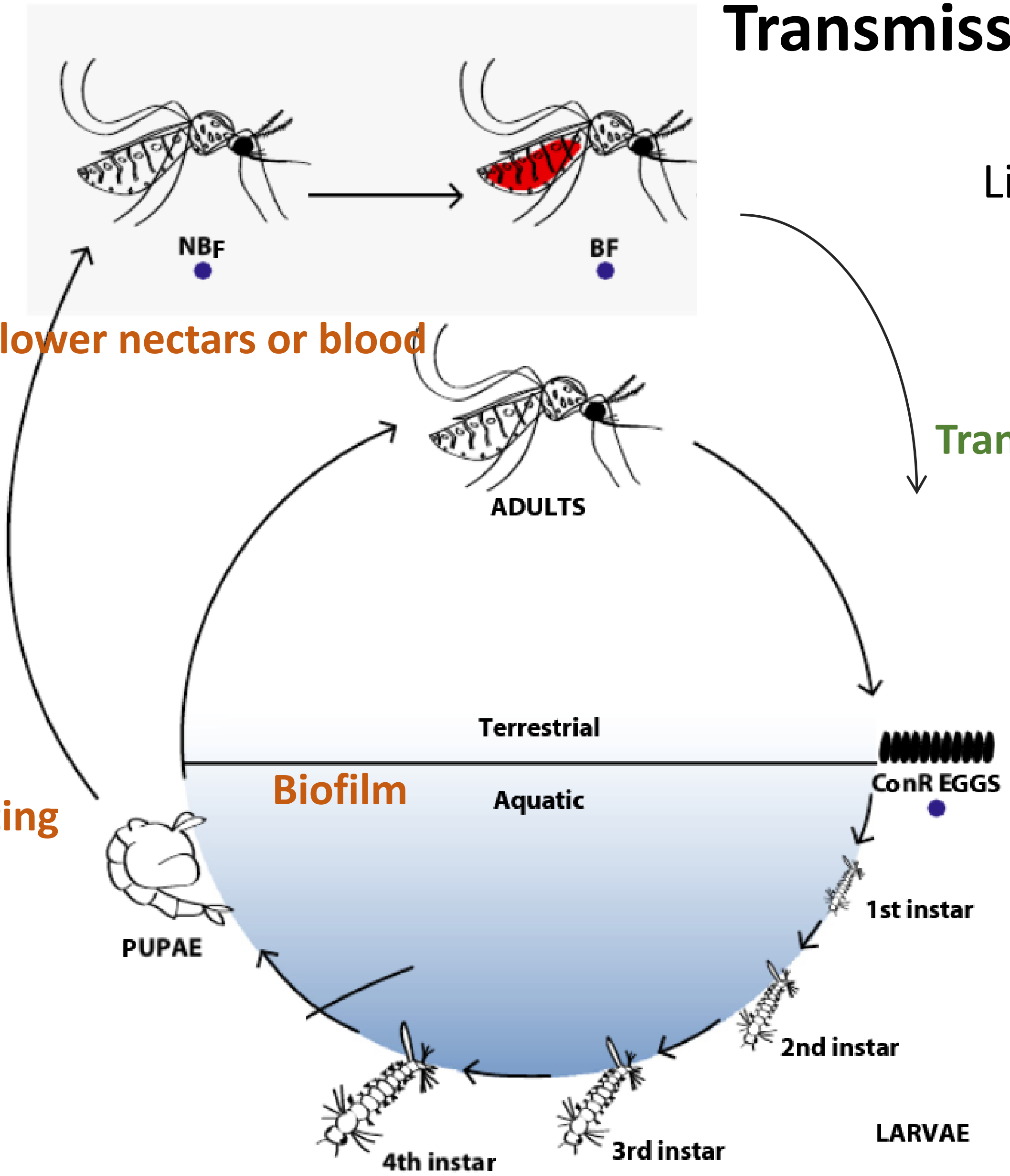
Perspectives de modulation du microbiote de l'anophèle pour limiter la transmission de *Plasmodium*

Vectopole Sud, Montpellier, 15 octobre 2020

Isabelle MORLAIS, IRD MIVEGEC

Transmission routes

Life cycle stage and diet affect bacterial communities



Feeding on flower nectars or blood

Mating

Moulting

Biofilm

Trans-ovarial transmission

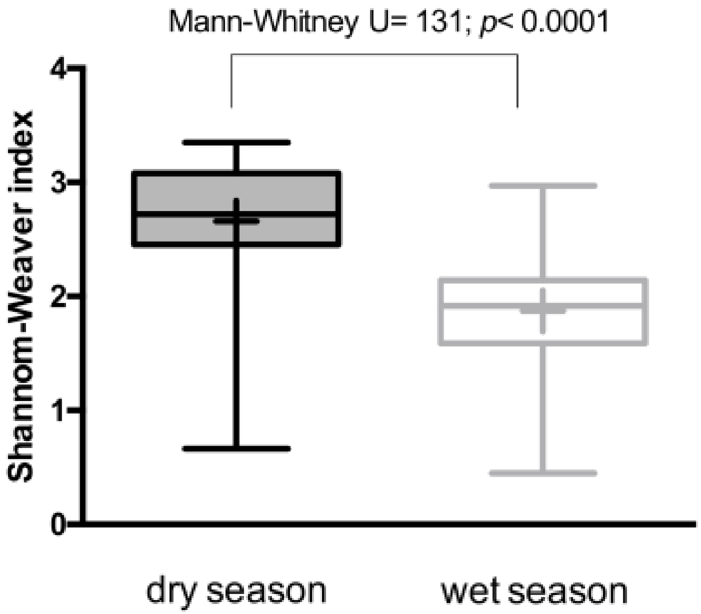
Egg smearing

Trans-stadial transmission

Microbiota acquisition

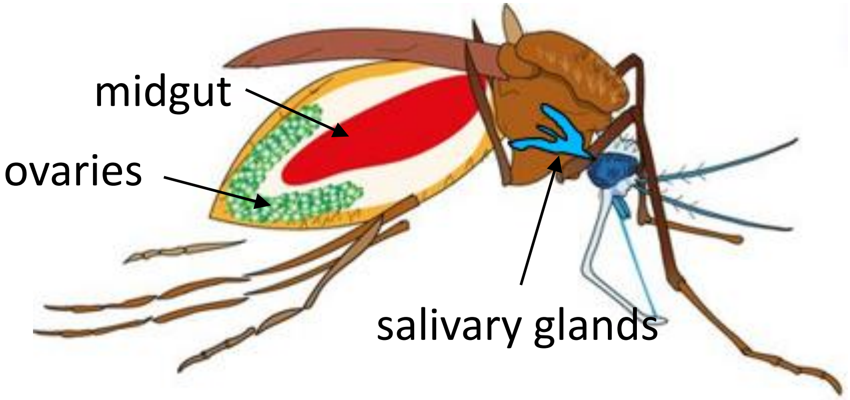
The breeding site is a crucial factor shaping the community composition in adults and the season affects microbiota diversity

Gimonneau *et al*. 2014



Akorli *et al*. 2016

Microbiota composition

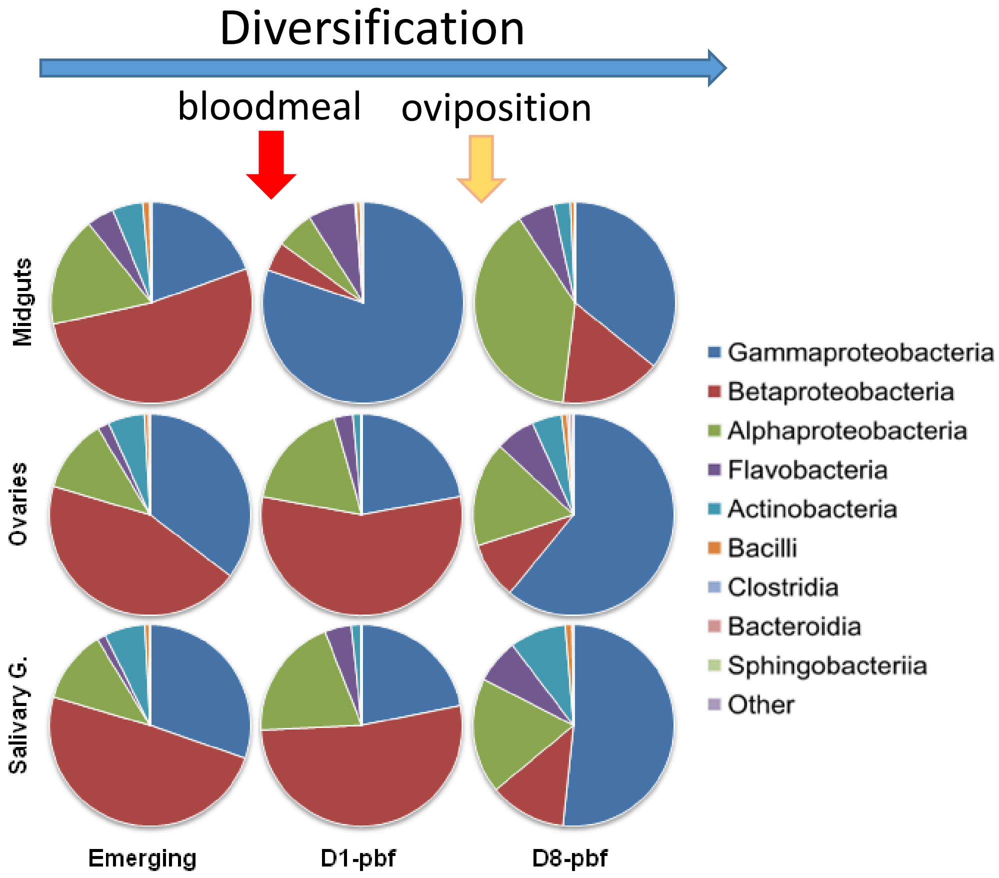


Changes with physiological state or aging

Mosquito epithelia share a core microbiota

Pseudomonas, Comamonas, Acinetobacter, Rhizobium, Burkholderia and members of the Enterobacteriaceae

Large individual variability



Taxonomic classification of bacterial reads retrieved from the mosquito tissues at the different developmental stages.

Tchioffo et al., 2014

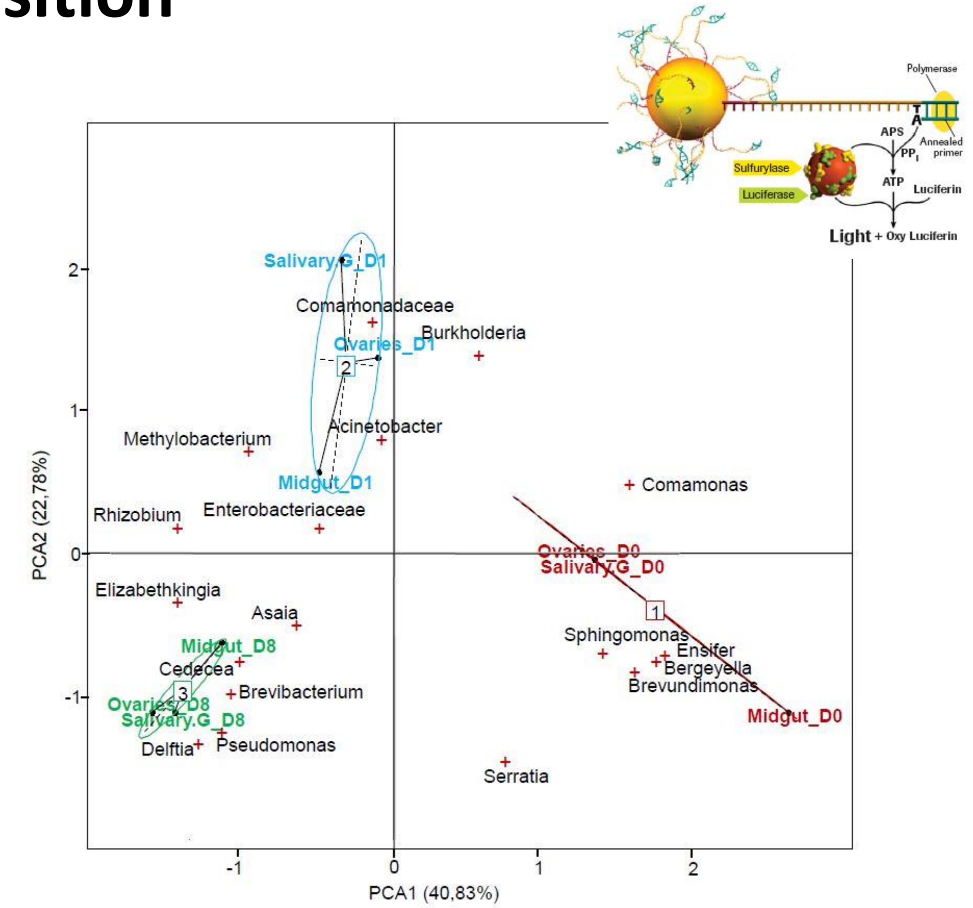
■ Pseudomonas
■ Serratia
■ Intrasporangiaceae_Y
■ Arthrobacter
■ Ralstonia
■ Comamonas
■ Brevibacterium
■ Ensifer
■ Ramlibacter
■ Citrobacter
■ Acinetobacter
■ Comamonadaceae
■ Bergeyella
■ Bacillus
■ Gluconacetobacter
■ Rhizobium
■ Enterobacteriaceae
■ Asaia
■ Streptococcus
■ Enterobacter
■ Burkholderia
■ Sphingomonas
■ Escherichia-Shigella
■ Staphylococcus
■ Chryseobacterium
■ Elizabethkingia
■ Delftia
■ Sphingobium
■ Propionibacterium
■ Micrococcus
■ Cedecea
■ Brevundimonas
■ Devosia
■ Nocardioides
■ Bacteroides
■ Methylobacterium
■ Microbacterium
■ Corynebacterium
■ Parabacteroides
■ Aeromonas

Microbiota composition

The bacterial flora was mainly composed of Gram-negative communities

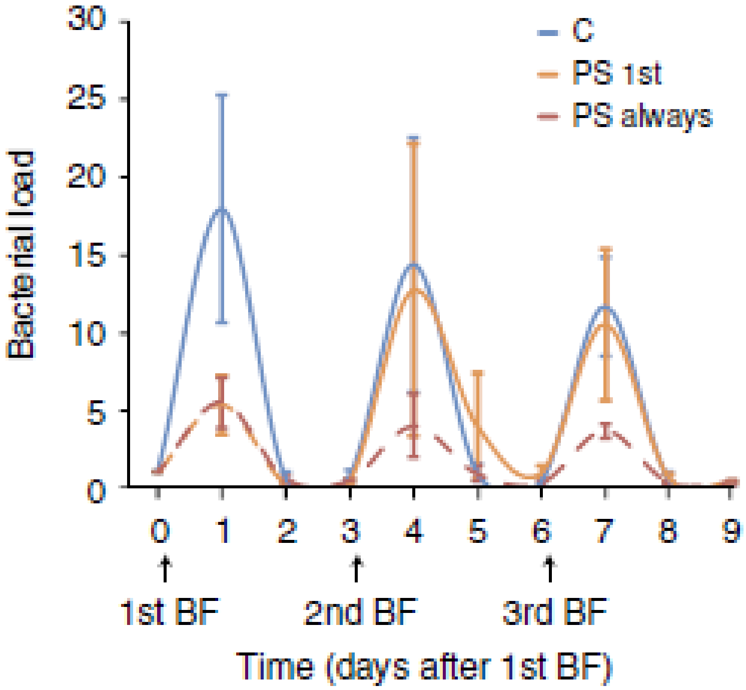
The microbial communities in the epithelia are structured according to the physiological status of the mosquito.

Some bacteria taxa were more associated with one or another tissue at a particular time point



Tchioffo et al., *2014*

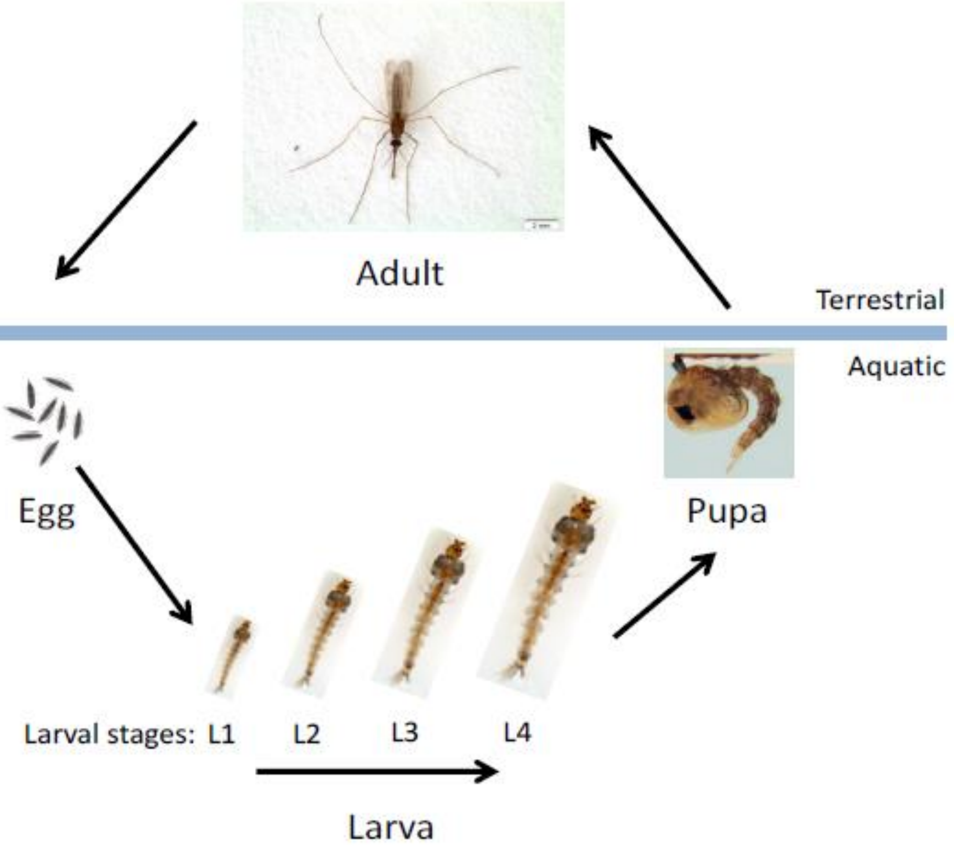
The bloodmeal reshapes bacterial communities



Upon blood feeding:

- reduction in diversity/richness
- bacterial proliferation, mainly Enterobacteriaceae (*Klebsiella, Serratia, Enterobacter,* …)

Impact of bacterial communities for the mosquito



From antibiotic treated mosquitoes to clear the microbiota

On development : axenic larvae fail to develop beyond the first instar, but bacterial recolonization rescues larval growth (Coon et al. 2014)

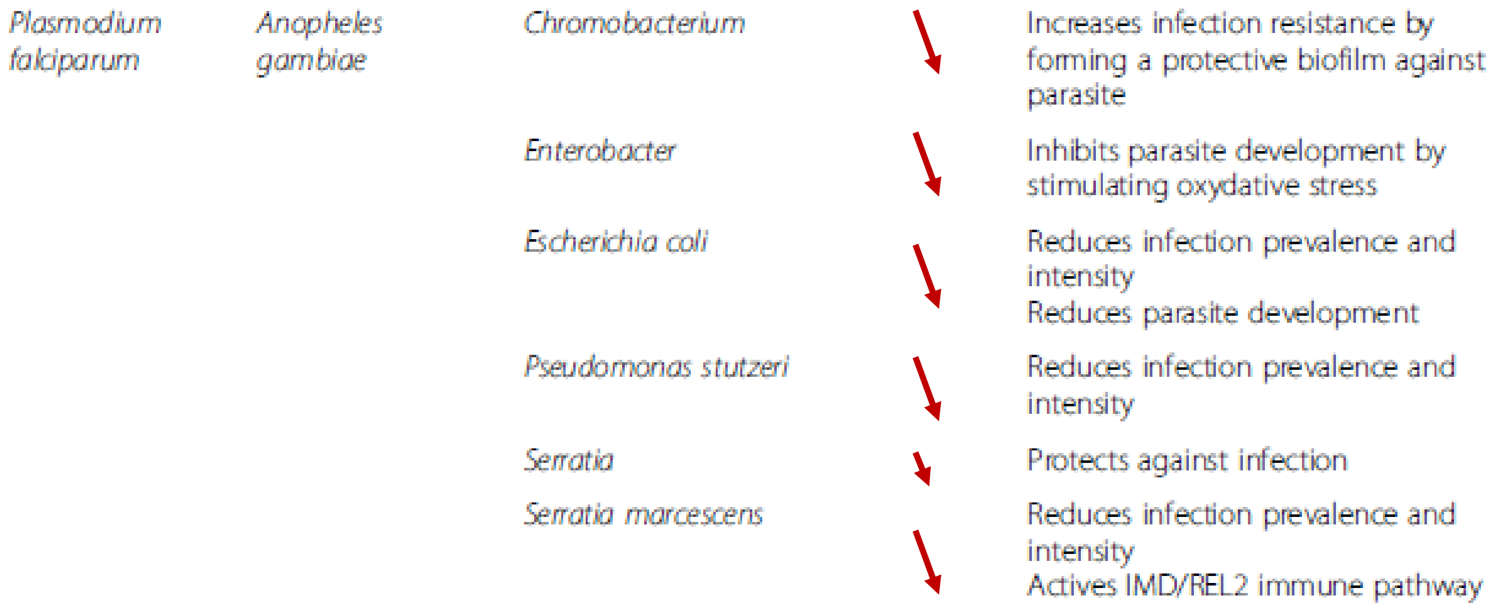
On lifespan and fecundity : supplementation of antibiotics in the blood meal increases fecundity and survival (Gendrin et al. 2015)

On mosquito resistance to insecticides : Antibiotic supplementation via sugar decreased tolerance to the insecticides (deltamethrin and malathion)

On infection : Antibiotic treatment increases mosquito susceptibility to *Plasmodium* infection (Beier et al. 1994, Dong et al. 2009, Gendrin et al. 2015)

➡ Microbiota protects the mosquito from *Plasmodium* infection but the natural microbiota proliferation may decrease mosquito population size and time available for parasite transmission

Bacteria that interfere with *Plasmodium* sporogonic development



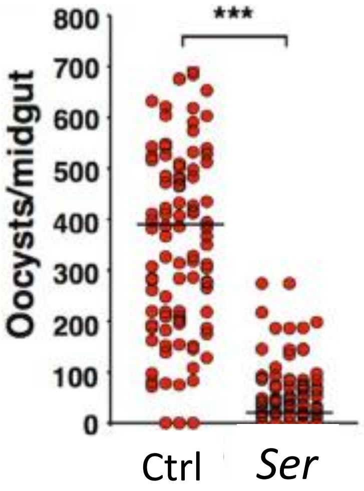
Plasmodium falciparum	*Anopheles gambiae*	*Chromobacterium*	↘	Increases infection resistance by forming a protective biofilm against parasite
		Enterobacter	↘	Inhibits parasite development by stimulating oxydative stress
		Escherichia coli	↘	Reduces infection prevalence and intensity Reduces parasite development
		Pseudomonas stutzeri	↘	Reduces infection prevalence and intensity
		Serratia	↘	Protects against infection
		Serratia marcescens	↘	Reduces infection prevalence and intensity Actives IMD/REL2 immune pathway

Asaia, *Enterobacter*, *Pantoea*, *Serratia* and *Pseudomonas* have been proposed as promising symbiotic agents for blocking malaria transmission

Discrepancies of results between laboratories or models

Contrasting effect according to experimental setups (experimental oral infection versus natural microbiota)

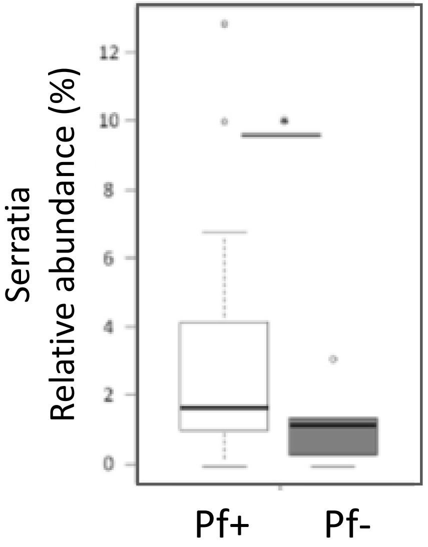
Oral challenge to bacterial cultures



Bando *et al*. 2013

Negative impact: *Serratia marcescens* reduces the *Plasmodium* parasite load

Correlation on field collected mosquitoes



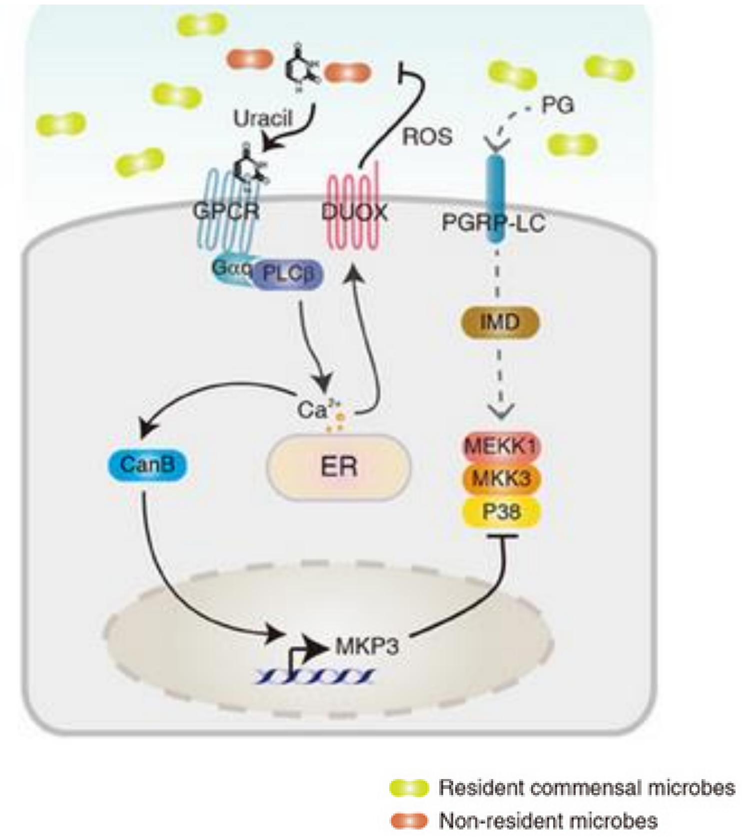
Tchioffo et al. *2014*

Serratia have a **protective role** on natural *P. falciparum* infections

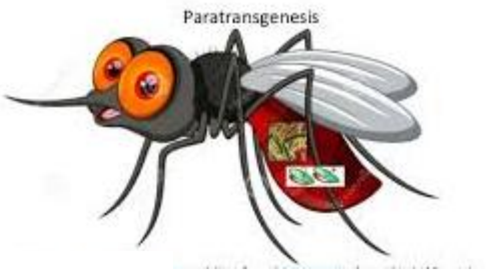
How mosquito microbiota protects against *Plasmodium* infection

- The natural microbiota stimulates basal immune activity that controls its proliferation, maintaining homeostasis
- Blood feeding induces high levels of oxidative stress, released heme reduces ROS generation and contributes to bacterial proliferation
- A dityrosine network (Duox/Peroxidase) decreases midgut permeability to bacterial elicitors, protecting the microbiota but also favoring *Plasmodium* development

Homeostasis



Fighting malaria with paratransgenesis



« An ideal paratransgenic candidate is one that can be genetically modified, colonizes vector mosquitoes in the same body compartments where pathogens develop, spreads through mosquito populations, and is not a human pathogen”

Basic requirements for paratransgenesis

i	Stable symbiotic relationship between microorganism and vector
ii	Symbiotic microorganism can be cultured *in vitro* and genetically manipulated
iii	Effector gene product should not impair symbiont and vector fitness
iv	Effector gene product should be secreted to assure interaction with the target pathogen
v	An efficient means of introducing the engineered symbiont into field must be devised

Elizabethkingia anophelis, a bacterium in the microbiome of lab-reared Anopheles mosquitoes

E. anopheles, Flavobacteria*,* is the predominant bacterial species in insectary-reared *Anopheles*, also found in field mosquitoes

Cultivable and amenable to genetic manipulation

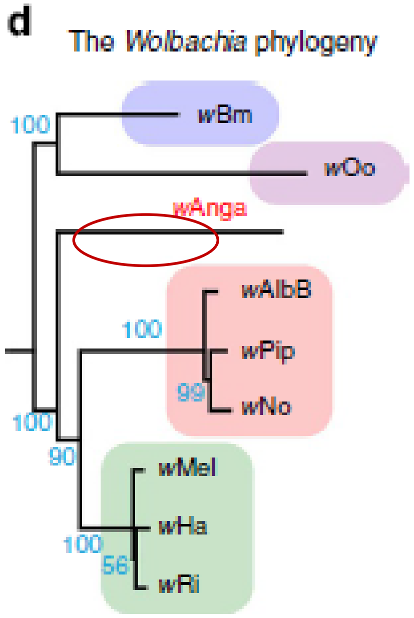
Could be a good candidate for paratransgenesis BUT

Outbreaks of *E. anophelis* infections in humans have been described in Singapore, in the Midwestern United States, Congo, (meningitis and respiratory infections in new born babies and people with weakened immune systems) …

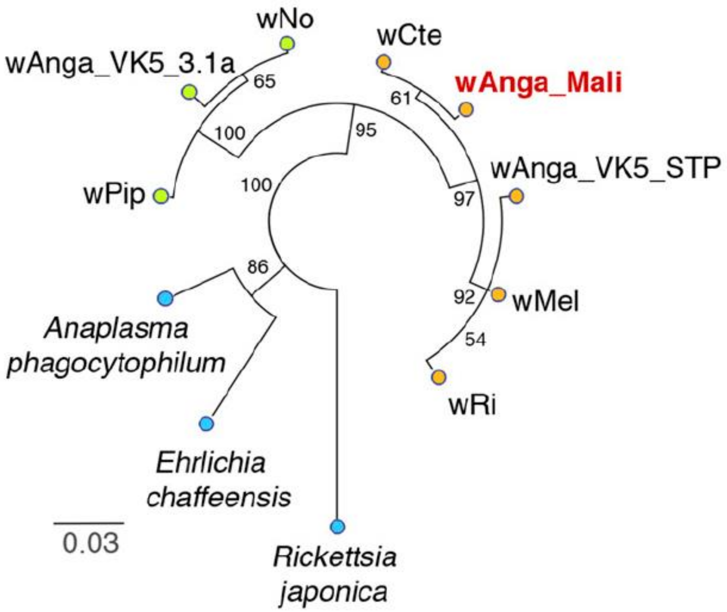
A multiresistant bacteria, an emerging nosocomial pathogen

Wolbachia and *Anopheles*

- Field populations of anophelines were thought to be resistant to *Wolbachia* infections
- In 2014 : first evidence of *Wolbachia* sequences in *Anopheles gambiae* in Burkina Faso (Baldini et al. 2014)
- A new strain, *w*Anga, highly divergent from *Wolbachia* strains isolated in other insects

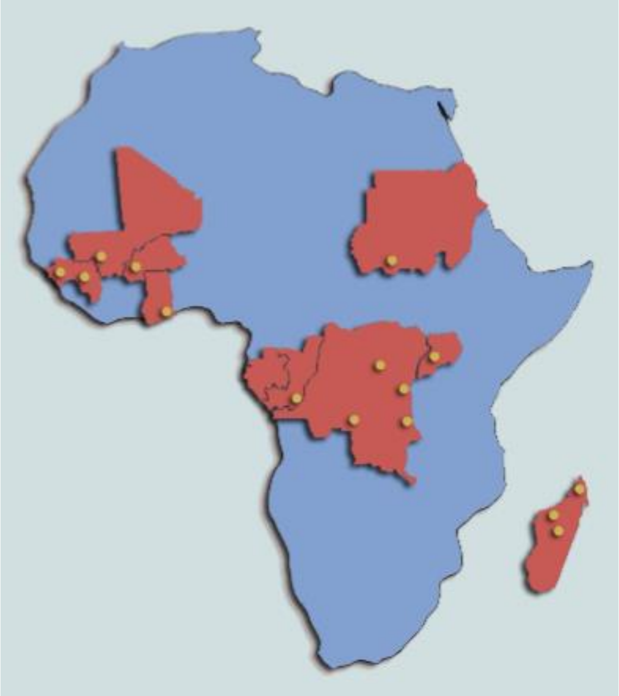


Baldini *et al.* Nature Com 2014



Gomes *et al.* PNAS 2017

Wolbachia prevalence in *Anopheles*



Wolbachia infection prevalence varies greatly among *Anopheles* species, country, season….

Wolbachia infection is commonly lower than 15% and
Wolbachia is present at low density (<1% of mosquito genome copies)

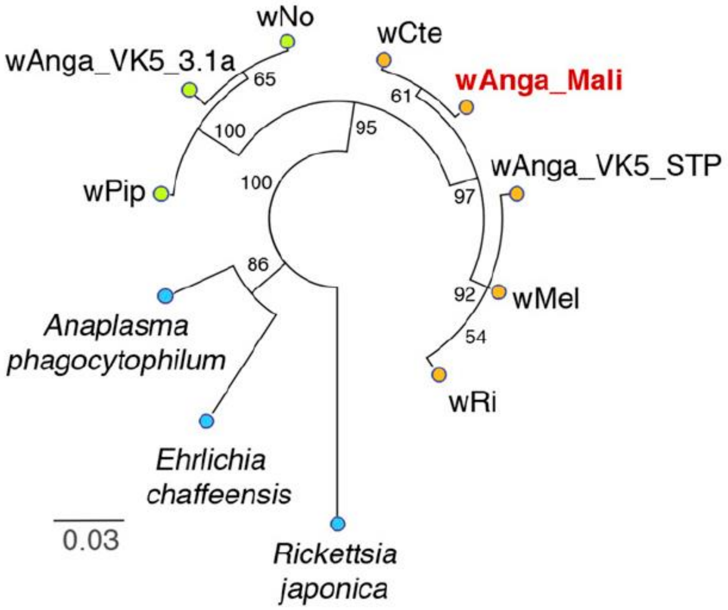
- Shaw *et al.* Nature Com 2016 : In BF, Vallée du Kou, wAnga at a frequency of 33% (16/49) in *A. arabiensis,* 46% (275/602) in *An. coluzzii*
- Baldini *et al.* Nature Com 2014: Prevalence of Wolbachia from *An. gambiae*: in VK7, 7,1% (3/42), Soumousso 4,2% (1/24), VK5 86% (6/7)
- Gomes *et al.* PNAS 2017 : In Mali, prevalence of Wolbachia infection in *A. gambiae* (53.6%)
- Niang *et al*. Mal J 2018 : In Senegal: frequency of Wolbachia infection in *A. funestus* is of 1.2% (3/121)
- Ayala *et al.* Mal J 2019 : In Gabon, 10.8% (70/648) *Wolbachia*-positive specimens that belonged to 16 different *Anopheles* species and *An. moucheti* and *An. nili* have higher infection rates (>50%)
- Baldini *et al. Parasites & Vectors* 2018 : In Tanzania: wAnga infection prevalence in *An. arabiensis* was 3.1% (n = 65, 2014) and 7.5% (n = 147, 2016) ; no infection was detected in *An. funestus* (n = 41)
- Sawasdichai *et al*. 2019 : in Myanmar, *Wolbachia* DNA was detected in 3.5% (13/370) samples

Phylogeny of *Wolbachia from Anopheles*

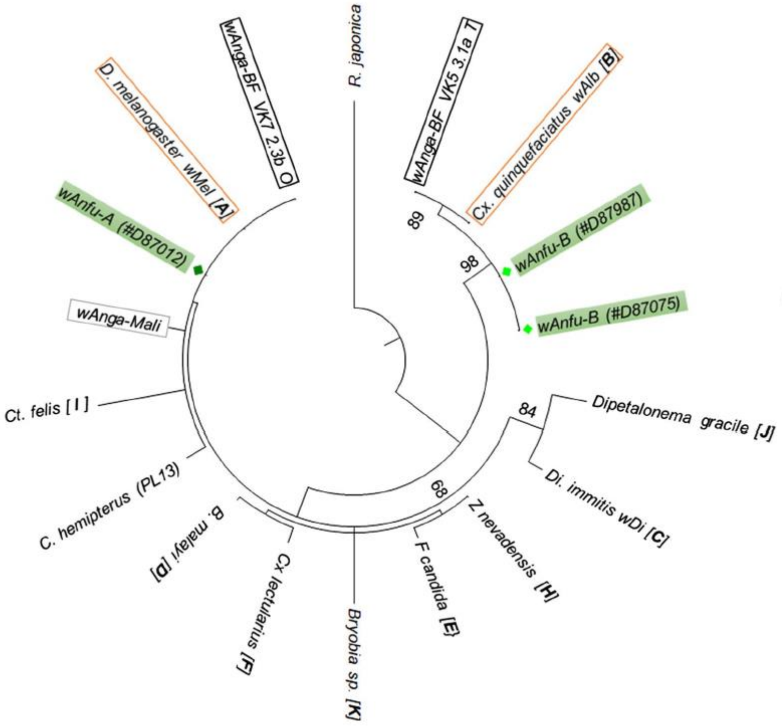
Anopheles-infecting *Wolbachia* bacteria show high genetic diversity

Anopheles species are infected by different *Wolbachia* strains

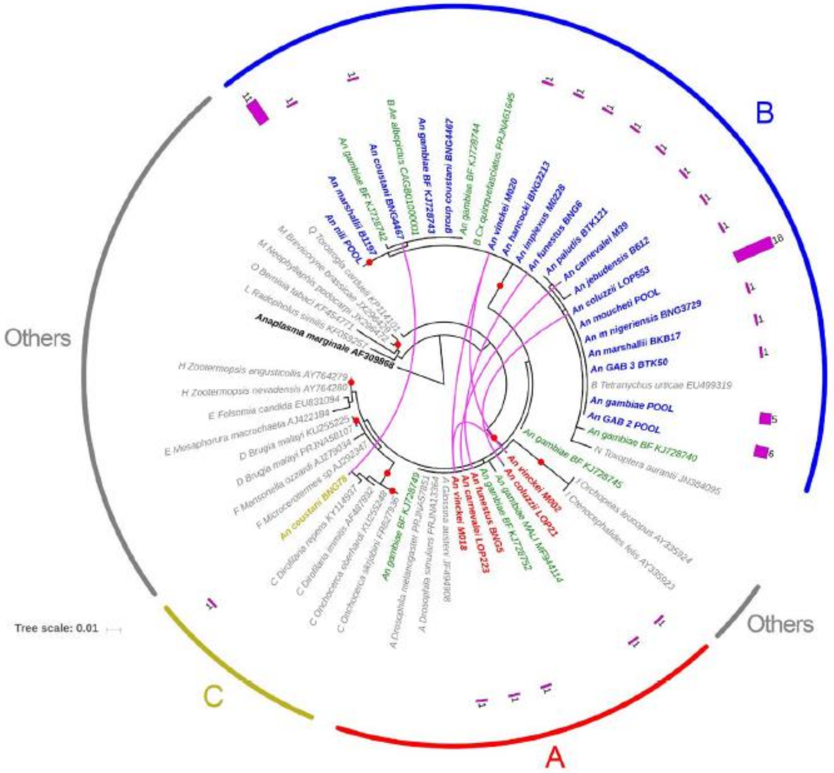
Wolbachia acquisition seems to be independent of the mosquito phylogeny



Gomes *et al.* PNAS 2017

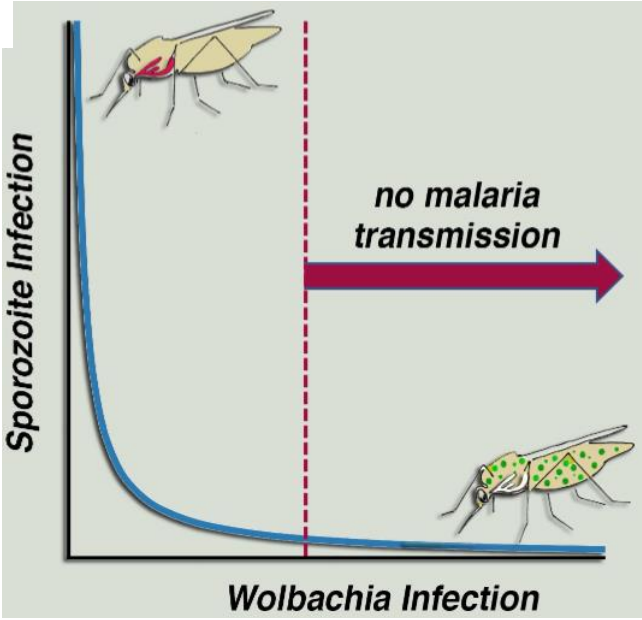


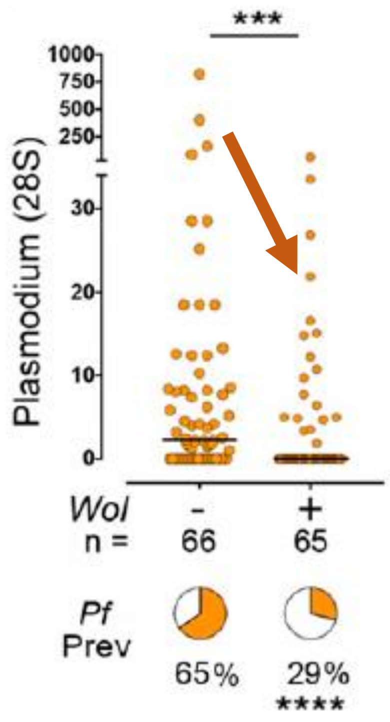
Niang *et al.* Mal J 2018



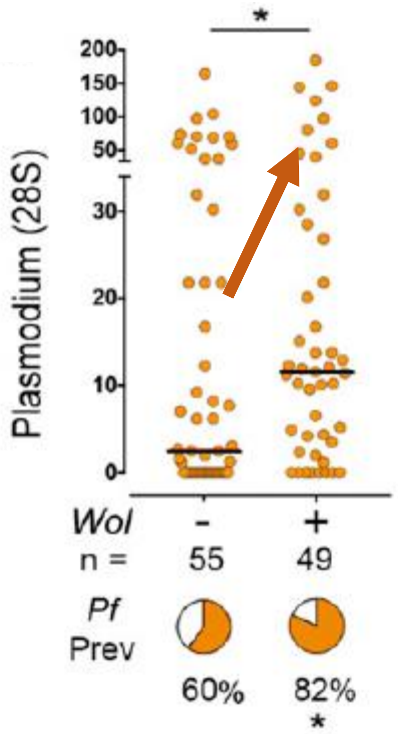
Ayala *et al.* Mal J 2019

Wolbachia and *Plasmodium* infections





Plasmodium sporozoite infection

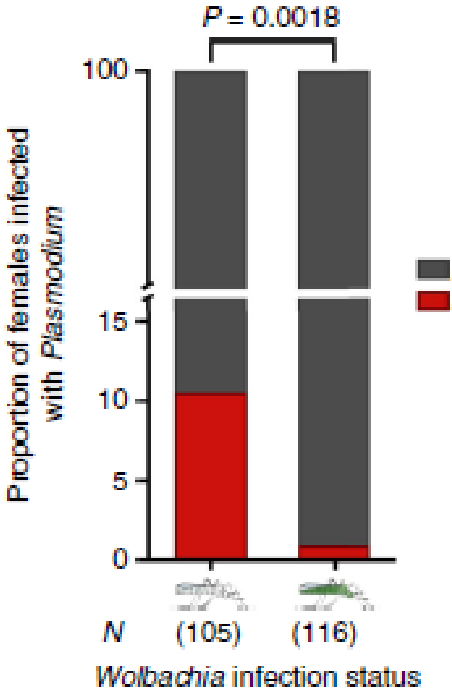


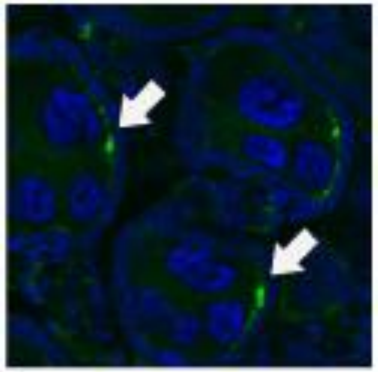
Plasmodium oocyst infection

Gomes *et al.* PNAS 2017

Wolbachia infection negatively impacts *Plasmodium* sporozoite infection in *A. coluzzii* but the effect is opposite at the oocyst stage, with an enhanced oocyst infection

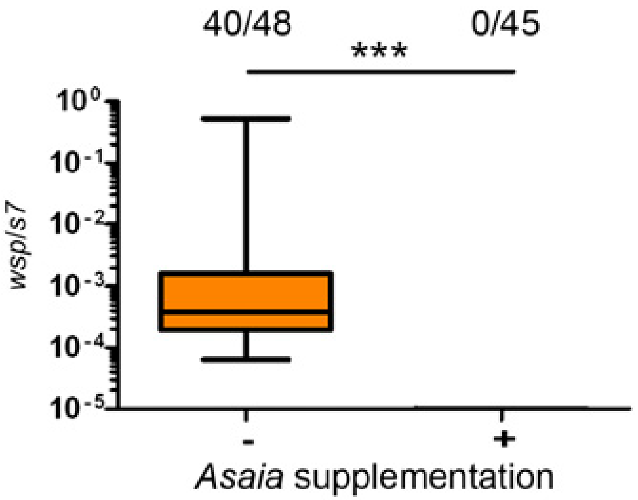
Wolbachia and *Anopheles*





In *Anopheles* (BF, VK5):
- *Wolbachia* infection significantly reduces the prevalence and intensity of sporozoite infection
- BUT no cytoplamic incompatibility, just accelerates egg laying

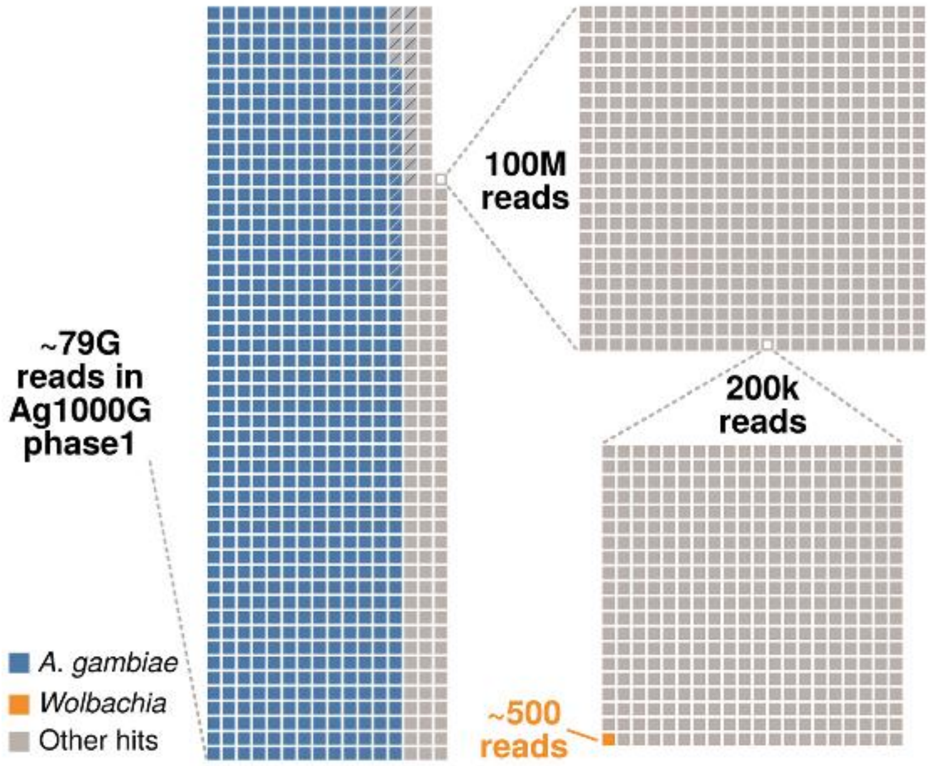
Shaw et al.2016



The native mosquito microbiota can impede *Wolbachia* transmission in *Anopheles*

Hughes et al.PNAS 2014

Is *Anopheles gambiae s.l.* a natural host of *Wolbachia*?



Chrostek mBio, 2019

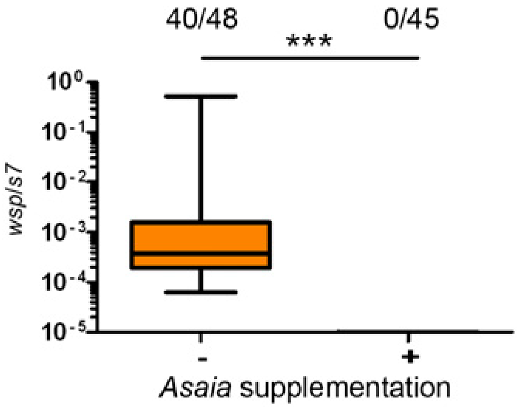
- ☐ From data generated in the Ag1000G project, (Illumina sequences of 765 wild-caught mosquitos from 8 African countries, 79G reads total): less than 500 reads map to *Wolbachia*
- ☐ No single *Wolbachia* strain associated with *Anopheles gambiae*
- ☐ Conflicting phylogenetic affiliations of the sequenced strains (span at least two *Wolbachia* supergroups)
- ☐ The presence of *Wolbachia* sequences in *An. gambiae* tissues can be a contamination

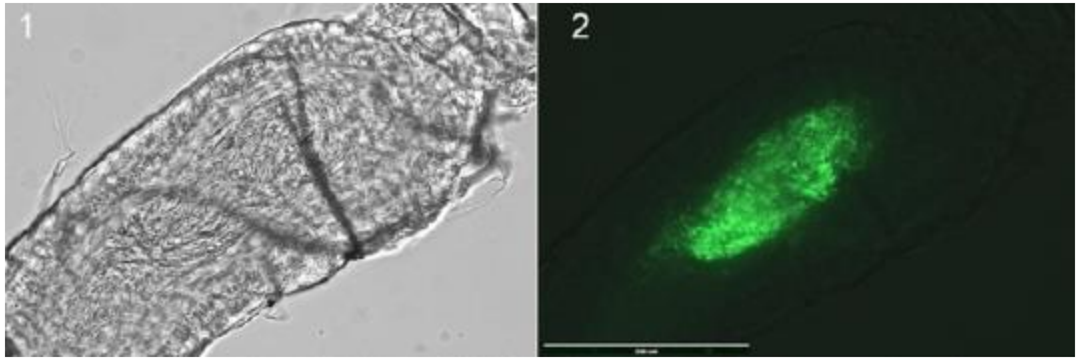
The mosquito microbiota can impede *Wolbachia* transmission ?

Asaia spp. and *Anopheles*

Asaia inhibits *Wolbachia* transmission in *Anopheles* (Hughes et al., PNAS 2014),

No *Wolbachia/Asaia* co-infections have been reported so far





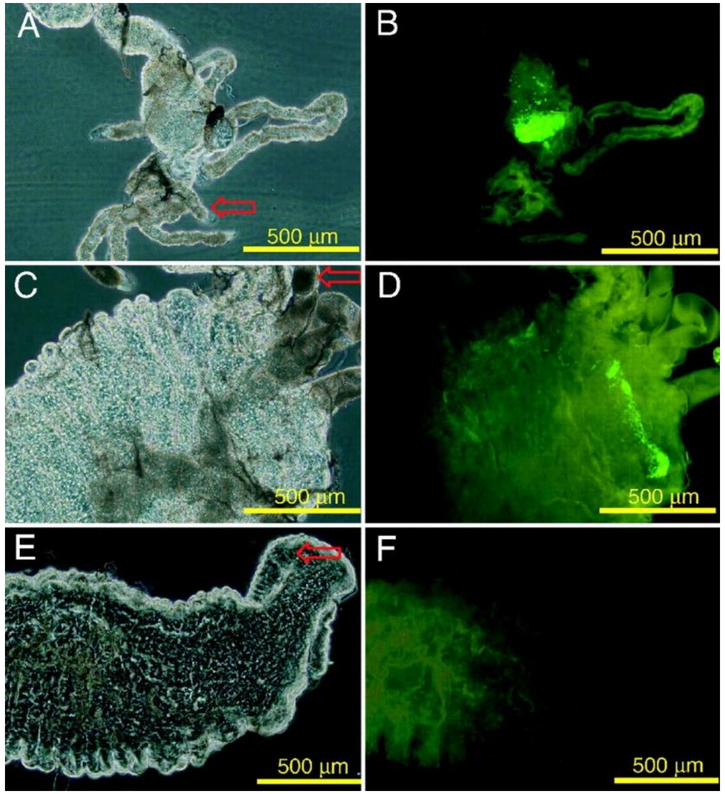
Asaia (Acetobacteraceae) : a genus of acetic acid bacteria, also isolated from tropical plants

Dominant bacteria colonizing mosquito midgut, ovaries/testis and salivary glands

Asaia is transmitted vertically from mother to progeny and horizontally through feeding on nectars

An easily cultivable and transformable bacterium (Favia et al. 2007)

Asaia, a suitable agent for the paratransgenic control ?



Favia et al. 2007

Asaia sp. SF2.1 isolated from *An. stephensi*

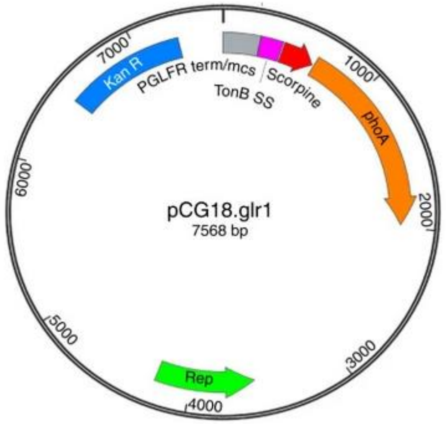
- Cultivable and transformable (Favia et al. 2007)

- Express antiplasmodial effector molecules constitutively (Bongio et al. 2015)

- Constructs with blood meal induced (BMI) promoters inhibit *Plasmodium* infection and transgenic bacteria compete effectively with wild type *Asaia* (Shane et al. 2018)

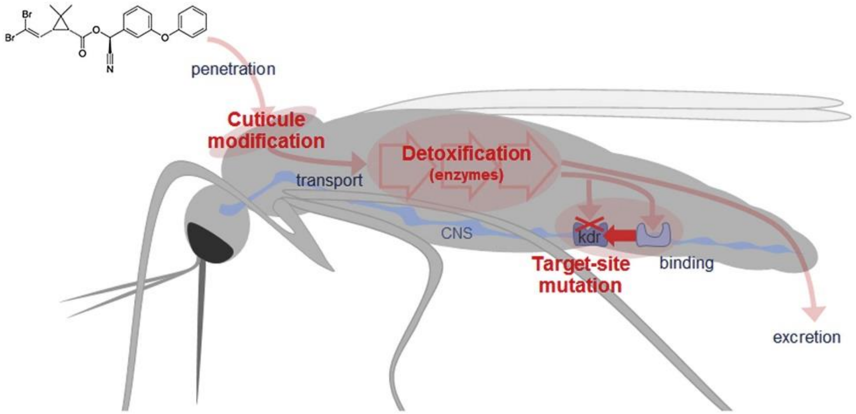
Next:

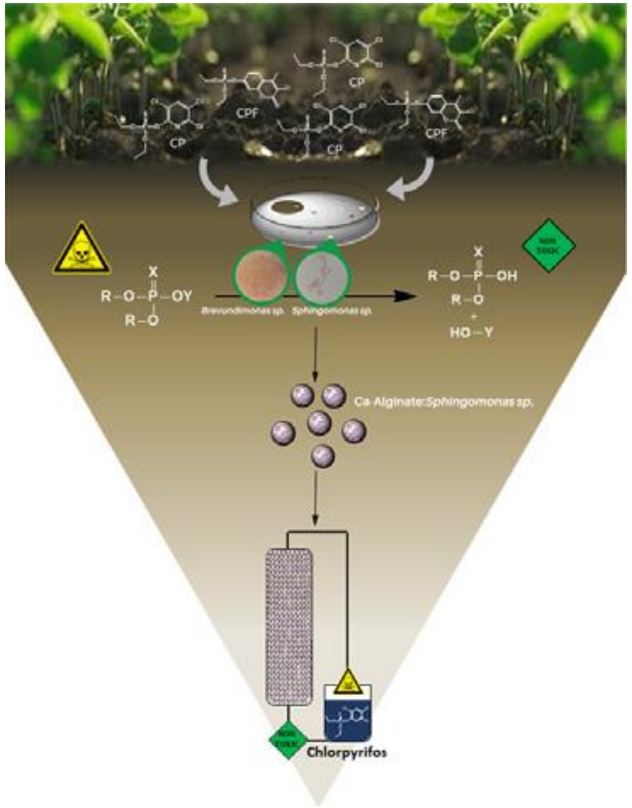
- Effector genes to be inserted into the *Asaia* chromosome of field strains where they can be inherited stably
- Express more than one antiplasmodial effector gene simultaneously to suppress the evolution of resistance by *Plasmodium* to the antiplasmodial products



Shane et al. 2018

Microbiota and insecticide resistance





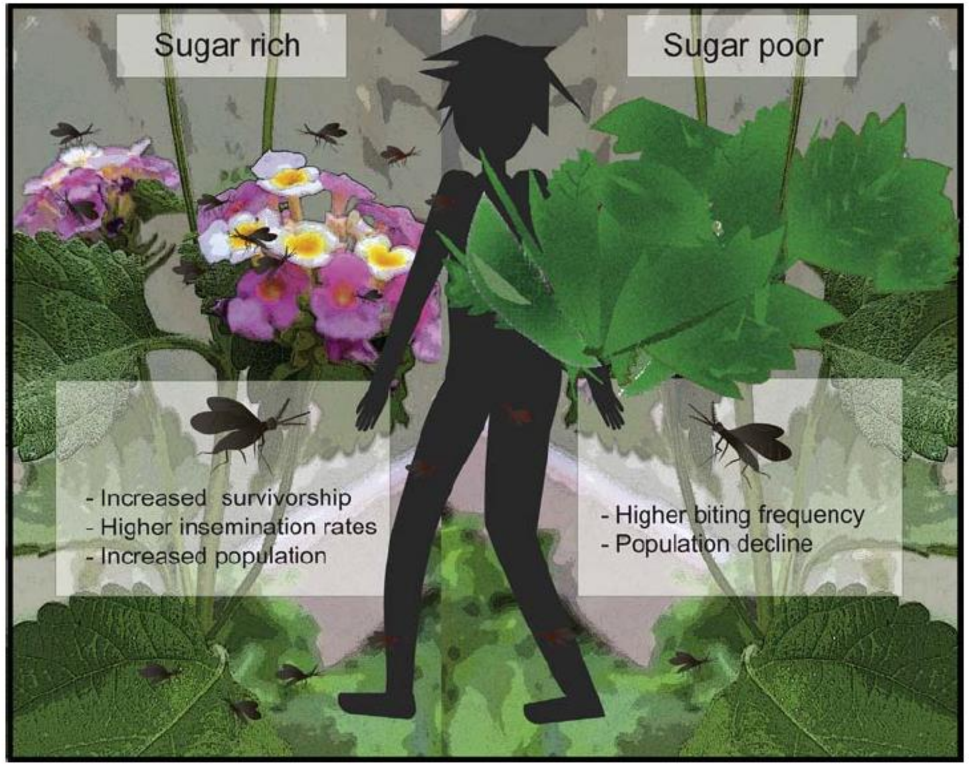
Mosquito microbiota is colonized by insecticide-degrading bacterial species :
Pantoea, Pseudomonas, Sphingomonas, Burkholderia, Bacillus, Klebsiella and *Serratia*

Insecticide exposure leads to shifts in microbial composition (Dada et al. 2019)

Insecticide resistant strains have lower bacterial diversity, but enrichment of insecticide degrading bacteria such as *Klebsiella* an OP-degrading species (Dada et al. 2018, Barnard et al. 2019)

Microbial communities have a role in insecticide resistance phenotype
Bacteria could be manipulated to produce toxins with novel insecticidal activity

Eco-holobiont approach, the microbiome of an entire ecosystem



Barredo et al. Trends Parasitol 2020

An. gambiae s.s. has a preference for specific plants : *Mangifera indica*, *Thevetia neriifolia*, *Senna siamea*, *Parthenium hysterophorus, Ricinus cummunis* (Manda et al. 2007, Gouagna *et al*. 2010)

The source of plant sugar meal affects

- Survival and fecundity
- Biting frequency

Pollen and nectar microbiomes may be important microbial contamination sources for mosquitoes (Asaia)

Plants can serve as a reservoir for horizontal transmission of bacteria (Caspi-Fluger et al.2012, Kenney et al. 2017)

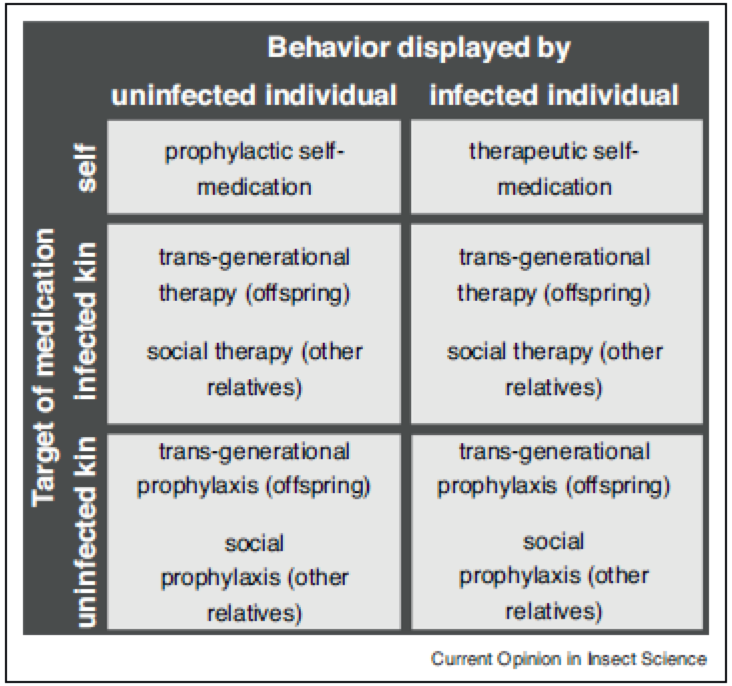
Plant bacteria could be used to express effector molecules interfering with the mosquito physiology

Self-medication as a defense against pathogen infection

The source of plant sugar meal affects mosquito fitness and also *Plasmodium* infection level (Hien *et al*. 2016)

Plant secondary metabolites may impact on the gut microbiota dynamics and contribute to pathogen development/inhibition

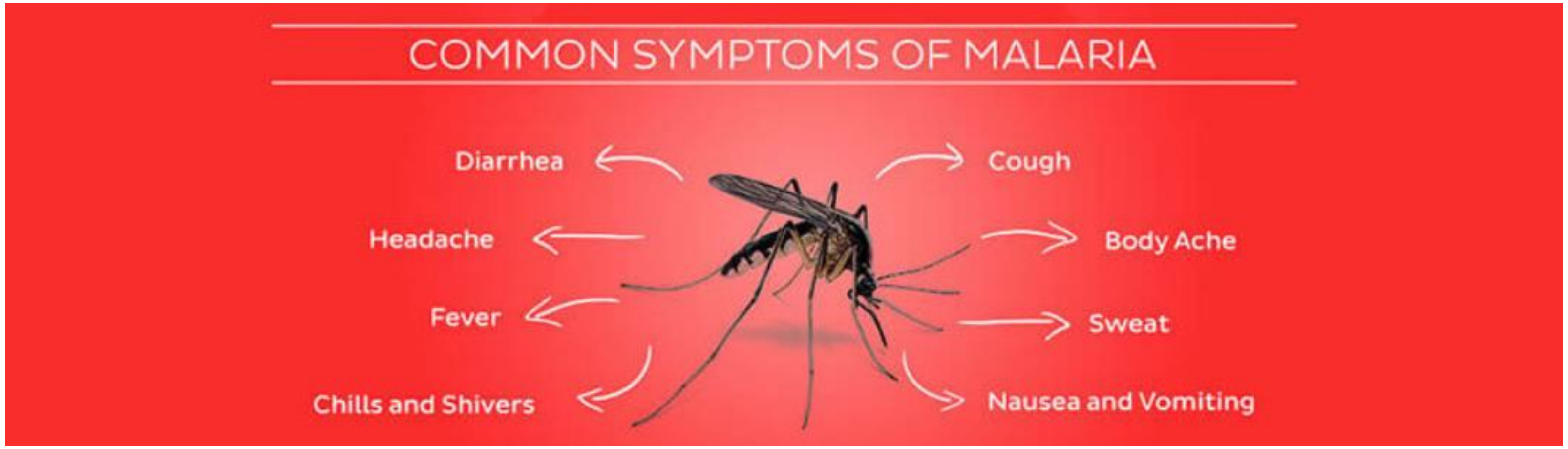
Can mosquitoes use medication to increase resistance or tolerance to infection ? (de Roode et al. 2019)



		Behavior displayed by	
		uninfected individual	infected individual
Target of medication	self	prophylactic self-medication	therapeutic self-medication
	infected kin	trans-generational therapy (offspring) social therapy (other relatives)	trans-generational therapy (offspring) social therapy (other relatives)
	uninfected kin	trans-generational prophylaxis (offspring) social prophylaxis (other relatives)	trans-generational prophylaxis (offspring) social prophylaxis (other relatives)

Current Opinion in Insect Science

The best microbiota : to cure or kill the mosquito ?



Thanks for attention !