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With the participation of UMR CNRS 5558 Laboratoire de Biométrie et  
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This book was designed by  
Christelle Mirebeau, EBI, UMR CNRS 7267, Poitiers  
and edited by Atlantique  
Editions de l'Actualité scientifique Poitou-Charentes,  
Espace Mendès France - 1 place de la Cathédrale, 86000 Poitiers  
Imprimerie Copy-Media - Mérégnac  
ISBN 978-2-911320-44-6

## PROGRAMME

### Thursday, June 7<sup>th</sup>

- 14:00-19:00 Meeting point: La Rochelle (Bus rides to St Pierre d'Oléron available) Registration
- 19:00-21:00 : Dinner
- 21:00-22:00 : Welcome and social hour at the Conference centre

### Friday, June 8<sup>th</sup>

- 8:30-9:00 : Official welcome and opening remarks

#### Session ECOLOGY & PHENOTYPES Chairman : Kostas BOURTZIS

- 9:00-9:20 : CHARLAT S., *Wolbachia* horizontal transfer
- 9:20-9:40 : GERTH M., Horizontal *Wolbachia* transfer among bees (*Anthophila*) and their kleptoparasites assessed with MLST data
- 9:40-10:00 : RIEGLER M., *Wolbachia* in drosophilid and tephritid fruit flies- same but different?
- 10:00-10:20 : DUMAS E., Structure of *Wolbachia* diversity in the *Culex pipiens* mosquito complex

#### **10:20-10:50 : Coffee break**

- 10:50-11:10 : XIAO JH., Rapid evolution of *Wolbachia* sequences inside the compact fig syconia of *Ficus benjamina* (Ficus: Moraceae)
- 11:10-11:30 : BOURTZIS K., Tsetse-*Wolbachia* symbiotic associations: from comparative *Wolbachia* genomics to mating incompatibility and applications
- 11:30-11:50 : SCHNEIDER DI., Scent matters: the impact of mutualistic *Wolbachia* on chemical communication and mate choice in the *Drosophila paulistorum* species complex
- 11:50-12:10 : BROWNLIE JC., Mind bending *Wolbachia* - exploring *Wolbachia*'s effect on *Drosophila* behavior

12:10-12:30 RICHARD FJ., Smelling from *Wolbachia*: sexual selection in terrestrial isopods

**12:30-14:00 : Lunch**

**Session ECOLOGY & PHENOTYPES**

**Chairman : Didier BOUCHON**

14:00-14:20 : JOHNSON KN., Density of *Wolbachia* in the host insect impacts antiviral protection

14:20-14:40 : HEATON L., *Wolbachia* increases disease resistance and wound healing in bedbugs *Cimex lectularius*

14:40-15:00 : TRUITT A., Investigating the affects of *Wolbachia* on population declines of a threatened butterfly species

15:00-15:20 : DITTMER J., Bacterial communities influenced by *Wolbachia*? Bacterial density and community structure in terrestrial isopods

**15:20-16:20 : Poster session (Coffee break)**

**Session EVOLUTION**

**Chairman : Sylvain CHARLAT**

16:20-16:40 : WERREN J., *Wolbachia* Diversity

16:40-17:00 : ZUG R., The *Wolbachia* pandemic among arthropods: re-estimating the proportion of infected species and modeling horizontal transmission in an epidemiological framework

17:00-17:20 : LE CLEC'H W., High virulence of *Wolbachia* after host switching: Tolerance versus Lethal autophagy

17:20-17:40 : LEFOULON E., A new *Wolbachia* type F from Splendidofilariinae (Onchocercidae) supports recent emergence of this supergroup

**19:00-21:00 : Dinner**

**21:00-22:00 : Poster session and social hour**

## Saturday, June 9<sup>th</sup>

### Session EVOLUTION Chairman : Takema FUKATSU

- 8:30-8:40 : Announcements
- 8:40-9:00 : MENDES C.S.M., Effects of heterogeneity in invasion by *Wolbachia*
- 9:00-9:20 : GJINI E., *Wolbachia* diversity, invasion and evolution
- 9:20-9:40 : MILLER WJ., The impact of *Wolbachia* on triggering sexual isolation and adaptive behavioral changes in Insects
- 9:40-10:00 : METCALF JA., Splicing the tree of life: Recurrent horizontal gene transfer of a *Wolbachia* lysozyme between Bacteria, Archaea, and Eukaryotes

#### **10:00-10:30 : Coffee break**

- 10:30-10:50 : MARTINEZ-RODRIGUEZ P., Phylogeographic analysis of European populations of *Wolbachia* infecting *Chorthippus parallelus* (Orthoptera)
- 10:50-11:10 : FUKATSU T., Lateral gene transfers underlie *Wolbachia*-mediated novel host phenotypes
- 11:10-11:30 : FLORES HA., Evolutionary analysis of the bag of marbles gene reveals an interaction with *Wolbachia*
- 11:30-11:50 : FUNKHOUSER LJ., Mom knows best: Maternal regulation of *Wolbachia* titers

#### **12:00-13:00 : Lunch**

- 13:00-19:30 : Excursion to La Rochelle city and aquarium (Bus rides available) or boat trip or visit of the oyster farming site

#### **19:30-21:00 : Dinner**

#### **21:00-22:00 : Poster session and social hour**

## Sunday, June 10<sup>th</sup>

### Session GENOMICS Chairman : Benjamin MAKEPEACE

- 8:30-8:40 : Announcements
- 8:40-9:00 : CORDAUX R., The impact of *Wolbachia* endosymbionts on the evolution of sex determination in the isopod *Armadillidium vulgare*
- 9:00-9:20 : GRÈVE P., The genome sequence of the feminizing *Wolbachia* wVulC from the isopod crustacean *Armadillidium vulgare* identifies multiple secretion/export systems in a bacterial endosymbiont
- 9:20-9:40 : KLASSON L., Comparative genomics of supergroup A and B *Wolbachia* strains infecting the same host species
- 9:40-10:00 : SHIRK P.D., Genomic sequencing of cell line cultured *Wolbachia* originating from *Ephestia kuehniella* and *Aedes aegypti*

### **10:00-10:30 : Coffee break**

### Session GENOMICS Chairman : Clément GILBERT

- 10:30-10:50 : MAKEPEACE BL., Global gene expression in *Wolbachia*: taking a sledgehammer to a supergroup C nut
- 10:50-11:10 : CERVEAU N., High abundance of insertion sequences in *Wolbachia* endosymbiont genomes: evolutionary causes and dynamics
- 11:10-11:30 : BORDENSTEIN S., Three Rules of Bacteriophage WO Evolution
- 11:30-11:50 : WOOLFIT M., Adaptation in *Wolbachia* genomes
- 11:50-12:10 : DUNNING HOTOPP JC., Lateral gene transfer from a *Wolbachia* endosymbiont to *Drosophila ananassae*: A tale of extensive duplication and epigenomic variation
- 12:10-12:35 : BARTON S., A Laterally Transferred Ferrochelatase Gene is Functional And Essential in Filarial Nematode Parasites

### **12:30-14:00 : Lunch**

**Session CELL BIOLOGY**  
**Chairman : Pierre GRÈVE**

- 14:00-14:20 : KOZEK W., *Wolbachia* and filarial hypodermis revisited
- 14:20-14:40 : GENTY L., *Wolbachia* colonization in *Armadillidium vulgare*: patterns and dynamics
- 14:40-15:00 : TOOMEY M., Men are messy: *Wolbachia* stem cell niche tropism in *Drosophila* is evolutionarily conserved only in females
- 15:00-15:20 : VORONIN D., Autophagy regulates *Wolbachia* populations across diverse symbiotic associations

**15:20-16:20 : Poster session (Coffee break)**

**Session CELL BIOLOGY**  
**Chairman : Abdelaziz HEDDI**

- 16:20-16:40 : MONÉ Y., Evolution and control of host-microbe symbiosis in arthropods: an RNAseq-based transcriptomics analysis
- 16:40-17:00 : SIMHADRI RK., Molecular mechanisms for *Wolbachia* tissue tropism in the gonads of *Drosophila melanogaster*
- 17:00-17:20 : SERBUS L., Host factors that influence *Wolbachia* titer and position in the *Drosophila* germline and somatic tissues
- 17:20-17:40 : LUSTIGMAN S., Mapping protein interactions between filaria and its *Wolbachia* endosymbiont
- 17:40-18:00 : PFARR K. Characterization of the peptidoglycan precursor Lipid II its biological role in *Wolbachia*

**19:00-21:00 : Dinner**

**21:00-22:00 : Poster session and social hour**

**Monday, June 11<sup>th</sup>**

**Session SYMBIONT DIVERSITY, DISTRIBUTION AND DYNAMICS**  
**Chairman : Olivier DURON**

- 8:30-8:40 : Announcements
- 8:40-9:00 : ZAKHAROV-GEZEKHUS IA., Symbiotic bacteria as a factor of gene pool dynamics in insect populations
- 9:00-9:20 : PARRATT SR., Near but far: Phenotype and genome comparisons reveal diversification between closely related members of *Arsenophonus nasoniae* clade
- 9:20-9:40 : WHITE J., Facultative symbiont distribution among global populations of *Aphis craccivora*: host plant is more important than geography

- 9:40-10:00 : MERVILLE A., Endosymbiont diversity among weevil sibling species competing for the same food resource
- 10:00-10:20 : MINARD G., *Acinetobacter* is more prevalent than *Asaia* in field populations of the mosquito vector *Aedes albopictus* with isolates showing diverse genetic rearrangements and metabolic profiles

**10:20-10:50 : Coffee break**

**Session PEST CONTROL**  
**Chairman : Patrick MAVINGUI**

- 10:50-11:10 : FAILLOUX AB., Differential effects of the native *Wolbachia* symbionts on transmission of arboviruses in the mosquito *Aedes albopictus*
- 11:10-11:30 : CHROSTEK E., *Wolbachia* wMel substrains confer differential protection to viruses
- 11:30-11:50 : ZÉLÉ F., Implication of *Wolbachia* on malaria (*Plasmodium* sp.) transmission by mosquitoes

**12:00-14:00 : Lunch**

**Session PEST CONTROL**  
**Chairman : Stephen DOBSON**

- 14:00-14:20 : RASGON JL., Eliminating barriers to *Wolbachia* germline infection and vertical transmission in *Anopheles gambiae*
- 14:20-14:40 : HERREN J., Mechanisms of *Spiroplasma* transmission to developing oocytes in *Drosophila melanogaster*
- 14:40-15:00 : DOBSON SL., Applied use of *Wolbachia* as a 'microbial pesticide' to control medically important *Aedes mosquitoes*
- 15:00-15:20 : CRAIN PR., Theoretical assessment of population replacement when *Wolbachia* negatively affects host life history
- 16:00-17:00 : Communication and poster awards & next *Wolbachia* congress (Coffee break)

**17:00-23:00 : Winery discovering, Pineau and Cognac degustation, and Gala Dinner, local foods and wines at La Cave des Alletières, La Brée les Bains (Bus rides available)**

**Tuesday, June 12th**

**7:30-8:20 : Breakfast**  
**Bus departure to La Rochelle**

## **ECOLOGY & PHENOTYPES**

**Chairmen : Kostas BOURTZIS, Didier BOUCHON**



## ***Wolbachia* horizontal transfer**

Simoes P., Mialdea G., Cariou M., Bailly-Bechet M. and Charlat S.

*CNRS (UMR 5558) - Université Lyon 1 - Laboratoire Biométrie & Biologie Evolutive - Bat. Mendel, 43 bd du 11 novembre - 69622 Villeurbanne - FRANCE - Email: sylvain.charlat@univ-lyon1.fr*

*Wolbachia* can jump across species, as evidenced by the lack of congruence between host and symbiont phylogenies. But “how?” and “how often?” are open questions that should be addressed to better understand the processes underlying *Wolbachia* distribution and evolution. Rather than focusing on particular ecological communities, where horizontal transfer is known to take place (e.g. *Drosophila*-Parasitoid communities) we approached this question without a priori, by extensively screening Arthropod species in one isolated geographical area: the Society Islands, in French Polynesia. Based on the distribution of the infections found in this sample (including over 5000 specimens and 1000 species, that is, an estimated 70% of the known biodiversity in this region), we will present a global picture of *Wolbachia* horizontal transfer and discuss the potential underlying ecological connexions.

*Keywords: Wolbachia, Horizontal transfer, Islands, Arthropods*

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## Horizontal *Wolbachia* transfer among bees (*Anthophila*) and their kleptoparasites assessed with MLST data

Gerth M., Rötke J. and Bleidorn C.

*University of Leipzig, Institute for Biology, Molecular Evolution & Systematics of Animals, Talstraße 33 04107 Leipzig, Germany, michael.gerth@uni-leipzig.de*

*Wolbachia* infect a wide range of hosts and may be the most common bacterial endosymbionts of arthropods. Much research has been focussed on how *Wolbachia* manipulate their hosts' reproduction and thereby promote their own vertical transmission. However, frequent horizontal transfer of the endosymbionts has also been demonstrated. The mechanisms responsible for this transmission are not well understood. In general, it has been proposed that *Wolbachia* spreads predominantly among closely related hosts or among hosts that are linked by their ecology.

In our study, we aim to test whether brood parasitism is a potential mechanism of horizontal *Wolbachia* transmissions in bees. Brood parasites or cuckoo bees infiltrate nests of solitary bees for oviposition. The cuckoo larva then feeds on the provisions provided by the bee host, thereby taking up salivary gland secretions of the bee host. If *Wolbachia* are transmitted this way, one would expect cuckoo bees and their corresponding hosts to share similar or identical *Wolbachia* strains. We tested for this pattern by sampling the German bee fauna with a focus on kleptoparasite-host pairs that bear *Wolbachia*. We then strain-typed the infections with five MLST genes and performed phylogenetic and statistical analyses.

Our results show that most bees bear quite similar *Wolbachia* strains, although there is no monophyletic clade comprising all *Wolbachia* strains from bee hosts. In some cases, kleptoparasite-host pairs carried near identical strains, suggesting that horizontal transfer of infections between bees and their kleptoparasites may occur. However, other pathways do not seem unlikely and need further exploration.

*Keywords: Horizontal transmission, bees (Anthophila), multilocus sequence typing*

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## ***Wolbachia* in drosophilid and tephritid fruit flies- same but different?**

Morrow J.L.<sup>1</sup>, Shearman D.C.A.<sup>2</sup>, Frommer M.<sup>2</sup> and Riegler M.<sup>1</sup>

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Drosophila fruit fly species (Diptera: Drosophilidae) are well-studied hosts of *Wolbachia* symbiosis - many are ideal laboratory models with an extensive repertoire of genetic tools and available information. There is an emerging body of work about *Wolbachia* in true fruit flies (Diptera: Tephritidae), including pest species of economic importance. *Wolbachia* associations of tephritids confirm many aspects of *Wolbachia* biology but also challenge others, with common multiple infections in individual hosts, a diversity of high and low bacterial titres as well as titre changes throughout a flies' life span, both contributing to difficulties in detection. In comparison with drosophilids, tephritids have developed different strategies to exploit resources, they are longer lived and, thus, may have extended opportunities for multiple species interactions. I will summarise and compare *Wolbachia* infections of tephritids from different biogeographic regions and present new findings of *Wolbachia* in the Australian species of the diverse genus of Bactrocera, in particular of the two sibling species of Queensland fruit fly, *Bactrocera tryoni* and *Bactrocera neohumeralis*. Newly detected *Wolbachia* infections in the latter may provide additional reinforcement for speciation of these two genetically very similar sympatric species.

*Keywords: Wolbachia, Tephritidae, Drosophilidae, Bactrocera, multiple infections*

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## Structure of *Wolbachia* diversity in the *Culex pipiens* mosquito complex

Dumas E., Atyame C., Weill M. and Duron O.

*Genomic of Adaptation, Institut des Sciences de l'Evolution (UMR CNRS 5554), Université Montpellier II. France*

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Addressing whether *Wolbachia* diversity can affect host population structure remains a challenging question. We investigated this issue in the *Culex pipiens* mosquito complex which is infected by a monophyletic clade of CI-inducing *Wolbachia* strains, called wPip. Crosses between *Cx. pipiens* mosquitoes of various origins are well known to exhibit a high frequency of uni- or bidirectional incompatibilities, contrasting with the low number of CI observed in other insects. Here we first characterized the wPip genetic diversity in 1600 field mosquitoes from 90 natural populations collected worldwide. Second, we investigated how wPip infections structure the diversity of coinherited host mtDNA and how they are distributed among the *Cx. pipiens* complex members: *Cx. p. quinquefasciatus*, *Cx. p. pipiens* and *Cx. p. molestus*. We found that five wPip phylogenetic groups coexist but that they are far from randomly distributed as showed by a remarkable geographic structuration. The spread of the different wPip strains was, however, strongly associated to cytoplasmic introgression, homogenizing mtDNA variations across the different *Cx. pipiens* taxa. As a result, although *Wolbachia*-induced CI may reduce gene flow between populations and has therefore been suggested as a possible mechanism of reproductive host isolation, there is no clear association between wPip diversity and the *Cx. pipiens* members. We will then discuss of the role of wPip in shaping reproductive isolation within the *Cx. pipiens* complex.

*Keywords: Wolbachia, Culex pipiens, cytoplasmic introgression, reproductive isolation*

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## Rapid evolution of *Wolbachia* sequences inside the compact fig syconia of *Ficus benjamina* (Ficus: Moraceae)

Yang C.-Y.<sup>1\*</sup>, Xiao J.H.<sup>2\*</sup>, Niu L.-M.<sup>3,2</sup>, Bian S.-N.<sup>1</sup>, Fu Y.-G.<sup>3</sup> and Huang D.-W.<sup>1,2</sup>

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Figs and fig wasps (Hymenoptera: Chalcidoidea) are a peculiar community in which the *Ficus* provides a compact syconium for the lives of a complex assemblage of Chalcidoid insects. The fig wasps have intimate ecological relationships within the syconia, and the latter may relatively become a barrier for the genetic communications (e.g. endosymbiotic bacteria genomes) of fig wasps with outside world. However, previous survey on the infection pattern of *Wolbachia*, maternally inherited endosymbiotic bacteria that widely infect a variety of arthropod and nematode hosts, proved that fig wasps have a high infection incidence of *Wolbachia*, even significantly higher than the average estimate based on a broad collection of insects. We ask whether the evolutionary patterns of *Wolbachia* sequences in the syconia are different from those in outside world. In the present study, by focusing on the 17 species of chalcidoid wasps living on *Ficus benjamina*, covering 4 families, 6 subfamilies, and 8 genera, we make a thorough survey on the *Wolbachia* infection pattern with *wsp* (*Wolbachia* Surface Protein) sequences. The high infection incidence of *Wolbachia*, frequent recombination events and prevailing horizontal transfer of *wsp* suggest a rapid evolution of *Wolbachia* within the syconia.

*Keywords: horizontal transfer, recombination events, Wolbachia surface protein, intimate ecological relationship*

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## Tsetse-Wolbachia Symbiotic Associations: from comparative *Wolbachia* genomics to mating incompatibility and applications

Doudoumis V.<sup>1</sup>, Brelsfoard C.<sup>2,3</sup>, Tsiamis G.<sup>1</sup>, Alam U.<sup>2</sup>, Malacrida A.<sup>4</sup>, Abd-Alla A.<sup>5</sup>, Aksoy S.<sup>2</sup> and Bourtzis K.<sup>1,6\*</sup>

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Tsetse flies (*Glossina* spp.) are the sole vectors of *Trypanosoma* spp., the causative agents of sleeping sickness in humans (human African trypanosomosis, HAT) and of nagana (African animal trypanosomosis, AAT) in livestock. Although epidemics have significantly declined during the last years, still tens of millions of people in Africa continue to be at risk of contracting sleeping sickness. There are several accepted environment-friendly methods to control the insect vector, including the sterile insect technique (SIT).

*Wolbachia* is a group of obligatory intracellular and maternally inherited symbionts infecting arthropod and filarial nematode species. These symbionts are able to manipulate the reproductive properties of their insect hosts by inducing parthenogenesis, male-killing, feminization and, most commonly, cytoplasmic incompatibility (CI). It has been proposed that the availability of *Wolbachia*-infected males that would be incompatible (through CI) with target natural populations can enhance the efficacy of the ongoing sterile insect technique (SIT) applications for tsetse flies.

In this study, we present the detection and characterization of *Wolbachia* infections in natural and laboratory populations of *Glossina* species, the genome sequence of both cytoplasmic and chromosomally inserted *Wolbachia* and also discuss how *Wolbachia*-based approaches could be used for the control of tsetse flies and trypanosomosis.

*Keywords: Wolbachia, tsetse flies, cytoplasmic incompatibility, genomics, vector control*

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## Scent matters: the impact of mutualistic *Wolbachia* on chemical communication and mate choice in the *Drosophila paulistorum* species complex

Schneider D.I.<sup>1</sup>, Chao T.<sup>2</sup>, Ehrman L.<sup>2</sup>, Däuble W.<sup>1</sup>, Kaltenpoth M.<sup>3</sup> and Miller W.J.<sup>1</sup>

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Mate recognition, a pivotal mechanism in sexual isolation, is an essential parameter in speciation. In *Drosophila*, some cuticular hydrocarbons (CHCs) are sex pheromones, playing a crucial role in conspecific communication. CHC composition, i.e., the pheromonal profile of an individual is characteristic, and it has recently been proposed that quantitative alterations in these profiles contribute significantly to sexual isolation in *Drosophila* and Lepidoptera.

Obligate mutualistic *Wolbachia* of *D. paulistorum*, a neotropical speciation complex *in statu nascendi*, manipulate host sexual behavior by influencing mate choice. In this model system, we have analyzed, via gas chromatography/mass spectrometry, CHC profiles of all six semispecies, which differ significantly in compound quantities but not in composition. In a proportion screen, we have evaluated potential influences of *Wolbachia* on pheromone signatures in host iso-female lines. Our results indicate massive changes in CHC profiles between naturally *Wolbachia*-infected (wt) and partially-depleted flies (*Wolbachia*-knockdown), suggesting strong influences exerted by this bacteria on *D. paulistorum* CHC signatures. Further, we have identified desaturase genes (desats), key players in the pheromone production pathway, as potential targets for *Wolbachia* to manipulate host pheromone production. We have hence performed desat expression analyses in wt and *Wolbachia*-knockdown lines of *D. paulistorum*. Finally, we demonstrate that partial depletion of obligate mutualistic *Wolbachia* from their *D. paulistorum* hosts is sufficient to induce the expression of de novo pre-mating isolation within members of the semispecies.

**Keywords:** *Drosophila paulistorum*, cuticular hydrocarbons, desaturase, de novo pre-mating isolation, speciation

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## Mind bending *Wolbachia* - exploring *Wolbachia*'s effect on *Drosophila* behaviour

Rohrscheib C.E., Weible II.M., Van Swinderen B. and Brownlie J.C.

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Complex behaviors allow animals to explore their environment for resources and successfully reproduce. *Drosophila melanogaster* has proved to be an versatile model organism for uncovering the genetic mechanisms that underpin behaviors such as sleep, aggression, courtship and attention. Previous studies showed *Wolbachia* influences *Drosophila* olfaction, the ability to smell, and reduced general locomotor activity under lab and field conditions. Recently we conducted a pilot study to determine if a natural *Wolbachia* infection could influence insect behavior - specifically visual responsiveness and arousal threshold. *Wolbachia* was shown to impair visual responsiveness in adult *Drosophila* when compared to *Wolbachia*-free fly lines. Arousal thresholds were increased in *Wolbachia*-infected flies, i.e. these flies showed a decreased response to mechanical stimuli when compared to *Wolbachia*-free flies, thus requiring greater stimulation before responding. The observed effects on behavior were equivalent in magnitude to known genetic mutations. Our preliminary work shows for the first time that a symbiont can influence visual responsiveness and arousal behaviors in an animal host, but poses several questions: is this a general effect on arousal or a narrow effect on different sensory modalities? What other behaviors does *Wolbachia* influence, how does *Wolbachia* manipulate behavior and do other *Wolbachia* strains influence host behavior?

*Keywords: Insect Behaviour, Non reproductive effects*

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## **Sexual selection in terrestrial isopods : Smelling from *Wolbachia***

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From prokaryotes to vertebrates, the use of chemical signals is widespread. Gregariousness and mate recognition have been found in some terrestrial isopods (Crustaceans). However, the underlying mechanisms that have led to these behaviors are poorly understood. *Armadillidium vulgare*, was used for this study and the presence of *Wolbachia*, an intracellular bacteria symbiont, results in feminization of genetic males into physiological and functional females. Previous results revealed that males interact more with uninfected females than feminized males. We focused on the ability of individuals to perceive other conspecifics at short-distance. To investigate biological parameters involved in individual attractions we tested individuals according to gender, moulting stage and *Wolbachia* infection status. Tested individuals were placed in a choice chamber separated by a mesh covered by a perforated opaque paper preventing visual and physical interactions. Males and females spent significantly more time close to a conspecific of the opposite gender than with those of the same gender. Moreover, males spent significantly more time close to females at the early moult stage than advanced moult or inter-moult stage. Finally, males were significantly more attracted by asymbiotic females compared to symbiotic females. Tested individuals perceived and used chemical cues for distance mate-finding as well as to discriminate infected female status. Our results provide clear evidence for chemical sex-recognition and preference to detect a female's reproductive status. The chemical signal of target individuals inform on the individual chemical pattern and led us to specific compounds involved in *A. vulgare* recognition cues.

*Keywords: chemical communication, behaviour, wolbachia, Armadillidium vulgare*

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## Density of *Wolbachia* in the host insect impacts antiviral protection

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Recently the maternally inherited endosymbiotic bacteria *Wolbachia* has been shown to protect insects from a range of microbial and eukaryotic pathogens. *Wolbachia*-mediated antiviral protection has been demonstrated in *Drosophila* and mosquitoes against RNA viruses, including *Drosophila C* virus and Dengue virus. To explore the mechanism of antiviral protection, we screened four diverse strains of *Wolbachia* within their naturally associated *Drosophila simulans* hosts, to determine if antiviral protection occurred across all *Wolbachia* strains. Although two of the *Wolbachia* strains delayed viral induced mortality, the other two strains did not. The two strains that mediated antiviral protection were more closely related and more abundant in the host than the two non-protective strains. We investigated the importance of *Wolbachia* density within the insect host on antiviral protection. To do this, we used low doses of antibiotic to decrease the density of a protective *Wolbachia* strain in its natural host, to levels that were similar to those observed in the two non-protective *Wolbachia* strains. Flies were then challenged with the pathogenic *Drosophila C* virus. It was found that when the density of the previously protective *Wolbachia* strain wAu was decreased to levels similar to the non-protective strains, *Wolbachia*-mediated antiviral protection was substantially decreased or lost completely. This data indicates that the density of *Wolbachia* in the insect host is important for the mechanism of antiviral protection and suggests that insects carrying a low load of *Wolbachia* will likely not be protected. These findings may facilitate prediction of *Wolbachia*-mediated protection in host-*Wolbachia* associations relevant for the biocontrol of insect-borne viruses.

*Keywords: antiviral, protection, virus, Drosophila*

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## ***Wolbachia* increases disease resistance and wound healing in bedbugs *Cimex lectularius***

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Female bedbugs (*Cimex lectularius*) die from repeated exposure to opportunistic bacteria transferred during mating. However bedbugs are armed with mutualistic *Wolbachia* that reduces mortality from these assaults. Two components make mating costly: i) wounding that occurs when the male pierces the female's abdomen with his intromittent organ during traumatic insemination, and ii) infection by the introduction of bacteria from the external environment. Here we present the results from experiments that show that *Wolbachia* reduces the survival costs associated with both of these components.

All experiments compare the response of *Wolbachia*-free females with that of females which have their *Wolbachia* complement. To create *Wolbachia*-free females we used a standardised heat treatment protocol in preference to antibiotics, as heat treatment allows us to explore the effects of eliminating *Wolbachia* without disrupting vital physiological (and immunological) processes. The ultimate effects of *Wolbachia* were determined by comparing measures of fecundity and survival. The proximate effects of *Wolbachia* were determined by assaying insect immune effector systems.

This study reports, for the first time, that *Wolbachia* increases host resistance against a *bacterial* insult.

*Keywords: Wolbachia heat symbiont-mediated*

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## Investigating the affects of *Wolbachia* on population declines of a threatened butterfly species

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The endosymbiotic bacteria, *Wolbachia* can affect population dynamics in its host species because, for many insect taxa, it can cause cytoplasmic incompatibility between its hosts and uninfected conspecifics. Such an effect could greatly reduce reproductive output and population size, especially at intermediate levels of infection in a population. Here, we examine whether *Wolbachia* served as a stressor to the imperiled Oregon silverspot butterfly, (*Speyeria zerene hippolyta*, OSB). Despite management that includes population supplementation, the few remaining populations of this species have not been increasing in numbers. Therefore, we set out to determine if this species is infected with *Wolbachia* and whether infection affects population growth of this butterfly. We conducted a screen for *Wolbachia* infection by collecting samples from archived female butterflies (from 1999 and 2001-2011, n=234), extracting DNA from the samples, and employing PCR protocols using *Wolbachia*-specific primers. Reproduction data, eggs laid and eggs hatched, for infected versus uninfected individuals were analyzed. Proportion of infected individuals per year was compared to population indices.

Our results revealed a *Wolbachia* infection in OSBs that changed over time. Infected individuals, on average, laid more eggs than uninfected individuals ( $p=0.0968$ ). Infected individuals successfully hatched more eggs than uninfected individuals ( $p=0.0472$ ). The event of an individual laying zero eggs was more prevalent in uninfected than infected samples. Our data shows evidence of *Wolbachia* being a potential contributor to population declines likely due to incompatibility between infected and uninfected mating pairs. Further investigations into the potential of multiple infections and speciation of OSB are underway.

*Keywords: Oregon silverspot butterfly (OSB), cytoplasmic incompatibility*

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## **Bacterial communities influenced by *Wolbachia*? Bacterial density and community structure in terrestrial isopods**

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*Wolbachia* acts as a reproductive parasite in terrestrial isopods, inducing either cytoplasmic incompatibility or the feminisation of genetic males. To date, three different feminising *Wolbachia* strains have been identified in the terrestrial isopod *Armadillidium vulgare*, presumably representing different co-evolutionary histories. However, the presence of other bacteria as well as their respective evolutionary implications remain as yet largely unexplored. This is of interest since symbioses with co-adapted bacteria are essential for survival in the vast majority of animal species, mainly via symbiotic contributions to digestion or by mediating resistance to pathogens. More recently, research focuses rather on entire endosymbiotic communities, the interactions between the different community members and their synergistic effects on host fitness. In this context, the association *Wolbachia*-terrestrial isopods represents an excellent model since *Wolbachia* interacts not only with its host but also with other bacteria, thus enabling the analysis of multipartite symbioses.

In order to get a first picture of the symbiotic bacterial community in *A. vulgare*, we characterised the microbial communities present in several laboratory lineages as well as in individuals from field populations using Temperature Gradient Gel Electrophoresis (TGGE). Bacterial density and community structure were analysed depending on (i) presence or absence of *Wolbachia*, (ii) infection with different *Wolbachia* strains and (iii) different host tissues. A better knowledge of these microbial communities might indicate a potential effect of *Wolbachia* on other members of the bacterial community and, more generally, result in a better understanding of multipartite endosymbioses.

*Keywords: Bacterial Communities, Symbiosis, Metagenomics*

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## **EVOLUTION**

**Chairmen : Sylvain CHARLAT, Takema FUKATSU**

## ***Wolbachia* Diversity**

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*Wolbachia* are common and widespread bacteria that readily move between host taxa on evolutionary time scales. Data are presented investigating the *Wolbachia* diversity in different host taxa, and how these bacteria change at the genomic level in closely related *Wolbachia* found in different hosts. Lateral gene transfers (LGTs) from *Wolbachia* to insect genomes are also common. Recent examples of LGTs are discussed along with speculations on the evolutionary significance of bacterial lateral gene transfers.

*Keywords: Diversity, Genomics, Lateral Gene Transfer, Evolution*

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## The *Wolbachia* pandemic among arthropods: re-estimating the proportion of infected species and modeling horizontal transmission in an epidemiological framework

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*Wolbachia* are considered the most common endosymbiotic bacteria on earth, infecting a vast number of arthropod species. Recently, a statistical analysis estimated the infection frequency of *Wolbachia* among arthropods to be 66%. The authors pointed out, however, that in order to improve the estimate, a larger number of individuals per species should be assayed and species be chosen more randomly. Here we analyze a more appropriate data set and indeed find a substantially different infection frequency. We now estimate the proportion of *Wolbachia*-infected species to be around 40%. This proportion still points to a surprisingly high number of arthropods harboring *Wolbachia* and it is significantly higher than our estimates for other reproductive parasites.

Despite the evolutionary success of *Wolbachia*, there is broad evidence for loss of infection in host lineages over evolutionary time, probably due to selection for resistance. The resulting paradox suggests that horizontal transmission between host species has been a key factor in shaping the global *Wolbachia* pandemic. In an unorthodox approach, we use epidemiology in evolutionary time to investigate *Wolbachia* horizontal transmission dynamics between arthropod species. Thus, species rather than individuals are regarded as infectious agents, and infection spreads on an evolutionary timescale. Our model can explain the high abundance of *Wolbachia* among arthropods even though selection for resistance within hosts frequently leads to loss of infection. We also find that the number of infected species may still be increasing over evolutionary time, and that, finally, transmission over large phylogenetic distances can be decisive for the high infection frequency of *Wolbachia* among arthropods.

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**Keywords:** *infection frequency, horizontal transmission, epidemiology, evolution of resistance, host phylogeny*

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## High virulence of *Wolbachia* after host switching: Tolerance versus Lethal autophagy

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*Wolbachia* are intracellular alphaproteobacteria that are widely distributed among arthropods and nematodes. These endosymbionts exhibit low virulence in their native hosts in accordance to what predicted in co-evolutionary process between host and vertically transmitted symbionts. However, some phylogenetic studies suggest that horizontal transfers to new host species occur frequently in nature. In transfer situations, virulence variations can be predicted since hosts and symbionts are not co-adapted to each other. An interesting case of increasing high virulence of symbiont after host switching is observed when wVulC, one *Wolbachia* strain of *Armadillidium vulgare* is transferred to another terrestrial isopod, *Porcellio dilatatus dilatatus*. Such transfer kills all recipient animals within 75 days. Before death, animals suffer symptoms such as growth slowdown and nervous system disorders. Neither those symptoms nor mortalities were observed after injection of wVulC into its native host *A. vulgare* which is tolerating well its native symbiont. Analyses of wVulC's densities in nerve cord cells of both naturally infected *A. vulgare* and transfected *P. d. dilatatus* and *A. vulgare* individuals revealed a similar pattern of host colonization suggesting an overall similar resistance of both host species towards this bacterium. However, for *P. d. dilatatus* infected by wVulC, we observed a huge accumulation of autophagic vesicles in the nerve cord cells. The nervous disorder symptoms and the following mortalities could therefore be explained by this lethal autophagic response against wVulC in *P. d. dilatatus* that is not triggered in *A. vulgare* which co-evolved with this *Wolbachia*. Our results show that *Wolbachia* can become pathogenic when transferred horizontally into species that are phylogenetically close to their native hosts. This change in virulence is likely resulting from the autophagic response of the new host, strongly altering its tolerance to the symbiont and turning it into a deadly pathogen.

*Keywords: co-evolution, horizontal transfers, virulence, Wolbachia, Porcellio dilatatus dilatatus, Armadillidium vulgare*

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## A New *Wolbachia* Type F from Splendidofilariinae (Onchocercidae) Supports Recent Emergence of this Supergroup

Lefoulon E., Gavotte L., Junker K., Barbuto M., Uni S., Landmann F., Laaksonen S., Saari S., Nikander S., De Souza Lima S., Casiraghi M., Bain O. and Martin C.

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The endosymbiotic bacteria *Wolbachia* exhibit a broad host spectrum, including numerous families of arthropods and only one family of nematodes, the Onchocercidae, including agents of human filarial diseases, e.g. lymphatic filariasis and onchocerciasis. These bacteria are vertically transmitted and behave as mutualists in Onchocercidae. *Wolbachia* infect the female germline and the hypodermis. It is commonly accepted that they co-evolved with their filarial hosts, and that the infection may have been acquired as a single event in the Onchocercidae with events of losses in few species. However most of the data on the relationship *Wolbachia*/Onchocercidae come from studies on two sub-families, the Dirofilariinae and the Onchocercinae which includes parasites of humans. Within the last few years, the analysis of more diversified material has suggested that lateral gene transfers occurred and that some groups of Onchocercidae do not have *Wolbachia*, i.e. Oswaldofilariinae from reptiles and Splendidofilariinae from birds. Two other Splendidofilariinae, *Rumenfilaria andersoni* from a Finnish reindeer and *Madathamugadia hiepei* from a South African gecko, are studied using PCR, immunohistochemical staining and whole mount fluorescent analysis, to detect *Wolbachia* and describe its strains. A new type F *Wolbachia* is depicted in *M. hiepei*; it is the first *Wolbachia* described in a non-mammalian filaria host. Intestinal cells are infected, but not the hypodermis. The analysis confirms a recent emergence of the type F; it also suggests several events of horizontal transmission between nematodes and arthropods in this supergroup, and the existence of different metabolic interactions between the filariae and their symbionts.

**Keywords:** *Onchocercidae*, *Splendidofilariinae*, *symbiosis*, *phylogeny*

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## Effects of heterogeneity in invasion by *Wolbachia*

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Population genetics predicts bistability and an invasion threshold for *Wolbachia*-carrying insects attempting to invade a population of non-carriers. This threshold is a function of fecundity costs associated to *Wolbachia*, and the intensity of cytoplasmic incompatibility it induces, which are measures of selection for and against *Wolbachia*. It has also been proposed that interference between a vertically-transmitted (such as *Wolbachia*) and horizontally-transmitted parasites (such as any pathogen against which *Wolbachia* confers protection) can facilitate persistence of *Wolbachia*, and possibly remove the threshold. Ecoepidemiological models, however, include many additional demographic parameters and populations, which greatly complicates their analytical interpretation - especially if protection against pathogens is only partial. To our knowledge, no treatment, analytical or otherwise, has been given to the invasion threshold in this kind of model.

We argue that, regardless of its dynamics, the presence of a pathogen ultimately amounts to additional mortality that is experienced differently by *Wolbachia* carriers and non-carriers - which is why it facilitates invasion. We were able to derive an expression for the invasion threshold, which in the absence of pathogens is the same as that predicted by population genetics, and in their presence decreases proportionally to the product disease burden and relative protection conferred by *Wolbachia*. This partial protection can also be heterogeneously distributed among subpopulations of carriers, which further lowers the threshold, with selection of more resistant variants.

*Keywords: invasion threshold, partial susceptibility, heterogeneity, interference*

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## ***Wolbachia* diversity, invasion and evolution**

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*Wolbachia*-mediated resistance to other pathogens is a crucial factor in *Wolbachia* invasion of natural populations. Such resistance, however, can be associated with fitness costs on the part of the host, including reduced fecundity or shorter lifespan. Here we investigate a continuum of *Wolbachia* strains, which impose varying levels of fecundity costs on their hosts and confer varying levels of protection from natural enemies. In order to explore evolutionary dynamics of *Wolbachia* during and after invasion, we allow mutation to act between these different strains. Using the Price Equation framework, we track the evolution of average fecundity cost and average protection in *Wolbachia* carriers, coupled with the ecological dynamics of invasion. By treating *Wolbachia* cost and benefit as distinct traits with potential covariance between them, such an approach opens the avenue for making concrete predictions on the magnitude of evolutionary change in response to natural environmental conditions or manipulations. We find that the background force of infection is a major selective force for the evolution of high *Wolbachia*-mediated resistance. Even without an intrinsic trade-off between fecundity and induced protection, mutation between different strains can yield intermediate trait values at equilibrium, without necessarily dominance of the fittest. The non-equilibrium dynamics, as well as the evolutionary equilibrium depend on the range of diversity that is possible for a given *Wolbachia* species and the mutation rate. We analyze the effects of different trait distributions in the introduced *Wolbachia* population, including a unimodal and bimodal distribution, and discuss their importance for predicting the outcome of biological interventions.

*Keywords: Price equation, diversity, mutation, fecundity-protection trade-off*

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## The impact of *Wolbachia* on triggering sexual isolation and adaptive behavioral changes in Insects

Miller W. J.<sup>1</sup>, Schneider D.<sup>1</sup>, Lind A.<sup>1</sup>, Däuble W.<sup>1</sup> and Ehrman L.<sup>2</sup>

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Detailed studies on long-term evolutionary interactions between conspecific *Wolbachia* strains and their native hosts systems, which are currently under incipient speciation, offer unique opportunities to decipher their biological complexities and dynamics in insects.

Here we will report on our latest results on monitoring *Wolbachia* titer dynamics, tissue tropisms and their effects on sexual behavior in native hosts and interspecies hybrids of neotropical *Drosophila* and tsetse flies, respectively. We further will present data on uncovering quite unorthodox transmission modes of both *Wolbachia* plus mitochondria in hybrids where obligate mutualistic *Wolbachia* have evolved intimate symbiotic associations and hitchhike host mitochondria.

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## Splicing the Tree of Life: Recurrent Horizontal Gene Transfer of a *Wolbachia* Lysozyme between Bacteria, Archaea, and Eukaryotes

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Horizontal gene transfer is surprisingly common and is increasingly recognized as an important player in eukaryotic evolution, mixing genes between different species and even different domains of life. We describe here the likely horizontal transfer of a *Wolbachia* bacteriophage WO gene between various viruses, bacteria, archaea, and eukaryotes. This gene encodes a lysozyme, an enzyme that digests peptidoglycan, whether for bacterial cell wall remodeling, phage-mediated bacterial lysis, or antimicrobial defense. Phylogenetic analysis suggests the lysozyme encoded by WORiA is present in such diverse organisms as the archaea *Aciduliprofundum boonei* and the plant *Selaginella moellendorffii*, as well as numerous bacteria and fungi in addition to the previously reported insect *Acyrtosiphon pisum*. Genomic integration of the lysozyme gene was confirmed by PCR and sequencing in all three domains of life. Phylogenetic analysis and protein modeling show that many of the residues lining the putative active site of this lysozyme are highly conserved amongst all members of the phylogeny. We therefore hypothesize that the lysozymes are functional and may be used by the non-bacterial species for antimicrobial defense. The lysozymes from three species are being cloned and purified to test their antimicrobial properties *in vitro*. If the lysozyme is antimicrobial in these species, this will be to our knowledge the first proven instance of functional gene transfer between all three domains of life.

*Keywords : horizontal gene transfer, WO, lysozyme, evolution, antimicrobial*

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## Phylogeographic analysis of European populations of *Wolbachia* infecting *Chorthippus parallelus* (Orthoptera)

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The grasshoppers *Chorthippus parallelus parallelus* (Cpp) and *Chorthippus parallelus erythropus* (Cpe) form a Pyrenean hybrid zone as a consequence of secondary contact between endemic Iberian (Cpe) and continental European populations (Cpp) that diverged genetically in allopatry.

*Wolbachia*, an obligate endosymbiont of an enormous variety of invertebrates, has been reported in *C. parallelus*. B and F bacterial supergroups have been detected based on sequence analyses of 16S *rRNA*, *wsp* genes, and a “Multi Locus Typing System” (MLST).

Previous studies based on 16S *rRNA* sequences allow us to discriminate between European populations (Northern pattern), Iberian populations (Southern pattern) and a high coinfection peak in a single population of the Pyrenean hybrid zone (Sallent de Gállego pattern). Current phylogenies based on the MLST cited above show several different “Sequence Types” (STs) and ST complexes along European and Iberian populations of this grasshopper, confirming and adding data about these geographical patterns.

F strain infection in *C. parallelus* is extremely variable. However, the B supergroup has a homogeneous distribution along Europe. The allelic profiles are associated with the geographical distribution of the *C. parallelus* populations and reveal data about the origin of *Wolbachia* infection in this species, a key point to understand the role of *Wolbachia* in the grasshopper hybrid zone dynamic, where it generates either unidirectional- and bidirectional cytoplasmic incompatibility, and certain cytogenetical effects.

Furthermore, we report recombination between strains of *Wolbachia*, suggesting a possible context for this recombination. Recombinant strains appear in the centre of the grasshopper's hybrid zone, where infection patterns converge; genomic shock in hybrid grasshoppers could lead this bacterial recombination. This offers an exciting situation with an orthopteroid hybrid zone enclosing a bacterial hybrid zone, evoking “matryoshka's dolls” in a scenario of concerted evolution (coevolution) of their genomes.

*Keywords: Wolbachia, Chorthippus parallelus, phylogeography, recombination*

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## Lateral Gene Transfers Underlie *Wolbachia*-Mediated Novel Host Phenotypes

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Lateral gene transfers from *Wolbachia* endosymbionts to their hosts have been reported from a wide range of arthropods such as a seed beetle, a fruit fly, parasitoid wasps, a longicorn beetle, mosquitoes, etc. (Kondo et al. 2002b; Dunning Hotopp et al. 2007; Aikawa et al. 2009; Klasson et al. 2009; Woolfit et al. 2009). However, biological significance of such lateral gene transfers has been elusive, except for the mosquito case wherein *Wolbachia*-derived genes have acquired tissue-specific expression patterns in the host insect (Klasson et al. 2009; Woolfit et al. 2009). Here we report an unprecedented case of lateral gene transfer in a *Wolbachia* endosymbiont with biological significance: the *Wolbachia* has laterally acquired foreign genes from other bacteria, and the genes play a pivotal role in affecting adaptive phenotypes of the host arthropod.

*Keywords: Wolbachia genome ; lateral gene transfer ; adaptation ; evolution*

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## Evolutionary analysis of the bag of marbles gene reveals an interaction with *Wolbachia*

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*Drosophila* germline stem cells (GSCs) can both self-renew and differentiate to generate oocytes or sperm. We have shown that multiple genes involved in GSC regulation are experiencing rapid, adaptive protein evolution in *Drosophila melanogaster* and the closely related species, *D. simulans*. We have focused on one of these adaptively evolving genes, *bag of marbles* (*bam*), to understand the functional consequences of this adaptive evolution. The best characterized function of *bam* is initiating GSC differentiation. We are using interspecies complementation to test whether adaptive evolution of *bam* has caused detectable functional differences. We assayed the ability of a *bam* ortholog from *D. simulans* to complement the male and female sterility associated with a *bam* mutation in *D. melanogaster* and found that the *D. simulans* *bam* ortholog can complement male sterility but fails to fully complement the female sterility. These data suggest that the evolutionary force driving the diversification of *bam* is focused on the female germline. We hypothesize this force may be conflict with *Wolbachia* due to its maternal inheritance and reproductive manipulation. To determine if any interaction exists between *bam* and *Wolbachia*, we tested the ability of *Wolbachia* to suppress *D. melanogaster* *bam* hypomorphic mutants and found that the presence of *Wolbachia* can enhance the fertility of the mutants. We also found that *Wolbachia* can enhance the female fertility in flies with *D. simulans* transgenic *bam* in our complementation assay. We are currently examining the nature of the interaction between *bam* and *Wolbachia* to understand the mechanism of enhancement.

*Keywords: Drosophila, evolution, germline*

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## Mom knows best: Maternal regulation of *Wolbachia* titers

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Maternal transmission of bacterial symbionts by transovarial diffusion is pervasive in invertebrates. Regulation of the symbiont inoculum within an oocyte is important in preventing host pathogenesis while also maintaining efficient maternal transmission. Co-evolution between a host species and its resident endosymbiont could produce unique host-symbiont interactions that govern the regulation of endosymbiont titers. In the parasitoid wasp *Nasonia*, *Wolbachia* strain *wVitA* maintains a low infection density in its natural host, *N. vitripennis*, but has an extreme and stable infection density 100-fold higher in the naïve host, *N. giraulti*. We have exploited this interspecific difference to determine the number of candidate regions affecting this trait. We report two key results. First, low *Wolbachia* density native to *N. vitripennis* is regulated by host factors that act dominantly through a maternal effect to establish the infection level in resulting offspring. Second, microarray and quantitative trait loci analyses indicate that three significant *Nasonia* QTL regions on chromosomes 1, 2, and 3 work additively to explain at least 40% of the interspecific variation in *Wolbachia* densities. Several candidate genes with important roles in innate immunity and oogenesis are located within these regions.

*Keywords: Maternal effect, transovarial transmission, symbiont titers, Nasonia*

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## **Genomics**

**Chairmen : Benjamin MAKEPEACE, Clément GILBERT**

## The impact of *Wolbachia* endosymbionts on the evolution of sex determination in the isopod *Armadillidium vulgare*

Giraud I., Badawi M., Lafitte A., Bouchon D., Greve P. and Cordaux R.

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In animals, the most common system of sex determination is genetic. Sex determination can also be affected by inherited bacterial endosymbionts. In the isopod *Armadillidium vulgare*, genetic sex determination follows female heterogamety. However, many *A. vulgare* populations harbour *Wolbachia* bacterial endosymbionts which can invert genetic males into phenotypic functional females. Other sex-determining factors have been identified in *A. vulgare*: a feminizing *f* element which may be a *Wolbachia* genome fragment carrying feminization information inserted into the host nuclear genome, and a masculinizing gene which can restore the male sex in the presence of the *f* element, as a result of a genetic conflict. Thus, sex determination mechanisms in *A. vulgare* seem to be largely driven by *Wolbachia* endosymbionts. However, the molecular genetic basis and evolutionary history of these sex-determining factors is unknown. The latest developments of molecular genetics technologies, such as next-generation DNA sequencing and high throughput genotyping, now make it possible to address these exciting questions.

*Keywords: Armadillidium vulgare, isopod, sex determination, Wolbachia, feminization*

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## The genome sequence of the feminizing *Wolbachia* wVulC from the isopod crustacean *Armadillidium vulgare* identifies multiple secretion/export systems in a bacterial endosymbiont

Pichon S.<sup>1</sup>, Liu L.<sup>2</sup>, Lanming C.<sup>2</sup>, Garrett R.<sup>2</sup>, Felix C.<sup>1</sup>, Cerveau N.<sup>1</sup>, Leclercq S.<sup>1</sup>, Lesobre J.<sup>1</sup>, Bourtzis K.<sup>3</sup>, Cordaux R.<sup>1</sup>, Bouchon D.<sup>1</sup> and Greve P.<sup>1\*</sup>

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*Wolbachia* are obligate intracellular alpha-Proteobacteria that infect filarial nematodes and many arthropod species including crustaceans. A recent survey showed that 47% of isopod species tested are infected, a prevalence that reached 61% when only terrestrial isopods were considered. In isopod hosts, *Wolbachia* mainly induce feminization of genetic males. Molecular mechanisms involved in the manipulations of host reproduction are still unknown. As *Wolbachia* endosymbionts are uncultivable bacteria, comparative genomics represents a particularly useful alternative for deciphering how *Wolbachia* interact with their hosts. The ability of *Wolbachia* to induce so many different phenotypes may depend on its ability to secrete virulence factors which are either displayed on the bacterial cell surface or secreted in the host cytoplasm. Here we report the analysis of the 1,663,852 bp genome sequence of the *Wolbachia* strain wVulC which infects the isopod crustacean *Armadillidium vulgare*. This represents the first genome sequence of a *Wolbachia* strain inducing feminization. The analysis of the wVulC genome sequence revealed the existence of 6 different secretion/export systems and numerous putative translocated effectors. We further show that these systems are also present in all *Wolbachia* genomes sequenced to date.

**Keywords:** crustacean, feminization, mobile element, secretion system, bacterial effectors

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## Comparative genomics of supergroup A and B *Wolbachia* strains infecting the same host species

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The genus *Wolbachia* currently consists of only one species, with several divergent lineages called supergroups. Given the significant divergence between the supergroups, it has been debated whether or not these supergroups actually constitute independent lineages and as such should be referred to as different species. Although the bacterial species concept is generally not well defined, large differences in gene content and low levels of recombination between the supergroups would suggest that the *Wolbachia* supergroups might be reclassified as species.

We have sequenced the complete genomes of the two *Wolbachia* strains wHa and wNo, which belong to supergroup A and B, respectively. Both strains are associated with *Drosophila simulans*, where they occur as natural double infections in populations on the Seychelles and in New Caledonia. Comparing the genomes of wHa and wNo to each other and already sequenced genomes of *Wolbachia* strains from both the A and B supergroups, we have investigated the level of genetic exchange and differences in gene content between and within supergroups. Since lack of recombination or variability in gene content can be attributed to physical separation and host specificity, using the genomes of wHa and wNo that are co-infecting the same host, controls for these parameters.

We show that supergroup specific genes that could be of relevance for general host interaction do exist and that genetic exchange occurs across supergroups, albeit with a much lower frequency than within supergroups.

*Keywords: Genomics, Wolbachia-Drosophila, Recombination*

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## Genomic Sequencing of Cell Line Cultured *Wolbachia* Originating from *Ephestia kuehniella* and *Aedes aegypti*.

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A procedure for preparing *Wolbachia* genomic DNA from cultured insect cells was utilized for genomic sequencing. *Wolbachia* infecting a laboratory strain of the Mediterranean flour moth, *Ephestia kuehniella*, and the mosquito, *Aedes albopictus*, were transfected separately into the *Bombyx mori* Bm5 cell line and expanded. *Wolbachia* from *E. kuehniella*, wKue, were a new isolate. Characterization by MLST showed wKue was a match to St-92 in supergroup A, a strain which had not been observed previously in *E. kuehniella*. *Wolbachia* from *A. albopictus* wAlbA were from isolates previously described (O'Neill et al., Insect Mol. Biol., 1997). The *Wolbachia* were isolated from the Bm5 cells essentially as described by Rasgon et al., (Appl. Environ. Micro., 2006). Following isolation, genomic DNA was extracted from the *Wolbachia*. Partial digests of the genomic DNA were inserted into vectors were submitted to the National Center for Genomics Resources, Santa Fe, New Mexico for sequencing with the Illumina Genome Analyzer II platform. Nucleotide sequence reads were obtained using standard protocols developed for the Illumina Genome Analyzer II platform. Assembly of the sequence reads of both wKue and wAlbA was performed using DNASTAR NGen 2.3 software. Assembled sequence contigs were exported as a FASTA format file for bioinformatics analysis using BLAST2GO software (Götz et al., Nucleic. Acids. Res. 2008) and annotated. The genomic organization of both strains will be presented and discussed.

**Keywords:** transinfection, insect cell culture, genomic organization

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## Global gene expression in *Wolbachia*: taking a sledgehammer to a supergroup C nut

Darby A.C.<sup>1</sup>, Armstrong S.A.<sup>1</sup>, Bah G.S.<sup>2</sup>, Blaxter M.L.<sup>3</sup>, Trees A.J.<sup>1</sup>, Cordaux R.<sup>4</sup>, Wastling J.M.<sup>1</sup>, and Makepeace B.L.<sup>1</sup>

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The *Wolbachia* strains of supergroup C, found exclusively in filarial nematodes, have been little studied. Moreover, the field as a whole has suffered from a paucity of data regarding the regulation of global gene expression in *Wolbachia*. Thus, we sought to investigate the relationship between *Onchocerca ochengi* (a parasite of cattle representing the closest relative of the major human pathogen, *O. volvulus*) and its supergroup C endosymbiont (strain wOo). First, we sequenced and closed the complete genome of strain wOo, and determined that it is the smallest described to date for the genus (0.96 Mb). Second, applying RNA-seq, we obtained global transcriptomes for strain wOo from both somatic hypodermal cord and female gonad host tissue. Third, we identified the most abundant endobacterial proteins using a gelLC-MS proteomics approach. Our data indicate that unlike wBm (supergroup D), the genome of wOo exhibits a dearth of insertion sequences, and it has lost the riboflavin biosynthetic pathway. Approximately 4% of wOo genes displayed significant differential expression between gonad and somatic tissue, with increased representation of translation and DNA replication processes in the former, compared with membrane transport and respiration in the latter. The wOo proteome reflected a constitutive heat-shock response, and the most abundant proteins included pathogen-associated molecular patterns that can stimulate the mammalian innate immune response. These findings suggest that (a) *Wolbachia* may play a mitochondrion-like role in the hypodermal cords by provisioning ATP, and (b) endosymbiont proteins probably contribute to the ineffective local neutrophilic response in onchocerciasis, facilitating filarial immune evasion.

*Keywords: filarial nematode, onchocerciasis, RNA-seq, mutualism*

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## High abundance of insertion sequences in *Wolbachia* endosymbiont genomes: evolutionary causes and dynamics

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The most abundant and simplest transposable elements in prokaryote genomes are insertion sequences (IS). Evolutionary models predict that IS density in bacterial genomes varies according to bacterial lifestyle. Recent intracellular bacteria tend to possess in their genome a higher IS number than free-living bacteria, whereas old endosymbiont genomes generally lack IS due to reductive evolution which eliminates non essential genes like IS. However several old bacterial endosymbionts, such as *Wolbachia*, possess an unusually high IS density. We combined *in silico* description, with the analysis of the four currently available complete *Wolbachia* genomes, and molecular biology experiments to identify causes of the high IS density and their evolutionary dynamics. We showed that *Wolbachia* genomes contain between 52 and 171 IS copies. Surprisingly, a high proportion of copies were non functional and degraded (>70%). These degenerated IS copies constitute genomic fossil records that allowed us to investigate IS evolutionary dynamics. We showed that IS transpositional activity was variable across time. Two phases of high IS activity were detected in *Wolbachia* genomes, one old and one more recent (and maybe ongoing), which were separated by a phase of relative quiescence. Simulations suggest that IS activity phases need to be preceded by new functional IS incoming through horizontal transfers, which we inferred to be frequent via experimental screening of 22 *Wolbachia* strains. Our results provide direct empirical evidence on the evolutionary dynamics of IS in bacteria and they also allow us to better understand the causes of high IS abundance in *Wolbachia* genomes.

**Keywords:** endosymbiont genomics, evolutionary dynamics, transposable elements, horizontal transfers

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## Three Rules of Bacteriophage WO Evolution

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Host-microbe symbioses involving bacterial endosymbionts comprise the most intimate and long-lasting interactions on the planet. While restricted gene flow might be expected due to their intracellular lifestyle, many endosymbionts, especially those that switch hosts, are rampant with mobile DNA and bacteriophage. *Wolbachia pipientis* infects a vast number of animal species and often has a significant portion of its genome dedicated to prophage sequences of a virus called WO. In this talk, I will discuss our research on phage WO and three findings that challenge fundamental theories of bacteriophage and endosymbiont evolution, namely the phage Modular Theory and endosymbiont genome stability. First, bacteriophage WO universally transfers between *Wolbachia* coinfections in the same host. Second, despite its rampant mobility, WO exhibits features of genomic constraint related to its intracellular niche, including gene deletions and infrequent acquisition of new genes. Finally, active and remnant fragments of phage WO retain an unusual core genome of head and baseplate genes; other genes are frequently deleted. In addition to these rules of phage genome evolution in *Wolbachia*, WO has also opened up new windows into the tripartite interactions between viruses, bacteria, and eukaryotes.

*Keywords: Genome Evolution, Horizontal Gene Transfer, Mobile DNA, Bacteriophage WO*

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## Adaptation in *Wolbachia* genomes

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*Wolbachia* interacts intimately with its hosts at the molecular level. These interactions are dynamic, as both partners in the symbiosis adapt and respond to one other, and the story of both historical and ongoing adaptation can be read from the genomes of *Wolbachia* strains. We have investigated the molecular signatures of adaptation at two levels in the publicly available *Wolbachia* genomes. First, we have tested for evidence of positive selection at the nucleotide level, to identify genes in the core *Wolbachia* genome that have been involved in adaptation in one or more strains. A surprisingly small proportion of the core genome shows significant evidence of adaptation, including genes encoding membrane proteins, transporters and components of secretion systems. Secondly, we have examined another source of adaptive genomic variation: the influx of new genes. *Wolbachia's* non-core genome is large and rapidly changing. We have identified genes that have been transferred into the genomes of one or more *Wolbachia* strains from diverse bacterial lineages, and characterised their evolution after insertion. These genes are involved in a wide range of metabolic pathways, and some may play roles in host interaction. The flow of these genes into *Wolbachia* genomes appears to have been occurring since before the divergence of the currently sequenced supergroups, and to be ongoing, contributing to differences between recently diverged strains. It is likely that the flexibility and adaptability of its genome contributes to *Wolbachia's* success in colonising and manipulating an extremely diverse range of hosts.

*Keywords: adaptation, selection, evolution, horizontal gene transfer*

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## Lateral Gene Transfer from a *Wolbachia* Endosymbiont to *Drosophila ananassae*: A Tale of Extensive Duplication and Epigenomic Variation

Dunning Hotopp J. C.\*, Klasson L., Kumar N., Andersson S., Sieber K., Bromley R., Tallon, Luke J., Flowers M. and Ott S.H.

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Lateral (or horizontal) gene transfer is the transfer of DNA between organisms in the absence of sex. Extensive LGT has now been described between *Wolbachia* endosymbionts and their invertebrate hosts. We call such nuclear *Wolbachia* LGTs “nuwts” using the established nomenclature for nuclear mitochondrial transfers (numts). We show through genome re-sequencing with qPCR validation that the nuwt in *Drosophila ananassae* is the largest nuwt known with multiple integrations of the 1.5 Mbp *Wolbachia* genome in the insect chromosome. As many as eight copies of large portions of the *Wolbachia* genome exist per insect chromosome in some lines, with as many as sixteen copies per nucleus as measured by genome sequencing and qPCR. This extensively duplicated transfer can be found in multiple lines of *Drosophila ananassae* from Asia and the Pacific indicating that it is widely distributed. Genomes of three of these flies have now been sequenced and each has its own unique properties. One line was identified that has a different duplication pattern as shown by genome sequencing and qPCR validation. We will present our analysis that these nuwts in a different line can become under-replicated in the genome through the life cycle and in the entire fly over time in a population. These results suggest that this LGT may move between the euchromatic and heterochromatic portions of the genome.

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## A Laterally Transferred Ferrochelatase Gene is Functional And Essential in Filarial Nematode Parasites

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A feature of the phylum Nematoda is the lack of the heme biosynthetic pathway and a metabolic dependency for extraneous heme or, in the case of most filarial nematodes, possible provision by the obligate endosymbiont, *Wolbachia*. Many filarial nematodes possess a ferrochelatase (FC) gene, the terminal step of heme biosynthesis, as a consequence of a lateral gene transfer (LGT) event. Sequencing of *Brugia malayi* revealed an open reading frame encoding a putative FC. The full-length FC transcript sequences from *B. malayi*, *Onchocerca volvulus* and the *Wolbachia*-free *Acanthocheilonema viteae*, provide predicted protein sequences that seem to be derived from  $\alpha$ -proteobacterial Rhizobiales, not of *Wolbachia* origin. BmFC contains 9 exons spanning ~ 4.5 kb and includes a mitochondrial-targeting domain (MTD). BmFC is functional based upon enzyme assay, complementation to an *E. coli* hemH-mutant, inhibitor studies with an FC-specific inhibitor in *B. malayi* and as a transgene in *C. elegans*. RNAi experiments show that BmFC is functional showing abnormalities in nuclei of germ cells and embryos. The MTD is required for mitochondrial location, but not for enzyme activity. FISH reveals the BmFC gene is almost universally expressed in both male and female tissues.

Phylogenetics suggests a non-*Wolbachial*, but  $\alpha$ -proteobacterial origin with the lateral transfer acquisition predating the split of the Rhabditida into the Spirurina and Rhabditiina clades. This is the first reported functional LGT gene in animal or human parasitic nematodes and its requirement for worm viability suggests it could play a role in the symbiotic relationship between the filarial nematode host and its symbiont and be a potential target for drug discovery against filariasis.

*Keywords: Wolbachia, filariasis, endosymbiont, heme, LGT*

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## **CELL BIOLOGY**

**Chairmen : Pierre GREVE, Abdelaziz HEDDI**

## ***Wolbachia* and Filarial Hypodermis Revisited**

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*Wolbachia* endosymbionts harbored by many filariae are principally distributed in the cells of the female germline, including embryos developing in the uterus, and in the lateral chords, where they occur in greatest numbers and concentration. Since recent reports suggested transmission of *Wolbachia* from the lateral chords to the ovarian tissues, we have re-examined, by transmission and scanning electron microscopy, the ultrastructure of the lateral chords of several adult filariae (*Onchocerca volvulus*, *Dirofilaria immitis*, *Brugia malayi* and *Litomosoides sigmodontis*) to seek structures that could explain the expansion and somatic invasion of *Wolbachia* within the lateral chords. The cuticular surface of the lateral chord is attached to the cuticle by hemidesmosomal junctions and bridges, but the coelomic surface, covered by an amorphous membrane, contains numerous fenestrations. Collection of actin bundles transverse the cytoms from the cuticular to the coelomic surfaces. Transmission electron microscopy reveals that the fenestrations of the coelomic surface are openings of cytoplasmic canaliculi which extend deeply into the cytoplasm of the lateral chord. These canaliculi could provide the means by which *Wolbachia* leave the lateral chords and, being limited by the amorphous membrane, be propelled by the coelomic fluid to distant, uninfected portions of the lateral chords. Contraction of the actin bundles could likewise assist in expulsion and uptake of *Wolbachia* by the cytoms. Infection of the ovary, would require that *Wolbachia* exit the lateral chord, disrupt the amorphous membrane, and proceed to penetrate the ovarian basal membrane that is much thicker and denser than the amorphous layer.

*Keywords: Wolbachia, filariae, lateral chords, transmission and scanning electron microscopy*

Supported, in part, by funds from the Rockefeller Foundation, World Health Organization, and RCMI Award RR-003051 from the NIH, USA.

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## ***Wolbachia* colonization in *Armadillidium vulgare*: patterns and dynamics**

Genty L.M., Raimond M., Bouchon D. and Bertaux J.

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*Wolbachia* is an intracellular bacterium mostly vertically transmitted, found not only in reproductive but also in somatic tissues. In the isopod *Armadillidium vulgare*, we showed with Fluorescence *In Situ* Hybridization (FISH) that both somatic and reproductive tissues were a mosaic of uncolonised and colonised cells. In addition the latter had varying *Wolbachia* cellular titers: One to 26 bacteria in haemocytes, one to 50 000 in oocytes. Consequently, we suspect mechanisms regulating *Wolbachia* presence in host cells and we would like to investigate what factors allow *Wolbachia* to remain and to proliferate in some cells and tissues. During ovarian maturation, the proportion of colonised oocytes increased from only 34% in immature ovaries, to 86% before laying, matching the known transmission rate. More precisely, while the number of colonized oocytes remained stable, the number of uncolonized cells decreased. Therefore oocytes without *Wolbachia* might be lost by apoptosis. We cannot exclude the possibility that uncolonized oocytes could acquire *Wolbachia* secondarily through a reservoir organ. But, so far, we did not witness *Wolbachia* penetrating in oocytes. Aiming to find an entry point for a possible cyclical invasion of the ovaries, we transfected *Wolbachia* to uninfected females, a strategy used in *Drosophila* to reveal the Somatic Stem Cell Niche. In order to understand how *Wolbachia* is carried and maintained in the host cell we developed a FISH mRNA method to monitor the activation of the host pathways in relation to the presence of *Wolbachia*.

*Keywords: Armadillidium vulgare, FISH, oocytes, transmission*

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## Men are messy: *Wolbachia* stem cell niche tropism in *Drosophila* is evolutionarily conserved only in females

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*Wolbachia* are mainly vertically transmitted, however there is also evidence of extensive horizontal transmission and the mechanisms by which this happens are poorly understood. Previous work has shown that *Wolbachia* target stem cell niches (the microenvironment that supports the stem cells) in *Drosophila*, *Cimex lectularius*, and *Zyginidia pullula*. We hypothesize that *Wolbachia* transmission is facilitated by stem cell niche tropism. Supporting this idea, here we show that somatic stem cell niche tropism in the ovary of the *Drosophila* genus is evolutionarily conserved in 11/11 *Wolbachia* strains investigated, and is present in approximately 92% of niches examined (N= 1194). *Wolbachia* also target the germline stem cell niche in a subset of species investigated, revealing different patterns of niche tropism in the ovary. Phylogenetic analysis shows a high degree of conservation of the same pattern of niche tropism in closely related *Wolbachia* strains. If the major role of niche tropism is related to *Wolbachia* transmission, evolutionary theory predicts that there should be no corresponding force to maintain niche tropism in males, due to strict maternal transmission. In agreement, niche tropism in the testis is present in only 6/10 *Wolbachia* strains analyzed and is observed in only 39% of niches analyzed (N=510). We also found that *Wolbachia* factors, rather than host factors, mediate niche tropism patterns in both females and males. However, in the testis the frequency of niche tropism is not as tightly regulated as in the ovary, indicating a disordered pattern in the absence of stronger selective forces.

*Keywords: stem cell, niche, tropism, transmission, germline*

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## Autophagy regulates *Wolbachia* populations across diverse symbiotic associations

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*Wolbachia* symbiotic relationships encompass obligate mutualism, commensalism, parasitism and pathogenicity. The consequence of the diversity of hosts and symbioses is that the bacteria must evade a broad range of immune defence mechanisms to ensure their survival and transmission and the host must regulate the bacterial population to avoid pathogenicity or fitness costs. Our work shows that autophagy, a conserved intracellular defence mechanism and regulator of cell homeostasis, determines *Wolbachia* population size. The regulation of *Wolbachia* populations by autophagy occurs across all distinct symbiotic relationships and can be manipulated either chemically or genetically to modulate *Wolbachia* population load in host cells. The recognition and activation of the host autophagy is particularly apparent in rapidly replicating strains of *Wolbachia* found in somatic tissues of *Drosophila* and filarial nematodes. In filarial nematodes, which host a mutualistic association with *Wolbachia*, the activation of host nematode autophagy reduces bacterial loads to the same magnitude as antibiotic therapy and identifies a novel mode-of-action targeting *Wolbachia*, which can be exploited for the development of new chemotherapeutic agents against onchocerciasis, lymphatic filariasis and heartworm.

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## Evolution and control of host-microbe symbiosis in arthropods: an RNAseq-based transcriptomic analysis

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Symbiotic interactions between microbes and eukaryotic organisms are widespread in nature and exhibit different features ranging from parasitism to mutualism. However, despite their different outcomes, parasites and mutualists microbes share mechanisms of infection, proliferation and defense within hosts: the molecular dialog between microbial mutualists or parasites and their hosts appears to have common bases.

To decipher common but also specific molecular mechanisms underlying the functioning of intimate interactions, we have developed a comparative transcriptomic approach using RNAseq in four symbiotic systems exhibiting different features, but all involving arthropod hosts associated with endosymbiotic vertically-transmitted bacteria. Three species are associated with *Wolbachia*: the hymenopteran wasp *Asobara tabida*, where *Wolbachia* appears necessary for oogenesis completion, the mosquito *Aedes albopictus*, where cytoplasmic incompatibility-inducing *Wolbachia* are close to commensal and the isopod *Armadillidium vulgare* where *Wolbachia* induces feminization generating a strong nucleo-cytoplasmic conflict. The fourth species is the cereal weevil *Sitophilus oryzae*, which shares an obligate trophic relationship with a gamma-proteobacteria called SPE (*Sitophilus* primary endosymbiont).

RNAseq analyses have been performed on ovaries of infected and uninfected individuals. In this talk, we will present the bioinformatics pipeline that has been set up for exploitation of transcriptomics data on these non-conventional organisms. The identification of genes differentially expressed between infected and uninfected individuals and their comparison across four invertebrate species will permit to unravel common and distant molecular mechanisms involved in symbiotic interactions.

**Keywords:** Bacterial-arthropod symbioses, Functional pathways in symbiosis, RNA sequencing

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## Molecular mechanisms for *Wolbachia* tissue tropism in the gonads of *Drosophila melanogaster*

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Microbes preferentially infect specific cell types in their hosts - which is termed as tissue or cell tropism. Here we probe into the molecular mechanisms of the tissue tropism of two strains of *Wolbachia* - *wMel* and *wMelPop*, in the testis of *Drosophila melanogaster*. The two strains differ considerably in their infection levels at the niche harboring the stem cells in the testis, located at the anterior tip of the testis. We profiled the transcriptional landscape of microdissected tips, containing the niche, associated stem cells and primary spermatocytes. Microarray analysis of the apical tip revealed 130 genes that are differentially expressed between the uninfected, *wMel* infected and *wMelPop* infected tissue. Most of these genes are involved in processes related to metabolism, including proteolysis, lipid metabolism, transport, oxidation-reduction and cell cycle. 62 of these genes are differentially expressed between *wMelPop* and *wMel*. In agreement with previous work indicating that *Wolbachia* depends on host amino acid pool as an energy source, our data indicates greater proteolytic activity in the *Wolbachia* strain with higher niche tropism - *wMelPop*. Utilizing the genetic tools available in *Drosophila*, we are currently testing the functional significance of the candidate genes for tissue tropism. This analysis will further our understanding of the molecular pathways relevant for *Wolbachia* targeting of specific host tissues.

*Keywords: Tissue Tropism, wMel and wMelPop, Hub cells, Microarrays, Proteolysis*

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## **A cell-based screen reveals that a common *Albendazole* metabolite, *Albendazole-sulfone*, targets *Wolbachia***

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*Wolbachia* endosymbionts carried by filarial nematodes give rise to the neglected diseases African River Blindness and Lymphatic Filariasis in millions of people worldwide. In effort to identify new anti-*Wolbachia* therapies, we conducted a cell-based chemical screen using a *Wolbachia*-infected, fluorescently labeled *Drosophila* cell line. This screen yielded several compounds that resembled *Albendazole*, a widely used anthelmintic drug that targets nematode microtubules. Follow-up studies demonstrate that a common *Albendazole* metabolite, *Albendazole sulfone*, reduces intracellular *Wolbachia* titer both in *Drosophila melanogaster* and *Brugia malayi*, the nematode responsible for Lymphatic Filariasis. Significantly *Albendazole sulfone* does not disrupt *Drosophila* microtubule organization, suggesting that the compound reduces titer through direct targeting of *Wolbachia*. Accordingly, high resolution imaging demonstrates that *Albendazole sulfone* disrupts *Wolbachia* binary fission. This suggests that the efficacy of *Albendazole* in treating filarial nematode-based diseases is attributable to dual targeting of microtubules and *Wolbachia* endosymbionts of the nematode.

*Keywords: titer, Albendazole, Drosophila, Brugia malayi*

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## Mapping protein interactions between filaria and its *Wolbachia* endosymbiont

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*Brugia malayi*, like most human filarial parasite species, harbors an endosymbiotic bacterium of the genus *Wolbachia*. The *Wolbachia* represent an attractive target for control as killing of the bacteria using antibiotics affects molting, reproduction, and survival of the worms. The molecular basis for the symbiotic relationship between *Wolbachia* and their filarial host, *B. malayi*, remains unclear. To identify proteins involved in this process, we focused on *Wolbachia* (wBm) surface proteins (WSPs), which include 7 outer membrane proteins (OMPs) and WSPs, known to be involved in bacteria-host interactions in other bacterial systems. We have found that four of these proteins (Wbm0054, Wbm0152, Wbm0284, and Wbm0432) bind specifically to *B. malayi* crude extracts and individual filarial recombinant proteins. Moreover, some of these OMP members also localize to various host tissues of the *B. malayi* female adult worms and/or are present in the excretory/secretory products of the worms. Interestingly, two of these OMPs (Wbm0152 and Wbm0432) were identified in the worm's excretory-secretory products. We have identified an interacting pair of proteins from wBm and its host (Wbm0284/Bm-G2) that localizes to the cytoplasm and nucleus of the parasite, suggesting that this complex may be acting as a transcriptional regulatory factor. We also have found that Wbm0432 interacts with several key enzymes involved in the host glycolytic pathway, while Wbm0152 interacts with the host cell's cytoskeletal proteins. Our ongoing studies aim to verify the binding and uncover the possible physiological role of these interactions for the endosymbiotic relationship.

**Keywords:** *Filaria*, *Wolbachia*, symbiosis, surface proteins, interaction

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## Characterization of the peptidoglycan precursor Lipid II its biological role in *Wolbachia*

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The endosymbionts in the genus *Wolbachia* found in filarial nematodes and many arthropod species have undergone gene reduction throughout their evolution, including most genes necessary to encode proteins for de novo amino acid synthesis. Despite the lack of a cell wall, these organisms have maintained many of the genes necessary to encode proteins for the biosynthesis of the cell wall precursor Lipid II. Using recombinant proteins, purified *Wolbachia* membranes and a mosquito cell line infected with *Wolbachia pipientis*, we had previously demonstrated that Lipid II is synthesized and essential for survival of the endobacteria. However, proteins of this biosynthesis pathway absent from the annotated *Wolbachia* genomes indicated that Lipid II cannot form peptidoglycan due to the lack of transglycosylases, leaving an open question as to its biologic role in the endobacteria. Because there are also no canonical L- to D-amino acid racemases, Lipid II might have a unique pentapeptide structure. To better understand the biology of *Wolbachia*, we have further characterized the Lipid II biosynthesis pathway and present recent results describing an alternative biochemical activity of the *Wolbachia* MetC protein and an essential role for Lipid II during cell division.

*Keywords: Lipid II, cell wall, racemase, cell division, MetC*

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# **SYMBIONT DIVERSITY, DISTRIBUTION AND DYNAMICS**

**Chairman : Olivier DURON**



## Symbiotic bacteria as a factor of gene pool dynamics in insect populations

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Our project aimed to test the hypothesis that symbiotic transovarially transmitted bacteria considerably affect population structure of the insect hosts. This hypothesis was confirmed by gene pool analysis of several species, including mosquitoes of the *Culex pipiens* complex and ladybirds *Adalia bipunctata*.

Genetic structure was studied in 34 populations of the *Culex pipiens* mosquitoes (*Wolbachia* infected) from geographically distant areas of the European part of Russia, and also in populations from Germany, Italy, and Tunisia, using mitochondrial genome markers; several Russian populations of coccinellid *Adalia bipunctata* L. (*Spiroplasma*, *Wolbachia* and *Rickettsia* infected) were also studied.

It can be concluded that *Wolbachia* and *Spiroplasma*, though affecting differently the reproduction of the studied species (the first induces cytoplasmic incompatibility, while the second kills the males) have similar impact on genetic structure of the infected populations sharply decreasing the mtDNA polymorphism.

*Keywords: Wolbachia, Culex, Adalia, gene pools*

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## Near but far: Phenotype and genome comparisons reveal diversification between closely related members of *A.nasoniae* clade

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Our understanding of symbiont evolution commonly derives from comparison of the phenotypic properties and genomic constitution of symbiont strains that are distantly related. Understanding how symbiont-host interactions evolve over shorter periods of evolutionary time requires us to compare strains that are recently diverged. In this paper we present a comparison of two strains of *Arsenophonus nasoniae*. This bacterium was first recognized as the 'son-killer' microbe in the gregarious wasp *Nasonia vitripennis*. Duron et al. (2010) demonstrated that the microbe could easily transfer between wasp species in the laboratory and had done so in nature. Here we report on the properties of a second strain of *Arsenophonus nasoniae* from the solitary wasp *Pachycrepoideus vindemmiae*. As expected for a symbiont in a solitary host, the strain does not distort the sex ratio. We present key features of the host-symbiont interaction and a draft of the genome of this related, but distinct, strain of *A.nasoniae*. We conclude that closely related strains can have very different biology in terms of both observed phenotype and properties inferred from the genome sequence.

*Keywords: Arsenophonus, genome, evolution, male-killer, host-shift*

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## Facultative symbiont distribution among global populations of *Aphis craccivora*: host plant is more important than geography

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The potential role facultative symbionts play in herbivore specialization remains contentious. In the pea aphid, *Acyrtosiphum pisum*, populations associated with different host plants consistently have different facultative symbiont communities. While some authors have found that these facultative symbionts facilitate *A. pisum* specialization on particular hosts, others have found that facultative symbionts don't affect host plant performance. We propose that the cowpea aphid, *Aphis craccivora*, may represent a suitable alternative system for investigating ecological interactions between facultative symbionts and herbivore host utilization. Like *A. pisum*, *A. craccivora* is a broadly polyphagous global pest. In a survey of populations collected off various host plants from around the world, we have found that *A. craccivora* is infected with at least 6 different facultative symbionts. Of these, *Arsenophonus* was found at consistently high levels in aphids collected from black locust, *Robinia pseudoacacia*, and was not prevalent in aphids from most other host plants. In contrast, *Hamiltonella* was almost exclusively found in aphids collected from alfalfa, *Medicago sativa*. We confirmed this pattern by conducting a local survey in Kentucky, USA: all *A. craccivora* populations collected from *M. sativa* had high prevalence of *Hamiltonella*, and all populations collected from *R. pseudoacacia* had high prevalence of *Arsenophonus*. Neither symbiont was fixed in populations of aphids from the respective host plants, indicating that the symbionts are not requisite for use of that plant. Experimental studies are ongoing, but our current hypothesis is that each symbiont provides *A. craccivora* with ecologically-mediated benefits that are somehow conditional on host plant.

*Keywords: Arsenophonus, Hamiltonella, Rickettsia, Serratia, Spiroplasma*

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## Endosymbiont diversity among weevil sibling species competing for the same food resource

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While the impact of endosymbionts on the population dynamic of their host is well recognized, their influence on the organization of communities of host species competing with each other is much less investigated. The mechanisms underlying the coexistence of competing sibling species remaining unclear, we hypothesize that endosymbiosis may contribute to structure communities of such species in two different ways: (i) distinct endosymbionts may promote accelerated diversification of the ecological niche occupied by their hosts and thus ensure their stable coexistence; (ii) endosymbionts manipulating host reproduction may accelerate speciation and thus increase the species richness in communities. In both cases, the endosymbiotic communities are expected to be distinct among competing host species. Here, we examined the endosymbiotic communities from four weevil species (*Curculio* spp.) that coexist and compete within the same individual oak trees (*Quercus* spp.). In addition to the primary symbiont that was found in all species, the weevil species clearly exhibited distinct secondary endosymbiotic communities, including *Rickettsia*, *Spiroplasma* and 2 strains of *Wolbachia*. Remarkably, three of the four weevil species were shown to house their own predominant facultative symbiont, while all species retained the remaining symbionts at a residual level. These data support a possible involvement of secondary endosymbiont communities in the organization of communities of sibling insect species competing for the same resource.

**Keywords:** Endosymbiosis, *Curculio*, oak weevil, niche partitioning, coexistence

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## ***Acinetobacter* is more prevalent than *Asaia* in field populations of the mosquito vector *Aedes albopictus* with isolates showing diverse genetic rearrangements and metabolic profiles**

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Insects harbor a great diversity of bacteria with which they established symbiotic relationships ranging from parasitism to mutualism. While hematophagous insects are involved in the transmission of many pathogens, only few studies described interactions with their associated symbionts. In the mosquito vector *Aedes albopictus*, recent studies highlighted the presence of two cultivable bacteria *Acinetobacter* and *Asaia* in insect organs but no systematic survey was conducted to explore the occurrence of such bacteria in field populations. Here, we investigated the prevalence of these two bacteria in mosquito populations from Madagascar by diagnostic PCR amplification of the 16S rRNA gene. Both genera were detected at relatively high frequencies, notably 46% for *Asaia* and 70% for *Acinetobacter*, with variation depending on the mosquito gender (*Acinetobacter*) or the interaction between the mosquito gender and the sampling site (*Asaia*). As *Acinetobacter* was the most prevalent genus, it was further fully characterized at genomic level and metabolic properties. Using pulsed field gel electrophoresis, no significant difference in genome size was found between mosquito *Acinetobacter* isolates compared to free-living relatives. However, a great diversity was observed in the number and size of plasmids, which correlated with the high diversity in substrate utilization. Further studies are still needed to determine which genes are encoded by plasmids of *Acinetobacter* mosquito isolates. Overall, these informations would be of great importance to assess the molecular foundations of the relationships between *Ae. albopictus* and *Acinetobacter*.

*Keywords: Acetic bacteria, Asian tiger mosquito, symbiosis, genomic diversity, metabolic repertoire*

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## **PEST CONTROL**

**Chairmen : Patrick MAVINGUI, Stephen DOBSON**

## Differential effects of the native *Wolbachia* symbionts on transmission of arboviruses in the mosquito *Aedes albopictus*

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The mosquito *Aedes albopictus* is considered as the principal vector of recent chikungunya (CHIK) outbreaks and behaves as a secondary vector of dengue (DEN) after *Aedes aegypti*, the main epidemic vector. While *Ae. aegypti* is free of *Wolbachia*, *Ae. albopictus* individuals are naturally super-infected with two *Wolbachia* strains, wAlbA and wAlbB. Considering the overlapping distribution of both *Wolbachia* and arboviruses in non-reproductive tissues of mosquitoes such as midgut and salivary glands, we conducted experiments to characterize the role of *Wolbachia* in viral transmission by *Ae. albopictus*. First, we demonstrated that after ingestion by *Ae. albopictus* of a blood-meal containing CHIK virus, the intensive phase of viral replication coincides with a decrease in *Wolbachia* densities. The *Wolbachia* decrease might result from competition for resources with replicating CHIKV in mosquito cells. Later, we showed that this pattern was not applicable to another arbovirus, DEN virus. Indeed, replication of DEN virus does not affect *Wolbachia* densities in *Ae. albopictus*. However, *Wolbachia* is able to reduce infection of *Ae. albopictus* salivary glands by DEN virus and to limit transmission. This suggests a role of *Wolbachia* to naturally restrict the transmission of DEN virus. Thus depending on the virus, *Wolbachia* may play a determinant role in shaping the ability of a naturally *Wolbachia*-infected mosquito such as *Ae. albopictus* to transmit CHIKV and DENV.

*Keywords: mosquito, chikungunya, dengue, bacteria, vector competence*

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## ***Wolbachia* wMel substrains confer differential protection to viruses**

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*Wolbachia* are able to protect various insect hosts from viral infections. This tripartite interaction was initially described in *Drosophila melanogaster* carrying wMel, its natural *Wolbachia* strain. Recently *Wolbachia* has been proposed as a tool for the control of vector-borne diseases. Mosquitoes harbouring wMel are more resistant to dengue virus and a release of these in dengue-endemic areas could reduce the prevalence of this disease in human populations. Despite its importance, the variability in the antiviral protection provided by natural variants of wMel has never been explored.

We have compared the antiviral protection conferred by wMel substrains in a genetically identical *Drosophila melanogaster* host. Our data reveal that different wMel variants confer different levels of protection upon infection with *Drosophila C* virus and Flock house virus. The titres of the endosymbiont are also substrain dependent. Furthermore the wMel substrains reaching higher levels in the host confer stronger antiviral protection. These phenotypes allow us to cluster the wMel substrains into two groups. One contains wMelCS and wMelCS2 and confers more protection to viruses than the one comprised of wMel, wMel2 and wMel3.

Our study strengthens the notion that the outcome of the disease depends not only on the genotypes of the host and the pathogen but also on the genotypes of the symbionts associated with them. As the polymorphisms within the genomes of the wMel substrains are described, the differences in the antiviral protection would allow dissecting the *Wolbachia* factors engaged in this phenomenon. Finally, our work helps to understand the interaction of wMel with its natural host and facilitates better planning of *Wolbachia* use in dengue control.

*Keywords: wMel, substrains, virus-protection, viruses, Drosophila*

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## Implication of *Wolbachia* on malaria (*Plasmodium sp.*) transmission by mosquitoes

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In recent years, there has been a shift in the one host one parasite paradigm with the realization that, in the field, most hosts are co-infected with multiple parasites. This question is particularly relevant when the host is a vector of diseases, because multiple infections can have drastic consequences for parasite transmission at both the ecological and evolutionary time scales. *Wolbachia pipientis* is the most common parasitic microorganism in insects and as such it is of special interest for understanding the role of coinfections in the outcome of parasite infections. I have investigated whether *Wolbachia* can modulate the effect of *Plasmodium* on different life history traits of mosquitoes, such as adult's size, fecundity and on what is, arguably, the most important component of the vectorial capacity of mosquitoes: their longevity. For this purpose, and in contrast to recent studies which have focused on mosquito-*Plasmodium* and/or mosquito-*Wolbachia* combinations not found in nature, we work on a *Wolbachia-Culex pipiens-Plasmodium* triad with a common evolutionary history. To explore different mechanistic explanations for my results, I have also carried out two different experiments in which I investigate whether *Wolbachia* or *Plasmodium* can modulate the energetic budget and the immune response of mosquitoes. Further, I discuss different evolutionary explanations as well as their consequences for *Plasmodium* transmission.

*Keywords: Culex pipiens, Plasmodium relictum, wPip, interaction, evolution*

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## Eliminating barriers to *Wolbachia* germline infection and vertical transmission in *Anopheles gambiae*

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*Department of Entomology, Center for Infectious Disease Dynamics and the Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA 16802, USA*

Many important arthropod species are uninfected by *Wolbachia*. *Wolbachia* can be artificially transferred between insects in the laboratory but this can be a laborious and sometimes fruitless process, and little is currently known about the factors governing successful horizontal transfer of *Wolbachia* between species. We have developed a simple in vitro assay to assess the ability for diverse *Wolbachia* strains to colonize arthropod germlines. Using this system, we identified a *Wolbachia* strain that is capable of infecting the germline of the major malaria vector *Anopheles gambiae*. After injecting this strain into the mosquito, we show by quantitative PCR and fluorescence in situ hybridization that germline infection and low levels of vertical transmission occur. We have also begun to identify specific barriers to *Wolbachia* vertical transmission in *An. gambiae*. By manipulating these barriers, 100% transmission can be achieved at densities comparable to *Wolbachia* titers in naturally infected hosts. These results are not only important for the creation of stably-infected *An. gambiae* strains for malaria control, but also provide insight into why some hosts are uninfected by *Wolbachia* in nature.

*Keywords: Anopheles gambiae, malaria, vertical transmission, Wolbachia distribution*

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## **Mechanisms of *Spiroplasma* transmission to developing oocytes in *Drosophila melanogaster***

Herren J.K., Paredes J.C. and Lemaitre B.

*Global Heath Institute, École Polytechnique Fédérale de Lausanne, Station 19, 1004, Switzerland*

*Spiroplasma poulsonii* exhibit highly efficient vertical transmission in their host *Drosophila melanogaster*. We find that mutations affecting the transport of yolk cause a drastic decrease in the vertical transmission of *Spiroplasma*. Using immuno-fluorescence microscopy we show that *Spiroplasma* are associated with yolk granules after entering the oocyte and that the lack of functioning yolk transport specifically inhibits the entrance of *Spiroplasma* during the vitellogenic stages of oogenesis (stages 8-10). Prior to its entrance into the oocyte, *Spiroplasma* can be observed in between follicle cells and in the extracellular space between follicle cells and the oocyte.

*Keywords: Spiroplasma, Yolk, Vertical transmission, Oogenesis*

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## **Applied use of *Wolbachia* as a 'microbial pesticide' to control medically important *Aedes mosquitoes***

Brelsfoard C.L., Mains J.W., Crain P.R., Andrews E.S. and Dobson S.L.

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An update will be provided on mathematical modeling and empirical work, toward the application of artificially generated *Wolbachia* infections against mosquitoes important to public health. The ability to generate transfected mosquitoes has resulted in patterns of cytoplasmic incompatibility that can be used for both the suppression and replacement of important mosquito species. This has led to the recent rapid progression from mathematical models to contained tests to field trials of applied strategies. We will provide a synthesis of this progression, discussing examples of recent work, complications and the potential of this new tool.

*Keywords: lymphatic filariasis, dengue, Aedes polynesiensis*

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## Theoretical assessment of population replacement when *Wolbachia* negatively affects host life history

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The evolutionary success of *Wolbachia* depends on the ability of its infection to spread through a population. Some *Wolbachia* infections cause fitness costs in hosts, which reduce the probability that such an infection can invade and establish. By manipulating populations, infections that may not be successful in nature can persist and spread despite being relatively poor competitors. Simulations of an explicit population dynamic model with stochastic female adults were used to calculate the likelihood of population replacement. Replacement scenarios include traditional population replacement concepts (*i.e.* a unidirectional crossing pattern of infected individuals that drives *Wolbachia* into an uninfected population), but also include competitive interactions of infected and uninfected individuals, reverse population replacement (*i.e.* conditions where uninfected populations can invade an infected population), and hypothetical interactions between competing infected and uninfected host populations with different reproductive rates. Results indicated the threshold parameter values required to achieve population replacement in each scenario. Generally, *Wolbachia* infections tend to achieve population replacement when costs are low, but can be successful at high costs under certain conditions. However, in populations where *Wolbachia* is fixed and relatively costly, the infection is stable despite large introductions of uninfected individuals. These results are discussed in relation to ongoing and future *Wolbachia*-based control strategies.

*Keywords: mathematical modeling, population dynamics*

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## POSTER



## **ECOLOGY & PHENOTYPES**

## The constant and high *Wolbachia* incidences in global community of fig wasps

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*Department of Genetics, University of Pretoria, Pretoria 0002, South Africa, zaheerento@gmail.com*

*Wolbachia* is a very common and widespread endosymbiont of arthropods. It can have considerable effect on its host's fitness. Putative global *Wolbachia* infection is 66% and particular taxonomic or ecological niches display significantly higher or lower incidences. In this study, we combined African samples with those of three previous studies from three continents. We reanalyzed *Wolbachia* incidence in 172 fig wasp species (including pollinators, non pollinators, gallers and non gallers) associated with 81 fig trees of six sub genera and revealed that *Wolbachia* infection in fig wasp species is 103/172 (60%). Importantly, its incidence is random with respect to host taxonomy, ecology or geography. The prevalence of high incidence of *Wolbachia* in fig wasp species of four different continents is randomly distributed and its more or less constant infections all over four continents may exhibit global equilibrium of *Wolbachia* infection.

*Keywords: Wolbachia incidence, fig trees, pollinators, non pollinators, sex ratio*

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## Evolutionary history of *Wolbachia* dynamics in global *Bemisia tabaci* cryptic species and their natural enemies

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*Wolbachia* is among the most common endosymbionts infecting arthropods. It invades host populations by manipulating its host reproduction with mechanisms such as cytoplasmic incompatibility and in so doing it increases the rate of host speciation. One species of insect, *Bemisia tabaci* (Homoptera: Aleyrodidae), is increasingly being considered as a cryptic species complex that contains at least 28 species, a number of which are infected with *Wolbachia*. It is therefore possible that *Wolbachia* may play a role in the evolution of these species. To appreciate the role that it may have played, it is first necessary to reconstruct the relationships between *Wolbachia* genotypes across their *B. tabaci* hosts. To this end we surveyed *Wolbachia* across different *B. tabaci* cryptic species as well as their natural enemy community, and after accounting for possible recombination, identified 34 different genotypes across *B. tabaci* and its natural enemies. Of these, 29 belong to the strain wBt1 which is predominant and occurs in *B. tabaci* cryptic species from Asia, Africa, Australia and Europe as well as their parasitoids and predators. The co-phylogenetic analyses revealed little evidence for cospeciation, but considerable evidence for host switches and duplications. In addition, the exploration of the relationship between *Wolbachia* genotypes and invading and indigenous *B. tabaci* in China found evidence for at least two host switches between invading and indigenous *B. tabaci*. Furthermore, as the invader and indigenous individuals are unable to copulate, we suggest that natural enemies may play a role in this horizontal transmission.

**Keywords:** *Wolbachia*, Whitefly, genetic networking, *wsp* gene, mitochondrial gene

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## De novo characterization of *Wolbachia* communities using a combination of HRM and Sanger sequencing

Arthofer W.<sup>1</sup>, Peschl P.<sup>1</sup>, Schuler H.<sup>2</sup>, Stauffer C.<sup>2</sup>, Steiner F.M.<sup>1</sup> and Schlick-Steiner B.C.<sup>1</sup>

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Large-scale screenings for *Wolbachia* usually assess whether the endosymbiont is present in the specimens examined, but do not draw inferences on strain diversity within individuals, populations or species. However, multiple *Wolbachia* infections are common, and strain composition may have strong influence on the expression of *Wolbachia* phenotypes. Recently, high-resolution melting analysis (HRM) was introduced as a tool for the fast identification of characterized *Wolbachia* strains, with the major limitation of ambiguous results in multiply infected individuals. We present a different approach and utilize HRM for the fast and cost-effective de novo characterization of *Wolbachia* communities. Completely omitting the traditional end point PCR diagnostic step, a large sample of specimens with unknown infection status is subjected to amplification of a ca. 125 bp stretch containing the *wsp* HVR4 region and HRM analysis. By this, (i) a preliminary *Wolbachia* diagnosis for each sample and (ii) a set of melting curve profiles is retrieved. Subsequent cloning and sequencing of *wsp* and MLST genes can be focused on a few specimens representative for the distinct HRM profiles. We demonstrate the suitability of this approach by the de novo characterization of the *Wolbachia* communities of two arthropod species.

*Keywords: diagnosis, high resolution melting analysis, endosymbiont communities, de novo characterization, multiple infection*

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## Does heterozygosity influence mate choice of the terrestrial isopods *Armadillidium vulgare*?

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Heterozygosity is a key factor that can influence mate choice. We experimentally determine the influence of this factor on mate preference in the crustacean terrestrial isopod *Armadillidium vulgare*. This biological model is gregarious which could increase the risk of inbreeding by mating with closely-related conspecifics. Mechanisms of inbreeding avoidance during mate choice can be thus expected. Moreover, a female bias sex ratio induced by *Wolbachia* (feminising males) predicts that males could be the choosy sex with mechanisms of infection avoidance which have been ever shown.

We perform Y-choice tests giving to male the choice between two genetically different females. This experiment is performed using genetically controlled individuals with genetic heterozygosity predetermining before behavioural tests. The first results seem to go in the sense of inbreeding avoidance according to the heterozygosity of females proposed to male. Indeed, the male seems to prefer to spend time with the more heterozygous female.

This male preference should result in more heterozygous offspring, especially when the male's heterozygosity is low. Because genetic heterozygosity might partly determine the ability of host to resist to parasite, adaptative mate choice could be an important factor in the evolutionary arms race between *A. vulgare* and its sexual parasite *Wolbachia*.

*Keywords: Heterozygosity, mate choice, Armadillidium vulgare, Wolbachia*

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## Assessing the effects of *Wolbachia* on mosquito life history traits

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*Wolbachia* is a well-documented reproductive manipulator of its hosts, but has been recently implicated in modification of host life history traits. *Wolbachia* affects physiology and behavior, but its life history effects can influence host population dynamics. Furthermore, the spread of *Wolbachia* can depend on fluctuations in population dynamics. To examine proximate and ultimate costs/benefits of *Wolbachia*, we have conducted theoretical and empirical studies. Empirical studies quantified life history traits of mosquito strains with novel *Wolbachia* infections and calculated respective net reproductive rates  $R_0$ . No difference in development time was found, but larval survival was significantly different across strains. The differences in larval survival were mirrored in net reproductive ratios. Scenarios above were then assessed theoretically in an explicit population dynamic model. The results generally resemble those from previous theoretical studies, except for newly documented response to reduced larval viability, which inhibits population replacement. Further theoretical and statistical tests identified key parameters that differ between infected and uninfected populations. Results here suggest the need for additional empirical studies, focused on the life table of insects with various *Wolbachia* infections. With the advancement and success of open field trials using *Wolbachia*, experiments examining such effects can help elucidate applied and evolutionary mechanisms of population dynamics and replacement.

*Keywords : life history, population dynamic modeling*

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## Cooperation between *Wolbachia* and leaf-miners to produce “green-islands”: insights into the plant-insect-bacteria interaction

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The life-cycles of many organisms are constrained by the seasonality of resources. This is particularly true for leaf-mining herbivorous insects who use deciduous leaves to fuel growth and reproduction even beyond leaf fall. Our results suggest that an intimate association with bacterial endosymbionts might be their way of coping with nutritional constraints to ensure successful development in an otherwise senescent environment. We show that the phytophagous leaf-mining moth *Phyllonorycter blancardella* (Lepidoptera) relies on bacterial endosymbionts, most likely *Wolbachia*, to manipulate the physiology of its host plant resulting in the “green-island” phenotype - photosynthetically active green patches in otherwise senescent leaves - and to increase its fitness. Curing leaf-miners of their symbiotic partner resulted in the absence of green-island formation on leaves, increased compensatory larval feeding, and higher insect mortality. Our results suggest that bacteria impact green-island induction through manipulation of cytokinin levels. All individuals analyzed so far are closely associated with *Wolbachia* and a key enzyme of the cytokinin biosynthetic pathway (ipt: isopentenyl transferase) has been isolated, cloned and sequenced from the *Wolbachia* genome (tRNA-ipt Wo). This key enzyme is clearly expressed in *Wolbachia*-infected insects. This is the first example of an insect bacterial endosymbionts associated with alterations of the plant physiology. Our results highlight (i) the intimate interactions between the bacterial symbionts and their insect host and (ii) the presence of cytokinin-producing *Wolbachia* and associated plant physiological alterations in different population of this species as well as in different plant-leafminer systems.

**Keywords:** *Leaf-miner, Wolbachia, Green-island, Cytokinin*

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## How many more arthropods may inherit *Wolbachia* F supergroup?

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We describe surveys conducted to detect *Wolbachia* in native *Agrilus* species; *A. anxius*, *A. bilineatus* and *A. liragus* as well as their parasitic wasps that have switched hosts and may have potential for biocontrol of *A. planipennis*. The most abundant parasitoid species was *Phasgonophora sulcata* followed by *Atanycolus* spp. and the less abundant parasitoids were *Balcha indica* and *Spathius* spp. Amplification of the *Wolbachia* surface protein gene (*wsp*) detected *Wolbachia* only in populations of *P. sulcata* and demonstrated that this endosymbiont was highly prevalent (100%) in sampled populations regardless of geographic location. This high prevalence suggests an obligatory symbiosis, which may result from co-adaptation and reciprocal dependence through long coevolution of a host and its symbiont. Sequencing revealed that the *Wolbachia* harboured in *P. sulcata* populations exhibited an *ftsZ* belonging to a unique subgroup within the F-supergroup of known *Wolbachia*. The newly identified strain may either belong to a different strain of *Wolbachia* from those previously found to infect other arthropods or may be the result of a horizontal transfer event. We would provide information on characterization of the *P. sulcata* inherited *Wolbachia*, discuss patterns of genetic diversity in demographic history and comment on possible selective sweep and its impact on *P. sulcata* reproduction.

*Keyword: Phasgonophora sulcata, Hymenoptera, Wolbachia, phylogeny, supergroup-F*

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## Endosymbiont screening in *Anastrepha* (Diptera: Tephritidae) flies revealed a novel *Wolbachia* strain present in *Anastrepha striata*

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Flies in the genus *Anastrepha* (Diptera: Tephritidae) are widely distributed in Mexico and are important fruit crop pests in tropical and subtropical regions. Bacterial endosymbionts are ubiquitous in arthropods and their effects are diverse, including reproductive parasitism, nutritional mutualism, defensive mutualism, and other forms of parasitism, commensalism and mutualism. Presence of endosymbiotic bacteria in Mexican populations of *Anastrepha* flies may be relevant for eradication efforts, because nutritional mutualisms could be used to enhance the production of sterile males and defensive mutualisms could limit the use of natural enemies (e.g., parasitoids) as an eradication measure. For this project, we collected *A. ludens* and *A. striata* females in the Mexican state of Chiapas (Southeast), from sour orange and guava plantations. DNA was purified from dissected ovaries and from whole bodies. We used PCR to screen for presence of endosymbiotic bacteria with three sets of universal bacterial primers, in addition to *Wolbachia*- and *Spiroplasma*-specific primers. The only heritable endosymbiont found was *Wolbachia* in *A. striata*, based on Multi-Locus Sequence Typing and phylogenetic analyses; this *Wolbachia* strain is new and belongs to the *Wolbachia* supergroup B. *Wolbachia* strains previously reported in members of the genus *Anastrepha* in South America belong to supergroup A. We discuss the potential implications for pest control of the presence of a different *Wolbachia* strain in southeastern Mexico.

*Keywords:* *Wolbachia*, MLST, Tephritidae, Incompatible insect technique, Chiapas

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## High-Resolution Melting technology: a new tool for studying the *Wolbachia* endosymbiont diversity in the field

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Many questions remain regarding the contribution of *Wolbachia* to host biology and ecology, and especially on environmental adaptation of their host. To better understand the influence of *Wolbachia* on host phenotypes and consequences of the manipulation of reproduction on the host genetic differentiation, it is crucial to be able to discriminate *Wolbachia* strains and determine their prevalence, which requires exhaustive screening. In the present study, we proposed the use of a new tool for population studies, based on the High Resolution Melting (HRM) analysis, less expensive and faster than the “classical” methods for large scale studies. We investigated the effectiveness of HRM to explore and characterize the diversity of *Wolbachia* strains. Results obtained showed that HRM is a powerful tool to identify strains and detect polymorphism in singly-infected hosts. When individuals harboured a mixture of *Wolbachia* strains (multiple infections), there is a risk of underestimation of the diversity if the proportions of the strains are highly different. However the same limitations exist for the other techniques commonly used. Overall, this study demonstrated that HRM analysis is a rapid and reliable technique useful for studying, without *a priori*, *Wolbachia* strains diversity in field populations.

*Key-words: HRM, screening, Wolbachia diversity, field populations*

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## The potential of *Wolbachia* to trigger cytoplasmic incompatibilities in tsetse fly inter-species hybrids

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In the 1940s, it was suggested to exploit cytoplasmic incompatibility (CI) to induce natural reproductive sterility in tsetse fly (genus *Glossina*) populations and consequently block transmission of *Trypanosoma*, the causative agent of human sleeping sickness [1,2]. In the light of a novel study, this idea is currently revived: strong unidirectional CI has been reported among crosses between *Wolbachia*-infected and antibiotic-treated tsetse flies of *Glossina morsitans morsitans*, pinpointing the biological significance of these symbionts in tsetse [3].

Natural hybridization between *Glossina* species in the field has been reported repeatedly, suggesting that pre-mating barriers to hybrid formation are rather weak between sympatric members of this genus [1,2,4]. However, under laboratory conditions female hybrids exhibit reduced fecundity and male hybrids are sterile. Hence, in the evolutionary point of view the *G. morsitans* group is considered a very young and highly dynamic species complex with weak pre-mating isolation but significant post-mating barriers. Since strong incompatibilities were observed in crosses of certain *G. morsitans* subspecies, where one crossing direction was less compatible than the reciprocal one, the basis of such mating incompatibilities was attributed to maternally inherited cytoplasmic factors, most likely *Wolbachia* [5].

Here, we report on intensive *Wolbachia* overreplication in inter-species hybrids belonging to the *G. morsitans* species group, quite similar to the situation we have recently described in *Drosophila paulistorum* [6]. We speculate that disturbance of the native host-symbiont equilibrium in hybrids with mixed genetic host backgrounds can transform symbiotic *Wolbachia* into pathogens by loss of replication control. This might consequently trigger hybrid incompatibilities between tsetse flies and thereby foster incipient speciation.

[1] Potts, W.H. 1944

[2] Vanderplank, F.L. 1944

[3] Alam, U., et al. 2011

[4] Gooding, R.H. 1993.

[5] O'Neill, S.L., et al. 1993

[6] Miller, W.J. et al. 2010

**Keywords:** *Glossina*, inter-species hybrids, pre- and post-mating isolation, speciation

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## Geographic variation of indirect flight muscle dimorphism in *Heptophylla picea* and its relationship to *Wolbachia* infections

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The yellowish elongate chafer, *Heptophylla picea*, is one of the major pest insects of tea fields in Japan. All adult males of this chafer have fully developed indirect flight muscles, whereas adult females show dimorphism in the flight muscles i.e., muscled and muscleless. Flight muscle dimorphism in *H. picea* females follows a simple Mendelian trait, with the muscleless form being dominant than the muscled one. Preliminary research indicated that *H. picea* was infected with intercellular symbiont *Wolbachia*. Occasionally, *Wolbachia* induces a selective sweep phenomenon in the host population. In this study, I intended to clarify the factors involved in the evolution of indirect flight muscle dimorphism. The geographic pattern of flight muscle dimorphism, haplotype variations of partial mitochondrial DNA, and *Wolbachia* DNA were investigated.

*Wolbachia* infected most populations and individuals of *H. picea*. The partial DNA sequence of the *wsp* gene showed that a single strain of *Wolbachia* infected *H. picea*. On the basis of results of mitochondrial DNA gene, two phylogenetic groups in *H. picea* can be distinguished. This grouping approximately reflects muscled and muscleless populations. The evolution of the muscleless form may have occurred many times in different populations, or *Wolbachia* infection may affect the spread of muscleless females in the Japanese Archipelago. In addition, environmental factors such as high altitude and latitude, and tea field may prove to detrimental for muscled and muscleless females, respectively.

*Keywords: Wolbachia, indirect flight muscle dimorphism, Scarabaeid beetle, geographi variation*

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## Genetic analysis of *Wolbachia* endosymbionts in Great Salt Lake Brine flies

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The genus *Wolbachia* comprises a group of endosymbiotic bacteria that have profound effects on the physiology and reproduction of their hosts. To date, at least ten major groups of *Wolbachia* have been identified, some of which correlate with the effect on the host. It is estimated that *Wolbachia* infect more than 65% of all insect species, but their presence in many taxa has not been determined. In particular, there is no description to date of *Wolbachia* in organisms inhabiting extreme environments. Great Salt Lake is the largest saline lake in the western hemisphere and is characterized by salinity levels in some regions approaching 27%. Among the few organisms that thrive in such conditions are brine flies, including *Cirrhia hians*, *Mosillus bidentatus*, and *Ephydra gracilis*. Using molecular genetic techniques, this study identifies *Wolbachia* in these three species, the first description of *Wolbachia* from a host that is associated with an extreme environment. The presence of *Wolbachia* in the brine flies was examined using the polymerase chain reaction with primers specific for *Wolbachia* 16S rRNA genes. DNA sequence comparisons were used to determine the phylogenetic affiliation of the brine fly *Wolbachia* samples. Determining the phylogenetic position of the Great Salt Lake *Wolbachia* is an important first step in assessing the contribution these bacteria may make to the incredible adaptability of brine flies. The results of this study may provide an indication of the precise effects these bacteria have on their brine fly hosts and will contribute to our understanding of *Wolbachia* diversification.

*Keywords: Great Salt Lake, brine flies*

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## ***Wolbachia-Drosophila* interactions in normal and stressful conditions: ultrastructural assay**

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Stressful conditions influence on *Wolbachia*-host association, but their effects have not been well investigated at the ultrastructural level. We have tested the morphology and distribution of *Wolbachia* wMelPop in ovaries of *Drosophila melanogaster*<sup>w<sup>1118</sup></sup> under normal conditions and after starvation and heat treatment of flies. At the normal conditions the new electron-dense form (0.2-0.5 µm in diameter) of *Wolbachia* was found which may represent dormant form of bacteria or minicell, containing RNA and protein, but little or no chromosomal DNA. Starvation and high temperature caused morphological changes in host cytoplasm which are related with stress response and presented in *Wolbachia*-infected and uninfected flies. Stressful conditions did not effect on *Wolbachia* localization in ovarian cells of *D. melanogaster*<sup>w<sup>1118</sup></sup>, however resulted in degeneration and morphological changes in bacteria. Starvation induced nonselective autophagic degradation of *Wolbachia* and host cytoplasmic organelles. High temperature initiated vesiculation process in *Wolbachia*. Bacteria did not change the contacts with the endoplasmic reticulum in ovarian cells in stressful conditions. The morphological heterogeneity of *Wolbachia* response to starvation and high temperature has been demonstrated. Such heterogeneity could represent the adaptive potential of endosymbiont population.

This work was supported by the Program of the Presidium of RAS "Biodiversity" (26.30).

**Keywords:** wMelPop, *Drosophila melanogaster*, ultrastructure, high temperature, starvation

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# EVOLUTION

## Is RAD-sequencing suitable to detect *Wolbachia*-driven introgression ? An in silico assessment

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Maternally inherited intracellular symbionts can spread across closely related species following hybridization events. Because of co-transmission with other cytoplasmic elements, this process can indirectly produce mitochondrial introgression. Using a large sample of Arthropods from French Polynesia, we aim at estimating the frequency of mitochondrial introgression and quantifying the contribution of *Wolbachia* to this phenomenon. Mitochondrial introgression can be detected through discordances between nuclear and mitochondrial phylogenies. Mitochondrial phylogenies, based on the CO1 gene, have previously been obtained ; we envisage to use Restriction Associated DNA sequencing to generate nuclear phylogenies. Here we assess the suitability of this particular technique for such a purpose. Resolution of nuclear phylogenies between closely related species can be hindered by several problems; lack of nuclear markers informative at short evolutionary timescale, incomplete lineage sorting and introgression for particular loci and lack of PCR-primer information in poorly studied taxa. In this context Restriction site Associated DNA sequencing (RADseq) seems promising (Davey et al. 2011). This technique can generate sequence data from DNA fragments flanking restriction sites, thus randomly distributed throughout the genome, from a large number of samples and without preliminary knowledge on the taxa under study. RADseq was first developed for population genetics and quantitative trait mapping. The suitability of this method for phylogenetic inference, relying on the presence of conserved restriction sites in different species, thus remains to be evaluated. We simulated a RADseq experiment using the 12 *Drosophila* genomes. Using these data, we were able to recover the expected phylogenetic relationships between the 12 *Drosophila* species, with strong statistical support. This study therefore validates the suitability of the RADseq technique to assess mitochondrial introgression.

### Références

Davey J. L. and M. W. Blaxter (2011) RADSeq: next-generation population genetics. *Briefings in functional genomics*. **9(5)** : 416-423

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## The associations among WO Bacteriophage, *Wolbachia* and *Corcyra cephalonica*

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WO phage is a bacterial virus infecting *Wolbachia* in arthropods. It is believed that WO phage may participate in the manipulation of genetic alteration of the hosts. *Wolbachia* strains and associated bacteriophage in *Corcyra cephalonica* was identified and characterized with which the presence of *Wolbachia* has been previously documented in *Corcyra cephalonica* but no the bacteriophage in this study. The relative densities of *Wolbachia* *wsp* gene and WO bacteriophage *orf7* gene in eggs, larva, pupa and adult insect were measured by Real-time quantitative-PCR. The amplification reaction was monitored using a SYBR green. The infection relationship between *Wolbachia* and WO phage, as well as the stability of WO phage in different generations and geographical populations of *Corcyra cephalonica* was observed. The results showed that WO phage was a special virus infecting *Wolbachia*, both stably existed in different generations and geographical populations of *C.cephalonica*. Phylogenetic tree established by *wsp* of *wolbachia* and by *orf7* of WO indicated that there was no consistency in topological structure. Phylogenetic analysis also revealed that WO phage and *Wolbachia* might have had a long and stable coevolution relationship. It is proved by the electron microscopic observation that WO bacteriophage was able to replicate its genome and, to form mature virus particle.

*Keywords: WO Bacteriophage, Wolbachia, Corcyra cephalonica, Co-evolution*

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## Evolution of *Wolbachia* infection in the tribe Naupactini (Coleoptera, Curulionidae): a mix of old associations and recent transfers

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*Wolbachia* infection has been detected in weevils of the tribe Naupactini. Although there is a correlation between reproductive mode and infection status (parthenogenetic species infected vs. sexual species not infected), a causal relationship was still not discovered. In the present contribution, we aimed to infer the events that shaped the current distribution of *Wolbachia* strains across the tribe Naupactini. With this purpose, we analyzed the history of the association between parasites and sexual and asexual weevil species.

We typified *Wolbachia* strains using MLST and *wsp* sequences and estimated host phylogeny through morphological and molecular (COI gene) characters. A cophylogenetic analysis was applied using the software Jane. We detected a basal cospeciation event that suggests a long history of association between both groups. We also detected at least three events of horizontal transfers. We ruled out cophylogenetic inertia as an explanation to multihost strains. Instead, recent host switchings could account for those bacterial lineages broadly distributed. However, cophylogenetic inertia could have occurred in young sister species and also in cryptic species. It seems *Wolbachia* takes a long time to evolve, since the complex of species in *status nascendi* *Naupactus cervinus* has the same strain since at least 540,000 - 1,270,000 years. On the opposite, the species complex *Aramigus tessellatus* harbours distantly related strains. In addition, pervasive recombination involving *wsp* gene was detected among strains infecting this group of weevils. Information about shared natural enemies and ecological traits could give some clues about the joint evolution of *Wolbachia* and these weevils.

*Keywords: Curculionidae, Naupactini, Cophylogenetic Inertia, Cospeciation, Horizontal transfer*

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## Recent horizontal transfer of *Wolbachia* between cherry infesting *Rhagoletis cerasi* and invasive *Rhagoletis cingulata* and its dynamic influence on the new host

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The wide distribution of *Wolbachia* in all major classes of arthropods and filarial nematodes evidence the ability of this heritable endosymbiont to switch horizontally between species boundaries. Several laboratory-studies where *Wolbachia* was successful transferred artificially by microinjection showed that *Wolbachia* is able to adapt to new genetic hosts backgrounds. However, there is still a lack of examples on the course of horizontal transfer in nature. Here the *Wolbachia*-infections of *Rhagoletis cerasi* are compared to those of the recently introduced *Rhagoletis cingulata*. Strain characterization by MLST and *wsp* showed that European and American *R. cingulata* specimens are infected with *wCin2*, a strain ident to *wCer2* of *R. cerasi*. Additionally some of the European individuals are infected with *wCin1*, a strain ident to *wCer1*. *wCin1* was never found in the USA and thus it is likely that European populations acquired this strain after their introduction to Europe from *R. cerasi*. Quantification of *wCin1* and *wCin2* by qPCR showed that *wCin1* positively influences *wCin2* titers. Our study system allows novel insights into the dynamics of interspecific *Wolbachia* transfer at an early stage.

*Keywords: Wolbachia density, horizontal transfer, invasive species, multilocus sequence typing, multiple infections*

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## **Men are messy: *Wolbachia* stem cell niche tropism in *Drosophila* is evolutionarily conserved only in females**

Toomey M.<sup>1,2</sup>, Panaram K.<sup>1</sup>, Fast E.<sup>1</sup>, Beatty, C.<sup>1</sup> and Frydman H.<sup>1,2</sup>

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Wolbachia are mainly vertically transmitted, however there is also evidence of extensive horizontal transmission and the mechanisms by which this happens are poorly understood. Previous work has shown that Wolbachia target stem cell niches (the microenvironment that supports the stem cells) in *Drosophila*, *Cimex lectularius*, and *Zyginidia pullula*. We hypothesize that Wolbachia transmission is facilitated by stem cell niche tropism. Supporting this idea, here we show that somatic stem cell niche tropism in the ovary of the *Drosophila* genus is evolutionarily conserved in 11/11 Wolbachia strains investigated, and is present in approximately 92% of niches examined (N= 1194). Wolbachia also target the germline stem cell niche in a subset of species investigated, revealing different patterns of niche tropism in the ovary. Phylogenetic analysis shows a high degree of conservation of the same pattern of niche tropism in closely related Wolbachia strains. If the major role of niche tropism is related to Wolbachia transmission, evolutionary theory predicts that there should be no corresponding force to maintain niche tropism in males, due to strict maternal transmission. In agreement, niche tropism in the testis is present in only 6/10 Wolbachia strains analyzed and is observed in only 39% of niches analyzed (N=510). We also found that Wolbachia factors, rather than host factors, mediate niche tropism patterns in both females and males. However, in the testis the frequency of niche tropism is not as tightly regulated as in the ovary, indicating a disordered pattern in the absence of stronger selective forces.

*Keywords: stem cell, niche, tropism, transmission, germline*

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## **Coupled infection dynamics of male killing bacteria and horizontally or vertically transmitted viruses**

Telschow A.

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Reproductive parasites are widely distributed in arthropods, with *Wolbachia* alone estimated to infect 70% of insect species. One of the most common forms of reproductive parasitism is male-killing (MK), a phenomenon where infected males are killed by the parasite. MK has received considerable attention as a driver for host evolution and as a mean for pest control. The present study is motivated by recent findings that in some arthropod hosts the viral replication is reduced by the presence of *Wolbachia*. We constructed a population genetic model in order to investigate the coupled infection dynamics of MK-bacteria and horizontally or vertically transmitted viruses. Crucial model assumption is that viral replication and transmission is impeded by the presence of MK-bacteria. Using a combination of analytical and numerical methods we demonstrated that the infection dynamics of MK-bacteria is heavily influenced by the presence of the virus and vice versa.

Our key findings are: (1) MK-infections reduce virus frequencies in most cases, sometimes resulting in virus extinction; this is true for both horizontally and vertically transmitted viruses; (2) vertically transmitted viruses facilitate the spread and persistence of MK infections by lowering invasion thresholds for transmission rate and fitness compensation; in contrast, horizontally transmitted viruses do not affect the spread of MK-bacteria. These results have important implications for the dynamics of both MK and virus infections in the field as well as for using MK-bacteria for pest control.

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## GENOMICS



## Enrichment strategy for sequencing of *Wolbachia* genomes from isopod crustaceans

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It has been known for decades that *Wolbachia* endosymbionts induce reproductive manipulations such as feminization and cytoplasmic incompatibility (CI) in their isopod crustacean hosts. To date, the genetic basis for these manipulations remains unknown. To determine candidate genes, we plan to compare the genomes of two closely related *Wolbachia* strains infecting isopod species: the feminizing strain wVulC (host: *Armadillidium vulgare*) and the CI strain wCon (host: *Cylisticus convexus*). The acquisition of genome sequences requires purifying *Wolbachia* DNA, which currently remains a technical challenge. This is because *Wolbachia* bacteria are unculturable and extraction of total DNA results in high levels of contamination with nuclear and mitochondrial DNA from hosts. Indeed, our analysis by quantitative PCR of wCon samples revealed that for 1 base pair (bp) of *Wolbachia* sequence, there is typically ~300 bp of nuclear and mitochondrial sequence. To improve the ratio of *Wolbachia* to nuclear and mitochondrial sequence, we performed an artificial enrichment of the bacteria by shredding the cells without damaging nuclei and then separating host nuclei from bacteria by filtration. This resulted in an improved ratio of 1 bp of *Wolbachia* sequence for 1 bp of nuclear and mitochondrial sequence. Consistent results were obtained in replicates of several experiments. This ~300-fold enrichment makes our wCon sample amenable to 454 pyrosequencing. The next step will consist in mapping wCon reads against the previously sequenced wVulC genome to identify candidate genes. To conclude, this method of selective enrichment of *Wolbachia* DNA is efficient, fast, cheap, reliable, avoids centrifugation and DNase treatment, and can easily be extended to other *Wolbachia* strains of isopod crustaceans.

*Keywords: Wolbachia ; Isopod ; DNA enrichment method; feminization, cytoplasmic incompatibility*

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## Unravelling the mutualistic symbiosis of *Wolbachia* and the filarial nematode *Brugia malayi*: a systems biology approach

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Abstract

The parasitic nematode *Brugia malayi* is a causative agent of lymphatic filariasis. *B. malayi* exists in a mutualistic symbiotic relationship with *Wolbachia*. Larval development, embryogenesis and adult worm survival are the key biological processes, entirely dependent on this symbiosis. We have applied an omics approach to investigate the molecular basis of this symbiosis. In order to study the role in larval and embryonic development, Illumina RNA Seq was used to produce a comprehensive transcriptome of four time points spanning the early post-infection development of *B. malayi* in the mammalian host *Meriones unguiculatus* and at multiple time-points post-antibiotic depletion from adult females. *Wolbachia*/worm ratios within developing larvae and adult worms at each time point were also monitored by qPCR and fluorescent microscopy. In parallel proteomic profiling of these selected nematode life cycle stages has been adopted to monitor protein expression of *Wolbachia* and *B. malayi*. In-solution tryptic proteolysis coupled with reversed phase liquid chromatography and analysis by high-resolution mass spectrometry provides a powerful tool for global proteome analysis. This initial shot-gun approach has been optimised to include an extensive pre-fractionation step to delve deeper into the proteome by increasing peptide and protein identification. Following basic analysis through established bioinformatics pipelines, transcriptomic, proteomic, and published datasets will be integrated in a systems biology approach with the objective of understanding the molecular basis of the mutualistic *Wolbachia*/*B. malayi* symbiosis.

*Keywords: Wolbachia, Brugia, filarial nematode, proteomics, transcriptomics*

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## Evolutionary dynamics and genomic impact of group II mobile introns in *Wolbachia* bacterial endosymbionts

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The streamlined genomes of ancient obligate endosymbionts generally lack transposable elements, as a consequence of their intracellular confinement. Yet, the genomes of *Wolbachia*, one of the most abundant bacterial endosymbionts on Earth, is littered with transposable elements, in particular insertion sequences (IS). Such a paradox raises the questions as to why there are so many transposable elements in *Wolbachia* genomes and whether or not IS elements are the only transposable elements in *Wolbachia* genomes. To address these questions, we conducted an exhaustive search for group II introns in completely or partially sequenced *Wolbachia* genomes. Bacterial group II introns are retroelements carrying reverse transcriptases, frequently found within genes. They mainly disperse through homing into free alleles of their host genes, but ectopic mobility has already been documented. Our results reveal that group II mobile introns are particularly abundant and diverse in most *Wolbachia* genomes. These genomes rank in the top 2% of the thousand completely sequenced prokaryotic genomes in terms of group II intron genomic density. In addition, we identified a high number of putatively functional intron copies in different *Wolbachia* strains. This suggests recent intron mobility in *Wolbachia*, which was confirmed by polymorphism assays. Moreover, group II introns were found to be involved in several genomic rearrangements. Overall, these observations highlight the ability of *Wolbachia* to exchange genetic material and to deal with acquired repeated elements in relation with genomic plasticity. Such characteristics may provide some clues on the exceptional evolutionary success of *Wolbachia* bacterial endosymbionts.

*Keywords: group II introns, transposable element, Wolbachia, genome evolution*

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## Lateral gene transfer from endosymbionts to the human filarial nematode *Brugia malayi*

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Lateral gene transfer (LGT) is a process by which organisms acquire DNA from other organisms in the absence of sex. *Brugia malayi*, an important human parasite causing lymphatic filariasis, has LGT from its obligate intracellular bacterial endosymbiont, *Wolbachia* strain wBm. *Wolbachia* is required for worm fertility so one of the methods for filariasis treatment is to eliminate *Wolbachia* by using antibiotics. In this study, we undertook resequencing *Wolbachia*-depleted *B. malayi* worms in order to generate a complete catalog of LGT events. Illumina GAll and HiSeq 2000 platforms were used resulting in high coverage of the *B. malayi* genome. The mean coverage of the 49.7 million, 54-bp GAll reads was 16' whereas that of the 138.6 million, 99-bp HiSeq reads was 131'. On the contrary, *Wolbachia* was covered at 1.6' and 13.8', respectively. This significant difference in coverage was exploited to differentiate from the low level *Wolbachia*-infection following depletion and LGT events between *Wolbachia* and *B. malayi*. In other words, all portions of the *Wolbachia* genome covered unusually high, were actually *Wolbachia* fragments transferred to *B. malayi*. We named these LGT fragments, nuclear Wolbachia transfers or *nuwts*, for short. As many as 115.4-kbp of the 1.08-Mbp wBm genome was present as *nuwts* in *B. malayi*. What is even more astonishing is that some of these *nuwts* have up to 10 copies. Their vast majority appears to be non-functional due to disruption of the original open reading frame. Some others, however, are intact and under investigation for functionality. Should any of them prove to be functional, development of new drugs that will be targeting these *nuwts* can offer alternative ways to treat filariasis that targets both the endosymbiont and the worm.

**Keywords :** *lateral gene transfer, nuclear Wolbachia transfers, lymphatic filariasis, drug development*

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## Targeted Genome Enrichment as a Method of Purifying Endosymbiont DNA from Host DNA

Geniez S.<sup>1,2</sup>, Foster J.<sup>1</sup>, Kumar S.<sup>1</sup>, Grève P.<sup>2</sup> and Barton E. Slatko<sup>1</sup>

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*Wolbachia* endosymbionts are widespread in arthropods and are considered reproductive parasites, inducing a variety of phenotypes including cytoplasmic incompatibility, parthenogenesis, feminization and male killing, which serve to promote their spread through populations. In contrast, *Wolbachia* infecting filarial nematodes that cause human diseases, including elephantiasis and river blindness, are obligate mutualists. These *Wolbachia* are subjects of drug discovery initiatives, but purification methods for efficient sequencing of these unculturable bacteria have proven difficult using a variety of techniques including chemical gradients, PFG purification, library construction followed by gene walking, etc. To examine the biology of symbiosis in worldwide natural populations, we have created a set of SureSelect™ (Agilent) 120-mer target enrichment RNA oligonucleotides (“baits”) for solution hybrid selection. These were identified from *Wolbachia* complete and partial genome sequences in Genbank, and were tiled across each genomic sequence with 60bp overlap. Baits were filtered for homology against host genomes that contain *Wolbachia* using BLAT, and sequences with significant host homology were removed from the bait pool. Filarial parasite *Brugia malayi* DNA was used as a test case, as the complete *Wolbachia* sequence is known. DNA eluted from capture was size selected (200-bp fragments) and sequencing samples were prepared using the NEBNext® Sample Preparation Kit. One-third of a 50nt paired-end sequencing lane on the HiSeq™ 2000 (Illumina) yielded 53 million reads and the entirety of the *Wolbachia* genome was captured. We then used the baits to isolate >95% *Wolbachia* DNA from *Armadillidium vulgare*, a distantly related *Wolbachia*, demonstrating that the method can be used to enrich target DNA from unculturable microbes over large evolutionary distances.

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## Single tube nested-PCR (STN-PCR): A sensitive detection technique for *Wolbachia* that is less prone to contamination

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Low density *Wolbachia* infections are abundant among insect species but challenging to characterize. In these low titre associations, *Wolbachia* densities are often below the detection threshold of conventional PCR. The shortcomings of more sensitive amplification techniques, such as nested PCR, are that they are easily contaminated due to the need to transfer amplified products between reactions. This makes gauging the infection prevalence of low density associations troublesome especially when a population has a small number of infected individuals. Using single tube nested PCR (STN-PCR) we have developed sensitive PCR-based detection techniques for *Wolbachia*. To combine the two PCRs in one closed tube, which minimizes the chance of contamination, STN-PCR exploits the higher annealing temperatures of longer primers in the first round of amplification, and then completes the second round using traditional PCR dynamics. The longer outer primers in the first round of PCR are limited in supply and as such, are used to exhaustion in the first reaction. Therefore, these outer primers do not interfere with the amplification of the second round of PCR. We have developed STN-PCR protocols targeting three different *Wolbachia* genes and achieved higher detection sensitivities for multiple strains of *Wolbachia* compared to traditional PCR. STN-PCR will benefit studies focusing on low titre *Wolbachia* associations.

*Keywords: detection limit, low titer infection, STN-PCR*

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## Characterization of two transcriptional factors in *Wolbachia* of *Brugia malayi*

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*Brugia malayi* is a filarial nematode that infects 13 million people and is a cause of lymphatic filariasis. Since current methods of treatment are inadequate, there is an urgent need for new anti-filarial drugs. *B. malayi* harbors an endosymbiotic bacteria *Wolbachia* (*wBm*) which has an essential role as elimination of *wBm* results in either parasite sterility and/or death. Consequently, targeting *wBm* is a promising approach for anti-filarial drug development. Genome analysis indicates the presence of a Type IV secretion system (T4SS) in *wBm* with two operons and 3 individual genes encoding a total of 14 components of T4SS. The T4SS has been shown to play an important role in the bacteria-host interaction in various bacterial pathogens and endosymbionts where the expression of T4SS is under stringent regulation by various transcriptional factors. We are interested in investigating how the T4SS is regulated in *wBm* with a view to targeting the regulatory system for drug discovery.

In *Ehlichia chaffeensis* a transcriptional factor EcxR has been identified and shown to positively regulate T4SS gene expression. In *wBm*, we identified two potential EcxR homologs. We cloned, expressed and purified the putative transcription factors for use in gel shift assays to investigate their role in regulating the T4SS in filarial *Wolbachia*.

**Keywords:** *Filariasis, Chemotherapy, Wolbachia, transcription factor, T4SS*

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## Overexpression of *Jhl-26* in males may mimic the CI phenotype in *Drosophila*

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*Wolbachia* are intracellular microbes found frequently in insects. Cytoplasmic incompatibility (CI) is the common reproductive modification induced by *Wolbachia* and results in lethality of embryos derived from crosses between *Wolbachia*-infected males and uninfected females. Our previous microarray analysis in *Drosophila melanogaster* have identified 167 up-regulated genes ( $\geq 1.5$  fold changes, q-value  $< 5\%$ ) in the third instar larval testes when infected by *Wolbachia*. *Jhl-26* showed the largest up-regulation. *Jhl-26* gene can be rapidly and specifically induced by Juvenile hormone (JH). It has recently been identified as a sperm protein in *Drosophila*. To investigate the function of *Jhl-26*, we first analyzed the spatiotemporal expression patterns of *Jhl-26* in *D. melanogaster* by qRT-PCR. High expression levels were detected in larval stages and the testes of young males (1d), indicating the role of this gene in spermatogenesis. To explore the association of *Jhl-26* gene with CI, *Jhl-26* was overexpressed by using UAS-GAL4 system in *Wolbachia*-free male *Drosophila*. We found that when nos-GAL4/UAS-*Jhl-26* males (1d) were crossed with uninfected females, the hatch rate of the embryos was only  $32.29 \pm 1.25\%$ , significantly lower than that in control. Interestingly, this hatch rate could increase to  $64.27 \pm 0.55\%$  when older (5d) transgenic males were used, which is similar to the phenotype in CI crosses. These results suggest that *Wolbachia* induced overexpression of *Jhl-26* in the testes of *Drosophila* may impact on the early spermatogenesis, thus results in embryonic lethality when fertilized with wild type eggs. The mechanisms by which *Jhl-26* overexpression in testes damages spermatogenesis are being further studied.

*Keywords: cytoplasmic incompatibility, Wolbachia, Jhl-26, overexpression*

This work was supported by the National Nature Science Foundation of China (30970405; 31172155)

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## The comparison of gene expression and proteome between wMelPop and wMelCS in different tissues of *Drosophila melanogaster*

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The genomes of five *Wolbachia* strains have been sequenced and are now publicly available. Those strains are symbionts of a diverse range of hosts including *Drosophila melanogaster* (wMel), *Drosophila simulans* (wRi), *Culex quinquefasciatus* (wPip), *Aedes albopictus* (wAlbB) and *Brugia malayi* (wBm). Comparing the genome sequences provides a great tool to understand the genetic determinants of the different phenotypes induced by those strains. However, because these *Wolbachia* strains are genetically divergent and are symbionts of different hosts, associating genomic features with phenotypes induced by *Wolbachia* is very difficult.

In our lab, we have sequenced the genome of two very closely related *D. melanogaster* strains: wMelPop and wMelCS. The preliminary analysis of those genomes indicates no or very few genomic differences between those two strains. This contrasts with the very different phenotypes induced by these strains: wMelPop is pathogenic growing to very high density and shortening the lifespan of *D. melanogaster* while wMelCS doesn't.

If the two genomes are nearly identical, then the phenotypic difference must result from more subtle strain differences: for example differential epigenetic mechanisms, differential gene expression patterns or differential protein regulation.

To unravel those hypotheses we propose to use a combined approach that would look at wMelPop and wMelCS specific gene expression by RNA-Seq and global proteome by shotgun LC/MS proteomic in different tissues of *D. melanogaster*.

Comparing the tissue-specific transcriptome and proteome between wMelPop and wMelCS in the same host will provide valuable information to understand the mechanisms underpinning the phenotypes induced by these two strains.

**Keywords:** RNA-Seq, shotgun LC/MS proteomic, tissue specific analyses, life shortening

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## Tandem repeat markers as novel diagnostic tools for high resolution fingerprinting of *Wolbachia*

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*Wolbachia* strains are extremely diverse both genotypically and in terms of their induced phenotypes in invertebrate hosts. Despite extensive molecular characterisation of *Wolbachia* diversity, little is known about the actual genomic diversity within or between closely related strains that group tightly on the basis of existing gene marker systems, including Multiple Locus Sequence Typing (MLST). The genome of the wMel strain of *Drosophila melanogaster* contains inter- and intragenic tandem repeats that may evolve through expansion or contraction. We identified hypervariable regions in wMel, including intergenic Variable Number Tandem Repeats (VNTRs), and genes encoding ankyrin (ANK) repeat domains. We amplified these markers from 14 related *Wolbachia* strains belonging to supergroup A and were successful in differentiating size polymorphic alleles. Because of their tandemly repeated structure and length polymorphism, the markers can be used in a PCR-diagnostic multi locus typing approach, analogous to the Multiple Locus VNTR Analysis (MLVA) established for many other bacteria and organisms. The isolated markers are highly specific for supergroup A and not informative for other supergroups. However, *in silico* analysis of completed genomes from other supergroups revealed the presence of tandem repeats that are variable and could therefore be useful for typing target strains. These novel inexpensive high-throughput fingerprinting tools will be useful for studies of population genetics, horizontal transmission and experimental evolution of *Wolbachia*.

**Keywords:** *Wolbachia*, VNTR, ankyrin, fingerprinting, MLVA

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## Molecular mechanisms for *Wolbachia* tissue tropism in the gonads of *Drosophila melanogaster*

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Microbes preferentially infect specific cell types in their hosts - which is termed as tissue or cell tropism. Here we probe into the molecular mechanisms of the tissue tropism of two strains of *Wolbachia* - wMel and wMelPop, in the testis of *Drosophila melanogaster*. The two strains differ considerably in their infection levels at the niche harboring the stem cells in the testis, located at the anterior tip of the testis. We profiled the transcriptional landscape of microdissected tips, containing the niche, associated stem cells and primary spermatocytes. Microarray analysis of the apical tip revealed 130 genes that are differentially expressed between the uninfected, wMel infected and wMelPop infected tissue. Most of these genes are involved in processes related to metabolism, including proteolysis, lipid metabolism, transport, oxidation-reduction and cell cycle. 62 of these genes are differentially expressed between wMelPop and wMel. In agreement with previous work indicating that *Wolbachia* depends on host amino acid pool as an energy source, our data indicates greater proteolytic activity in the *Wolbachia* strain with higher niche tropism - wMelPop. Utilizing the genetic tools available in *Drosophila*, we are currently testing the functional significance of the candidate genes for tissue tropism. This analysis will further our understanding of the molecular pathways relevant for *Wolbachia* targeting of specific host tissues.

*Keywords: Tissue Tropism, wMel and wMelPop, Hub cells, Microarrays, Proteolysis*

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## **CELL BIOLOGY**



## Filaricidal drugs induce apoptosis in the filarial nematode-*Brugia malayi* and this effect is not primarily due to *Wolbachia* depletion

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Filarial nematodes harbor *Wolbachia* bacteria and depletion of *Wolbachia* in filarial nematodes results in defects in nematode development, fertility and viability. Induction of apoptosis in *Brugia malayi* upon *Wolbachia* depletion was shown and thus suggested the involvement of factors originating from *Wolbachia* (Landmann et al., 2011). To further confirm that apoptosis occurring during anti-filarial drug treatment is caused by *Wolbachia*'s depletion, we compared two types of drugs: a macrofilaricidal drug which doesn't target *Wolbachia* such as flubendazole and gentamycin, an antibiotic known to be ineffective in killing *Wolbachia* vs. tetracycline, which targets *Wolbachia*. Adult *B. malayi* worms were cultured in vitro and treated with 40 µg/ml of tetracycline, 20 µg/ml of flubendazole or 40 µg/ml of gentamycin. As a control for effects of death, we used untreated adult worms that were killed by freezing. Tetracycline and flubendazole killed *B. malayi* adult worms on day 5 and day 6 of treatment, respectively where as with gentamycin treated worms survived for 10 days and untreated worms survived for more 2 weeks. Treated worms were harvested at the point of death or alive (untreated worms) and were sectioned and stained for *Wolbachia* and a Tunnel assay was performed to detect apoptosis. Interestingly, minor degree of apoptosis was already found in untreated worms. These untreated worms harbored many *Wolbachia* based on immunohistology. In untreated worms killed by freezing also showed signs of apoptosis although *Wolbachia* could be detected in these worms. In tetracycline treated worms, there were extensive signs of apoptosis and there was also a marked reduction in staining for *Wolbachia*, however, worms treated with flubendazole although showed extensive apoptosis numerous *Wolbachia* could be still be detected. Apoptosis was also observed in gentamycin treated worms while numerous *Wolbachia* were still detected. Thus our results show that induction of apoptosis following anti-filarial drug treatment is mainly caused by worm death due to the drug's activity and its toxicity and is not necessarily due to the depletion of *Wolbachia* alone, as proposed earlier.

**Keywords:** *Wolbachia*, *Brugia malayi*, apoptosis

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## **What do we know about the *Wolbachia* impacts on *Armadillidium vulgare* immunity?**

Chevalier F., Sicard M., Bertaux J., Le clec'h W., Bouchon D., Grève P. and Braquart-varnier C.

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The feminizing *Wolbachia* in the pill bug *Armadillidium vulgare* (crustacean, terrestrial isopods) is associated to detrimental effects on fecundity and lifespan. In this isopod, *Wolbachia* infect all host tissues including immune cells (i.e., hemocytes). To understand the impacts of *Wolbachia* on *A. vulgare* immune system, we first had to characterize the immune effectors (haematopoetic organs, haemocyte types, antimicrobial peptides, immune genes...) of this unconventional host. Then we have investigated the impact of *Wolbachia* on these newly identified cellular and molecular immune effectors. By this multilevel approach from molecules to cellular phenotypes, we reveal an overall strong impact of *Wolbachia* on these effectors especially during aging. However, some results suggest immunodepression while others seem to indicate an immunostimulation, showing that *Wolbachia/A.vulgare* symbiosis navigates between conflicting and beneficial interactions.

*Mots clés: Wolbachia, hemocytes, Armadillidium vulgare*

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## The androgenic hormone of terrestrial isopods: a putative *Wolbachia* target

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In crustaceans, the androgenic gland (AG), thanks to the synthesis of the androgenic hormone (AH), controls the differentiation of the primary and secondary male sexual characters. In the isopod *A. vulgare*, the endosymbiotic bacteria *Wolbachia* induces the feminization of genetic males into functional females. *Wolbachia* is thought to target AH or its receptor early during development inhibiting male differentiation of embryonic gonads. In this study, we amplified AH cDNA of 12 new species belonging to five different families of the infra-order Ligiamorpha of terrestrial isopods. Essential sites for the production of a functional AH protein exhibit negative selection signatures and are strictly conserved including typical proteolytic cleavage motifs, a putative N-linked glycosylation motif on the A chains and the 8 Cys positions. An insulin like growth factor motif was also identified on *Armadillidium* AH sequences. The phylogenetic relationships of AH sequences allowed to distinguish two main groups, AH of species from the *Armadillidiidae* and the *Porcellionidae* families which is congruent with the narrow specificity of AG heterospecific grafting. This result may explain the host specificity of the *Wolbachia* feminization effect considering that bacterial effectors of a strain infecting one species are not able to interact efficiently with the AH or its receptor of another species. An in-depth understanding of AH endocrine regulation would get insights into the interaction between *Wolbachia* and *A. vulgare* sex differentiation.

*Keywords: isopod crustacean, androgenic hormone, male differentiation, Wolbachia, feminization*

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## Cellular Mechanisms of the Vertical Transmission of *Wolbachia* in *Culex pipiens*

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*Culex pipiens* are the major mosquito vector for diseases such as West Nile Virus and Japanese Encephalitis. Many *Culex* species are naturally infected with *Wolbachia*, which are passed down maternally through the egg cytoplasm. The cellular mechanisms of *Wolbachia* vertical transmission are poorly understood in this system. Here we show a pattern of *Wolbachia* transmission from the germarium (a structure that houses all the necessary cellular tools to create an egg) to the oocyte of a developing follicle. Developing follicles derive from a germline stem cell and undergo 3 rounds of incomplete mitosis to create an eight-cell cyst, one of which becomes the oocyte. *Wolbachia* are highly concentrated within the germarium upon adult eclosion and in the initial stages of oogenesis is evenly distributed among all germline cells. Shortly thereafter, *Wolbachia* preferentially accumulate in the developing oocyte providing a route for vertical transmission similar to what has been shown in *Drosophila*. Furthermore *Wolbachia* have been shown to modulate specific oogenenic events in *Drosophila* such as programmed cell death and germline stem cell division. We aim to identify *Wolbachia*'s role in oogenesis within *Culex pipiens* however major cellular components, such as the stem cells and corresponding niches, have yet to be identified. Towards elucidating the mechanisms of *Wolbachia*-host interactions within the ovary, we seek to classify specific cell types within the *Culex* ovary.

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## A screen for host genes interacting with *Wolbachia*

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*Wolbachia* are arguably the most widespread endosymbiotic bacteria known. Their presence can have profound effects on their hosts: from cytoplasmic incompatibility to protection from RNA viruses. However, little is known about the interaction of these bacteria with their hosts at the molecular level. We are approaching this problem taking advantage of the genetically tractable *Wolbachia*'s natural host *Drosophila melanogaster*. We are doing a genetic screen to identify host factors required by *Wolbachia* for a successful infection or required by the host to control or deal with this infection. We have screened approximately 700 lines and quantified the amount of *Wolbachia* in each of them. Interestingly, we do not observe a significant difference in *Wolbachia* titers between most of the different lines, suggesting that

*Wolbachia* levels are very well regulated. We are currently retesting some of the mutant lines where the *Wolbachia-Drosophila* tight regulation is disrupted.

*Keywords: Wolbachia, Drosophila melanogaster*

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## The impact of endosymbiosis on host sex-chromosome evolution

Gilbert, C.

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While the genomic consequences of endosymbiosis are relatively well understood in endosymbionts, fewer studies have so far investigated the impact of this phenomenon on the evolution of eukaryotic genomes. Terrestrial crustacean isopods show a wide diversity of chromosomal sex determination mechanisms, with male [XY/XX] and female [ZZ/ZW] heterogametic systems existing among different species of the same genus and even among different populations of the same species. Interestingly it has been proposed that this diversity of systems could result from the co-evolution between isopods and *Wolbachia* strains that are able to feminize male embryos, thereby increasing their transmission rate within host populations. The host sex-ratio distortion resulting from *Wolbachia*-induced feminization is predicted to generate strong selection pressure favoring the evolution of host genes restoring a balanced sex-ratio. Furthermore, it has been suggested that terrestrial isopod species harboring feminizing *Wolbachia* may have undergone recurrent changes of sex-determination mechanisms coupled to the emergence of new sex chromosomes. We are testing this hypothesis using a combination of cytogenetics and genomics tools.

*Keywords: Feminization, terrestrial crustacean isopods, sex-chromosomes*

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## Applying *in vivo* imaging to analyze *Wolbachia*-host interactions

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Much progress has been made in the *Wolbachia* biology, but molecular and cellular mechanisms underlying their host interactions are poorly understood. Notably, it has been shown that interactions with host microtubules (MTs) are involved in vertical transmission and cellular segregation of *Wolbachia* (Serbus et al. 2008 *Annu Rev Genet* 42, 683-707), indicating potential power of cell biological approaches to the issue.

We have been pursuing the mechanisms underlying the establishment of cell polarity by using quantitative *in vivo* imaging of *Drosophila* wings. During development of multi-cellular organisms, each cell establishes planar cell polarity (PCP) according to the organ or tissue axis, to fulfill specific cellular functions. In the wing epidermis where each cell develops PCP along the proximodistal axis, PCP regulators of a category, called “core group”, are localized at either proximal or distal cell boundaries to specify the site of wing hair formation. To elucidate a mechanism of this characteristic localization, we have analyzed MT dynamics and molecular transport by introducing quantitative *in vivo* time-lapse recording (Shimada et al. 2006 *Dev Cell* 10, 209-222; Harumoto et al. 2010 *Dev Cell* 19, 389-401).

In this presentation, we would like to show our live imaging analyses on PCP, and discuss the applicability of our method to studies on *Wolbachia*-host interactions in *Drosophila*.

*Keywords: Drosophila, Wolbachia, in vivo imaging, Microtubule, Planar cell polarity*

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## ***Wolbachia* in the larval stages of *Dirofilaria immitis***

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Filaria life cycle consist of two developmental phases: invertebrate intermediate host phase (development of ingested microfilaria to the infective larva (L3) and the vertebrate host phase (maturation of (L3) through adulthood and production of microfilaria). Since *Wolbachia* are transmitted transovarially, these endosymbionts are present in each developmental stage. We traced the location of *Wolbachia* in the larvae of *Dirofilaria immitis* maturing in the mosquito vector, to elucidate the role that *Wolbachia* may play during development of the larvae. Female *Aedes aegypti* (Liverpool strain) were infected by membrane-feeding on a dog's blood containing microfilariae. Six to ten mosquitoes were killed each day from day 1 through day 15 post infection, their Malpighian tubules containing larvae were isolated and processed for examination by confocal and transmission electron microscopy. Our results indicated that low numbers of *Wolbachia* were limited to the lateral chords of microfilaria and of the three larval stages. These results indicate that the distribution of *Wolbachia* persists in the lateral chords of each larval stage of the filarial life cycle; the distribution of *Wolbachia* in the larvae of *D. immitis* is similar to that observed by McGarry *et al.* (1) in *Brugia malayi*; suggest that the contribution of *Wolbachia* to the development of the larvae in the mosquitoes may be limited, as compared to the adult stages and may indicate that *Wolbachia* may have a different metabolic role in the development of the larva than they do in the development of the adult stages.

*Keywords: Wolbachia, Dirofilaria immitis, larval stages, mosquito*

*Acknowledgments: Supported, in part, by the RCMI Award RR-003051 from the NIH to University of Puerto Rico; NIAID/NIH Filariasis Research Reagent Resource Center (FR 3), and Bill and Melinda Gates Foundation Grant (A-WOL Consortium) to the Liverpool School of Tropical Medicine.*

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## Tissue tropism of *Wolbachia* in the *Drosophila paulistorum* species complex

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*Wolbachia pipientis* is a maternally transmitted, obligate endosymbiont found in a majority of all insects studied. In the old-world *Drosophila* host species, it has been found that these endosymbionts infect various tissues. While it is clear to why endosymbionts would infect germline cells, maintaining the vertical transmission mode, it is not known as to why somatic tissues harbour *Wolbachia*. In the *Drosophila paulistorum* species complex, *Wolbachia* helps trigger speciation by establishing bi-directional cytoplasmic incompatibility (CI) between semi-species infected with conspecific, although distinct, strains of *Wolbachia*. CI between semi-species causes post-mating isolation, where the progeny either dies or become infertile. In addition we recently have shown that *D. paulistorum* *Wolbachia* significantly affect pre-mating isolation by manipulating sexual behaviour of females by introducing strong mating avoidance towards males infected with incompatible *Wolbachia*.

This pre-mating isolation can be disturbed, however, by subjecting the flies to a mild antibiotic treatment, causing a partial depletion of the endosymbiont titer. Also, the mating behaviour of artificially created hybrid females is severely altered, causing them to reject all mates, including hybrid males.

The mechanism behind how the endosymbionts affect its host behaviour is yet to be elucidated. However, *Wolbachia* localized to the brain is a prime candidate for causing such behavioural changes. While localization to the brain is considered an evolutionary dead end for a maternally transmitted symbiont, here we show *Wolbachia* tissue tropism of neural tissues in both adult and larval flies.

*Keywords: Drosophila paulistorum, sexual behaviour, mate avoidance*

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## ***Wolbachia*-mediated virus resistance in *Drosophila melanogaster***

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*Wolbachia* induce resistance to lethal infection of RNA viruses in the host, which increases fitness of *Wolbachia*-infected insects and is expected to contribute to the wide spread prevalence of the bacteria. The virus resistance has been observed in *Drosophila* and *Aedes* species infected with competent *Wolbachia* strains. The density of *Wolbachia* in host cells is implicated to be important for induction of virus resistance from the study using *Drosophila* adults and *Aedes* cultured cells. However, some *Wolbachia* strains that highly infect somatic cells do not induce virus resistance, indicating that there may be qualitative differences between *Wolbachia* strains.

The induction of virus resistance is implicated to cell-autonomous from the observation that, in *Aedes* fatbody, only *Wolbachia*-infected cells suppress virus amplification. However, it remains to be determined that how *Wolbachia* confer virus resistance in host cells. Toward understanding the mechanism of *Wolbachia*-mediated suppression of virus amplification, I observed infection status of *Drosophila* C virus in several *Drosophila* tissues as a first step for RNAi screening aiming to identify host genes responsible for the virus resistance.

*Keywords* : Virus, *Drosophila*

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## Involvement of Toll and IMD pathways on *Wolbachia* density and dengue blocking

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The non-virulent *Wolbachia* strain wMel and the life-shortening strain wMelPop-CLA, both originally from *Drosophila melanogaster*, have been stably introduced into the mosquito vector of dengue fever, *Aedes aegypti*. Each of these *Wolbachia* strains interferes with RNA virus pathogenicity and/or dissemination in both their natural *Drosophila* host and in their new mosquito host. It has been recently demonstrated that activation of the Toll pathway by *Wolbachia* (strain wAlbB from *A. albopictus*) in *A. aegypti* induces the production of antimicrobial peptides (cecropin and defensin) that inhibit dengue proliferation (Pan *et al.*, 2012). However, in a previous study we showed that the strains wMel and wMelPop induce transcription of antimicrobial peptides genes via Toll and IMD pathways only in its new host *A. aegypti*, and not in its natural host *D. melanogaster*, while *Wolbachia* reduces dengue virus replication in both hosts (Rancès *et al.*, 2012). At this stage, it is not clear whether or not immune priming by *Wolbachia* is the only mechanism involved in dengue interference. Therefore, we tested the ability of *Wolbachia* to interfere with dengue virus in IMD and Toll *Drosophila* mutants. Those mutations disrupt the induction of antimicrobial peptides. In parallel we looked at the impact of those two pathways on *Wolbachia* density and cellular tropism in its natural host. This study should contribute to a better understanding of the fundamental mechanisms involve in dengue interference.

Pan X., Zhou G., Wu J., Bian B., Lu P., Raikhel A. S., Xi Z. (2012). P.N.A.S. 109(1): E23-31.

Rancès E., Ye Y. H., Woolfit M., McGraw E. A., O'Neill S. L. (2012). PLoS Pathog. 8(2): e1002548.

*Keywords: dengue blocking, immunity*

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## ***Wolbachia* modulates *Hira* gene expression in insects through manipulation of a host microRNA to generate cytoplasmic incompatibility**

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The underlying mechanisms that *Wolbachia* use to manipulate invertebrate hosts are poorly understood. The best-studied example, cytoplasmic incompatibility (CI), results in embryo mortality when *Wolbachia* uninfected eggs are fertilized by sperm from *Wolbachia*-infected males. In this study, we show that a clear relationship exists between *Hira* (Histone regulative gene) expression levels, larval development time and CI strength in crosses with *Drosophila melanogaster* infected with the wMelPop strain of *Wolbachia*. Reduced CI strength (higher hatch rates) in 'younger brothers' was associated with a decreased ability of *Wolbachia* to suppress *Hira* expression. Similarly, when *Wolbachia* was trans-infected into a heterologous mosquito host a reduction in *Hira* gene expression was also observed in males. RNAi-mediated silencing of *Hira* were performed using dsRNA for both *Drosophila melanogaster* and *Aedes aegypti*. Crossing experiments with *Hira*-silenced males mated to *Wolbachia*-uninfected females mimics the CI phenotype. When *Hira*-silenced males were mated to *Wolbachia*-infected females, a significant increase in the hatch rate was observed although this 'rescue' effect was more pronounced in flies than in mosquitoes. Also, we show that *Wolbachia* utilizes a host microRNA, aae-miR-12, binding to the 3'UTR of *Hira* to reduce mRNA transcript levels *in vitro* in *A. aegypti* cells. Overall, we demonstrate that *Wolbachia* modulates aae-miR-12 to modulate *Hira*. Decreased *Hira* expression in males correlates with CI expression in both *D. melanogaster* and *A. aegypti*. Silencing of *Hira* in adult males results in a phenotype that mimics CI and *Wolbachia* in adult females can partially 'rescue' this reduced fertility.

**Keywords:** *Wolbachia*, cytoplasmic incompatibility, *Hira* gene, RNAi, microRNA

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## PEST CONTROL

## **A·WOL drug discovery - screening of both focused anti-infective and diversity-based libraries for novel compounds with efficacy against *Wolbachia* endosymbionts of filarial nematodes**

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Filarial nematodes are an important group of pathogens infecting 150 million people throughout the tropics with over 1.5 billion at risk. Control of filariasis currently relies on mass drug administration (MDA) programmes using drugs which principally target the microfilarial life-cycle stage. Antibiotic targeting of filarial *Wolbachia*, an essential bacterial symbiont, provides a novel treatment with macrofilaricidal activity. In order to turn this therapy into a public health tool suitable for filariasis control programmes, the Anti-*Wolbachia* Consortium (A·WOL) utilises *in vitro* cell and nematode assays, followed by secondary *in vivo* assays, to screen both focused and diversity compound libraries against *Wolbachia*. Here we describe the screening of 5399 compounds, from five focused anti-infective chemical libraries, in a *Wolbachia* cell-based assay. We have identified 263 hits, with 117 compounds showing improved efficacy over doxycycline against *Wolbachia*. Hit compounds also show activity against nematode *Wolbachia in vitro* without exhibiting direct anti-nematode activity. Based on our hit criteria, 104 compounds have progressed down the screening funnel and been screened in a *Litomosoides sigmodontis* mouse model, where encouragingly, a number show equivalent or improved efficacy compared with doxycycline against *Wolbachia in vivo*. Our diversity-based screening approach has involved the procurement of 558,000 compounds from three large chemical libraries, of which 12,400 compounds have been screened *in vitro* with 130 hits identified. Hits are then scrutinised to determine suitability for further assessment of structure-activity relationships, with narrow-spectrum activity against *Wolbachia*, and to select the best candidates to take forward as part of the drug discovery program.

*Keywords: A·WOL, drug-discovery, filariasis, macrofilaricidal*

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## A new mosquito cell line to decipher *Wolbachia*/virus interactions

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*Wolbachia* (*Rickettsiales*, *Anaplasmataceae*) are maternally transmitted endosymbionts highly prevalent among insects, including vectors of pathogens. Recent findings showed that artificially injected *Wolbachia* strain wAlbB in the *Aedes aegypti* mosquito vector reduce dengue virus (DENV) and chikungunya virus (CHIKV) multiplication and dissemination *in insecta*. Despite these encouraging results, natural *Wolbachia* strains wAlbA and wAlbB of the tiger mosquito *Aedes albopictus* did not reduce significantly CHIKV transmission in orally infected females, even if the viral replication was shown to be more erratic in absence of the bacteria. To get more insights into *Wolbachia*-arbovirus interaction mechanisms in mosquito, we developed a new *Ae. albopictus* cell line stably infected with wAlbB strain, using the well-known C6/36 cell line.

After infection with wAlbB strain using Shell-Vial technique, cells were cultured for several generations. *Wolbachia* density was measured by quantitative PCR, and the infection pattern was monitored by fluorescence *in situ* hybridization (FISH) and electronic microscopy. Following infection, *Wolbachia* could be maintained at high density rate for over 30 passages. FISH results showed that the large majority of C6/36 cells harbour wAlbB strain.

This new C6/36 cell line stably infected with a natural *Wolbachia* strain of *Ae. albopictus* will be useful to decipher (i) *Wolbachia* infection, spread, and maintenance *in cellulo* and (ii) cellular processes involved during the interaction between *Wolbachia* and arboviruses.

**Keywords:** *Wolbachia*, *Aedes albopictus*, arboviruses, C6/36 cells

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## ***Wolbachia* wMel substrains confer differential protection to viruses**

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*Wolbachia* are able to protect various insect hosts from viral infections. This tripartite interaction was initially described in *Drosophila melanogaster* carrying *wMel*, its natural *Wolbachia* strain. Recently *Wolbachia* has been proposed as a tool for the control of vector-borne diseases. Mosquitoes harbouring *wMel* are more resistant to dengue virus and a release of these in dengue-endemic areas could reduce the prevalence of this disease in human populations. Despite its importance, the variability in the antiviral protection provided by natural variants of *wMel* has never been explored.

We have compared the antiviral protection conferred by *wMel* substrains in a genetically identical *Drosophila melanogaster* host. Our data reveal that different *wMel* variants confer different levels of protection upon infection with *Drosophila* C virus and Flock house virus. The titres of the endosymbiont are also substrain dependent. Furthermore the *wMel* substrains reaching higher levels in the host confer stronger antiviral protection. These phenotypes allow us to cluster the *wMel* substrains into two groups. One contains *wMelCS* and *wMelCS2* and confers more protection to viruses than the one comprised of *wMel*, *wMel2* and *wMel3*.

Our study strengthens the notion that the outcome of the disease depends not only on the genotypes of the host and the pathogen but also on the genotypes of the symbionts associated with them. The phenotypes associated with the different substrains indicate that the selection pressure driving the recent expansion of *wMel* substrain was not increased resistance to viruses. As the polymorphisms within the genomes of the *wMel* substrains are described, the differences in the antiviral protection will allow dissecting the *Wolbachia* factors engaged in this phenomenon. Finally, our work helps to understand the interaction of *wMel* with its natural host and facilitates better planning of *Wolbachia* use in dengue control.

**Keywords:** *wMel*, substrains, virus-protection, viruses, *Drosophila*

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## **SYMBIONT COMMUNITY**

## Comparative genomic analysis of bacteriophages associated with male-killing and non-male-killing spiroplasmas in *Drosophila*

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Members of the genus *Spiroplasma* belong to the class *Mollicutes* and are associated both endocellularly and extracellularly with a variety of plants and arthropods. Some spiroplasmas cause female-biased sex ratios of their host insects including *Drosophila* fruit flies, ladybird beetles and butterflies, as a result of selective death of the male offspring during embryogenesis. Although the male-specific lethality has been the focus of studies on the symbiotic spiroplasmas, the molecular mechanisms underlying the male-killing phenotype largely remain unknown. Male-killing spiroplasmas have been reported from *Drosophila nebulosa* and *D. melanogaster*, which are often referred to by the acronyms NSRO (*nebulosa* sex ratio organism) and MSRO, respectively. In addition, a non-male-killing variant, named NSRO-A, has been serendipitously isolated during maintenance of NSRO in a laboratory strain of *D. melanogaster*. Our preliminary experiments revealed that the genomes of NSRO and NSRO-A carry many copies of phage-like sequences. In this study, three closely related bacteriophages were isolated from the male-killing spiroplasmas, NSRO and MSRO, and the non-male-killing NSRO-A, and their whole genome sequences were determined. Each phage genome was 19 kb in size and encoded 23 open reading frames, which included five spiroplasma-unique sequences with similarity to putative *Spiroplasma citri* virulence genes P58, P12, P54, P123 and P18. These genes correspond to a 9.5-kb *S. citri* genome segment that is deleted in a non-transmissible line of *S. citri*. ORFs encoded in the three bacteriophages are almost the same but several exceptions. Biological functions of the encoded genes and differences between the spiroplasma strains will be discussed.

**Keywords:** *spiroplasma*, *Drosophila*, *bacteriophage*, *male killing*

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## ***Wolbachia* infection in *Podisma pedestris* hybrid zone**

Martinez-Rodriguez P.<sup>1</sup>, Bella J.L.<sup>1</sup> and Nichols R.<sup>2</sup>

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*Wolbachia* are gram-negative bacteria described as obligate endosymbionts of an enormous variety of invertebrates, including insects like *Podisma pedestris* (Orthoptera, Acrididae, Melanoplinae). This grasshopper is subdivided into two chromosomal races with different sex determining systems: one neo-XY, the other XO. They meet and interbreed along a narrow zone, which formed in Alps some time during the last 8000 years.

Recently, *Wolbachia* infection in *Podisma pedestris* has been detected. Because of the long-standing interest in the evolution of introgression across this zone, we sought to characterize the strains infecting *Podisma pedestris* populations on either side.

Our preliminary 454 sequencing detected *Wolbachia*-like sequences, which may be nuclear inserts. We have now followed up this lead to characterize this putative bacterial genome integration in *Podisma pedestris* and to study the genomic dynamics of these sequences in the *Podisma pedestris* genome. This evidence of extensive *Wolbachia* insertion events is similar to the evidence of a large number of pseudogenes of mitochondrial and rDNA origin in the *Podisma* genome, many of ancient divergent ancestry. This unusually extensive archive of integration events may be a consequence of the evolutionary dynamics that has led to an exceptionally large *Podisma* genome of 18,000 Mb; probably including a relatively low rate of DNA loss relative to substitution. This archive may provide particularly complete evidence of lateral gene transfer from *Wolbachia* into invertebrate genomes. This may help evaluate the frequency of lateral gene flow in eukariota, which has been considered rare - whereas emerging evidence suggests that it may not be unusual between an endosymbiont and its host.

*Keywords : Wolbachia, Podisma pedestris, hybrid zone, nuclear inserts, lateral gene transfer*

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## ***Rickettsia* dynamics in populations of the sweetpotato whitefly, *Bemisia tabaci*, in Israel and the USA**

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A new heritable bacterial association can bring a fresh set of molecular capabilities, providing an insect host with an almost instantaneous genome extension. Increasingly acknowledged as agents of rapid evolution, inherited microbes remain underappreciated players in pest management programs. A *Rickettsia* bacterium was recently tracked sweeping through populations of an invasive whitefly species, *Bemisia tabaci*, in the southwestern USA where it may be providing fitness benefits. In contrast, whiteflies in Israel show little or no apparent fitness benefits from *Rickettsia* infection. A survey of historical and current whitefly samples reveals the distribution of *Rickettsia* in *B. tabaci* populations across the cotton-growing regions of Israel and the USA. Ten sites from Israel and twenty-five sites from the USA were sampled, along with various preserved specimens. Across the USA, *Rickettsia* is generally at very high frequencies, close to fixation, whereas in Israel the infection rates are low and declining. Intermediate frequency may indicate a cost to infection in certain environments. The distinct outcomes of *Rickettsia* infection in these two countries present an opportunity to determine the mechanisms and consequences of symbiont infection in this serious agricultural pest.

**Keywords:** *Rickettsia*, whitefly, *Bemisia tabaci*, symbiont sweep, distribution

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## The impact of symbiotic associations in metapopulations

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Recent reports suggest that symbiotic organisms may infect up to 70% of all insect species. Either obligatory or facultative, symbionts have evolved a large range of phenotypes with different impacts on their host biology. Either mutualistic, parasitic or somewhere between these two extremes, these life-histories evolved to promote the successful spread of the symbionts across the host population. The Glanville fritillary butterfly, *Melitaea cinxia*, is considered as a model organism in the study of metapopulation and conservation biology in fragmented landscape. This butterfly species is the host of an endoparasitoid wasp, *Hyposoter horticola*, and the biology of both insects, as well as their interaction, has been intensely investigated in the last decades. Surprisingly, no study has yet investigated the role of symbionts in the dynamic of both insect metapopulations or on the outcome of their interaction.

The proposed research will (1) describe the infection profiles of both insect species, (2) assess the importance for conservation biology to consider symbionts, and their impacts on their hosts' life-history traits and dynamics within metapopulation contexts.

*Keywords: Symbiont, parasitoid, metapopulation, Conservation Biology*

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## Effects of the endosymbiont *Rickettsia* on whitefly flight

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Dispersal by flight is a key adaptation in the evolutionary success of insects, but to our knowledge the effects of symbiont infection on host flight have never been studied. We investigated the effects of the facultative symbiont, *Rickettsia*, on the free flight behavior of the whitefly *Bemisia tabaci*. Whitefly dispersal contributes to its success as a noxious agricultural pest in the warm southwestern USA, sustaining populations year round as adults move between crops. Our previous work showed that *Rickettsia* spread extremely rapidly in whitefly field populations in the southwestern USA, conferred dramatic fitness benefits to infected whiteflies, and caused strongly female biased sex ratios. Given the importance of dispersal in *B. tabaci* biology, we investigated the effects of *Rickettsia* infection on flight. We compared the relative migratory abilities of *Rickettsia*-infected and uninfected whiteflies in two genetic backgrounds in a vertical flight chamber, determining individual whiteflies' likelihood of flight and flight duration. Analysis revealed that *Rickettsia*-infected females were more likely to fly than uninfected females in one genetic background, but there was no difference in the second genetic background. Flight duration was also similar for infected and uninfected whiteflies. These data suggest that any metabolic cost of *Rickettsia* infection does not compromise flight. Further, quantitative PCR showed that the genetic background in which *Rickettsia* does not influence flight has a lower *Rickettsia* titer than the first background, contrary to what we would predict if *Rickettsia* exerts a metabolic cost on infected whiteflies. These results suggest that *Rickettsia* spread in whiteflies in the field may be unaffected or enhanced by its influence on whitefly flight. Further, our results indicate that the whitefly-*Rickettsia* interaction is not uniform across different whitefly genotypes.

**Keywords:** Symbiont, whitefly, *Bemisia tabaci*, *Rickettsia*, flight behavior

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## Characterization of *Arsenophonus*, a widespread inherited bacterial endosymbiont of leafhoppers across Canada

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*Arsenophonus* is a widespread yet little studied clade of bacterial symbionts of arthropods that includes sex-ratio distorters, mutualists, and plant pathogens. We screened 43 leafhopper species from 5 provinces and found that *Arsenophonus* infections are abundant in this group, with 13 species testing positive, including ~60% of individuals of *Macrostelus quadrilineatus*, the major vector of aster yellows disease. Different leafhopper species harbour identical *Arsenophonus* strains, suggesting that horizontal transmission is common, perhaps via shared host plants.

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## Interactions of *Arsenophonus* and Host-Plant Resistance on Soybean Aphid Performance

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Recent evidence suggests bacterial endosymbionts can mediate the intimate relationships between some insects and their host plants. However, deciphering the role of the symbiont is often confounded by complex interactions between the interwoven genotypes of plant, insect, and bacteria. The endosymbiont Candidatus *Arsenophonus* infects a wide taxonomic range of arthropod hosts, and is suspected of an uncharacterized mutualistic role in hemipterous insects. In the soybean aphid, *Aphis glycines*, a notorious invasive pest of soybeans in the United States, *Arsenophonus* is the sole facultative endosymbiont. Recently, resistant soybean genotypes, *Rag* plants, were developed to minimize aphid outbreaks. However, soybean aphid “biotypes” virulent to *Rag* plants have since been discovered. We hypothesized that *Arsenophonus* is partially responsible for overcoming the antibiosis effect of the resistant plants. This hypothesis was predicated on another system in which a *Rickettsia*-like symbiont was associated with virulence of the potato aphid, *Macrosiphum euphorbiae*, on tomato plants protected with a resistance gene. We cured five soybean aphid biotypes, virulent and avirulent, of their natural *Arsenophonus* infection through ampicillin microinjection, resulting in infected and uninfected isolines. These isolines were subjected to growth rate assays on resistant *Rag* versus susceptible soybean. Our preliminary results indicate that *Arsenophonus*-infected avirulent biotypes perform better than cured on both plant types. This trend was evident in virulent biotypes, but less pronounced. In contrast to the *M. euphorbiae* system, *Arsenophonus* may provide certain biotypes of soybean aphids a general fitness benefit regardless of the presence of the resistance gene in the host plant.

*Keywords: Arsenophonus, Aphis glycines, Aphid Biotypes, Host-Plant Resistance*

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