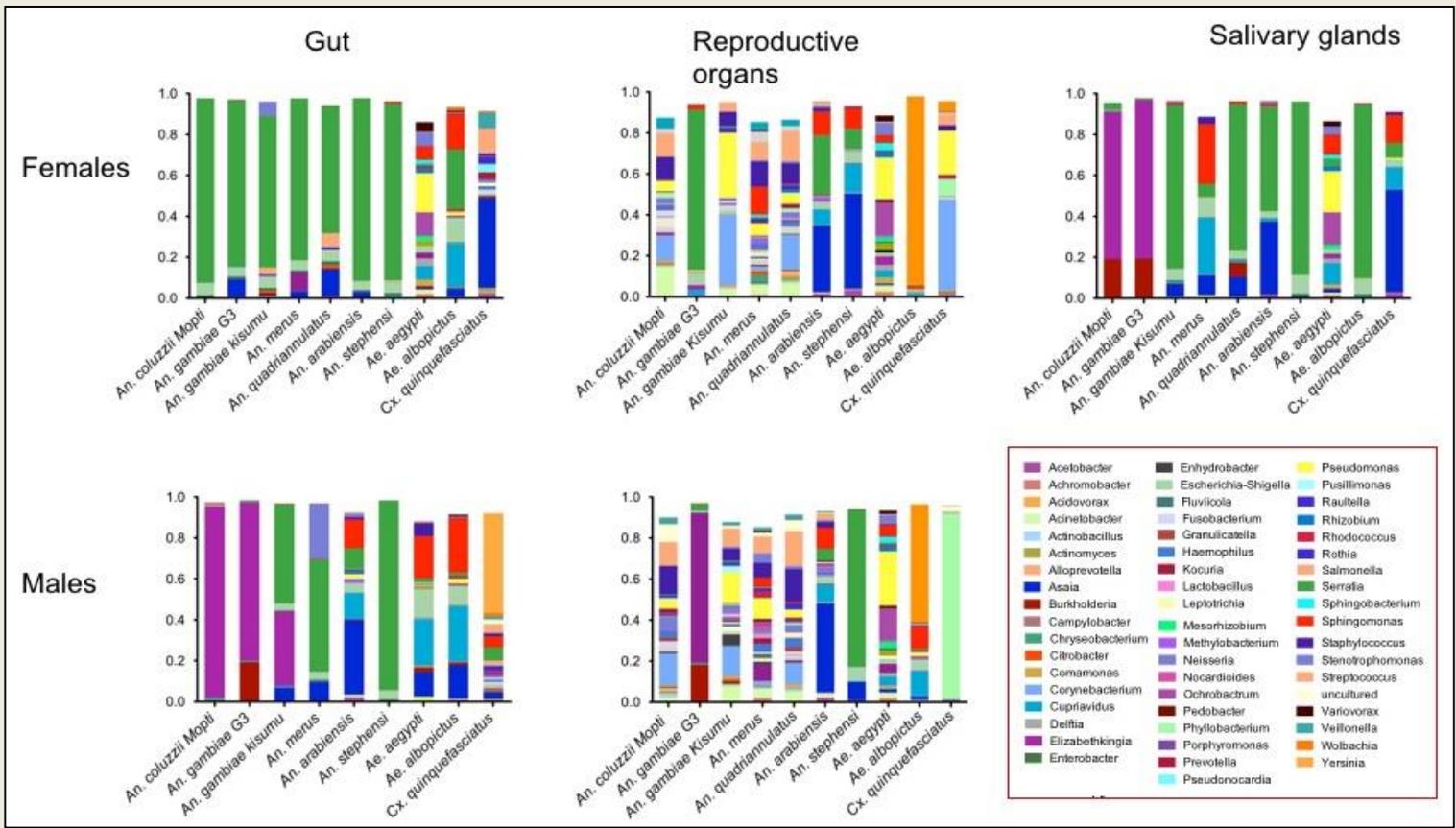
### Estimating bacteria diversity in different organs of nine species of mosquito by next generation sequencing



M V Mancini<sup>1,3†</sup>, C Damiani<sup>1†</sup>, A Accoti<sup>2</sup>, M Tallarita<sup>2</sup>, E Nunzi<sup>2</sup>, A Cappelli<sup>1</sup>, J Bozic<sup>1</sup>, R Catanzani<sup>2</sup>, P Rossi<sup>1</sup>, M Valzano<sup>1</sup>, A Serrao<sup>1</sup>, I Ricci<sup>1</sup>, R Spaccapelo<sup>2\*</sup> and G Favai<sup>1\*</sup>



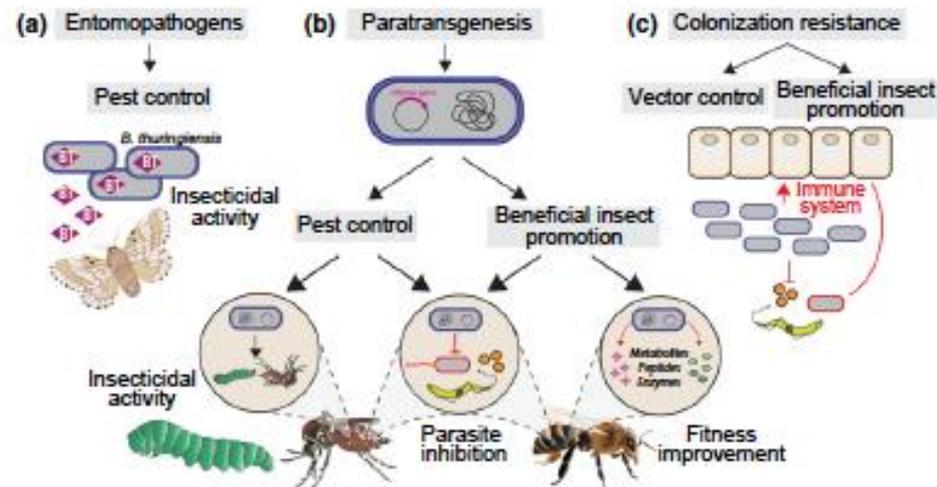
# Metagenomic analysis in three different organs / tissues of nine different mosquito species



Many other bacteria could be used in the SC of VBD's



# Take home message



**Fig. 5.** Different applications of gut bacteria for the management of insects. (a) Insecticidal potential of entomopathogenic gut bacteria can be used to control pest species. (b) In paratransgenesis, bacteria are used as vehicles to express molecules in the gut, which negatively or positively affect health of the host or suppress parasite colonization. These approaches could be applied for the management of pest species and beneficial insects. (c) Alternatively, gut bacteria that naturally inhibit parasite colonization could be disseminated in insect populations, for example, to prevent the spread of human disease via insect vectors or to protect beneficial insects from parasitic diseases.

thank you!

