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Abstract book

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Program

Friday, 6 June 2014

1600 - 2000	Registration
1900 - 2200	Welcome reception

Saturday, 7 June 2014

0814 - 0830	Wakeup call: Steve Reich (* 1936) Music for a large ensemble
0830 - 0900	Official welcome and opening remarks

Symposium EVOLUTION
Chair: Didier Bouchon

0900 - 0920	Charlat S.: Quantifying the *Wolbachia* turnover
0920 - 0940	Lindsey A.: Experimental evolution of *Wolbachia* in novel hosts
0940 - 1000	Gerth M.: Phylogenomic analyses of *Wolbachia* supergroup relationships
1000 - 1020	Martínez-Rodríguez P.: Understanding codivergence of hosts and their associated bacteria: *Wolbachia* infection in the *Chorthippus parallelus* hybrid zone
1020 - 1050	Coffee break

Symposium ECOLOGY, DIVERSITY, DYNAMICS
Chair: Sylvain Charlat

1050 - 1110	Truitt A.: Investigations into a case of *Wolbachia* and an endangered butterfly population
1110 - 1130	Ilinsky Y.: Maternal inheritance of long-established laboratory *Drosophila melanogaster* stocks
1130 - 1150	Jeong G.: Endosymbionts-induced microbiome disruption in *Vollenhovia emeryi* (Hymenoptera: Myrmicinae)
1150 - 1210	Bouvaine S.: Diversity of *Wolbachia* in African Cassava whiteflies
1210 - 1230	Hurst G.: Ecological and evolutionary impacts of male-killing bacteria
1230 - 1400	Lunch

Symposium DISEASE AND PEST CONTROL
Chair: Einat Zchori-Fein

1400 - 1420	Dobson S.: Application of *Wolbachia* as a pesticide against *Aedes albopictus* (Asian Tiger Mosquito) in the USA
1420 - 1440	Calvitti M.: Cytoplasmic incompatibility patterns between naturally and *w*Pip infected *Aedes albopictus*: implications for safety and long term effectiveness of a suppression strategy
1440 - 1500	Xi Z.: Interplay of novel *Wolbachia* strains with *Aedes* and *Anopheles* mosquitoes
1500 - 1520	McGraw E.: *Wolbachia* infection lengthens extrinsic incubation period in dengue infected mosquitoes
1520 - 1550	Coffee break

Symposium DISEASE AND PEST CONTROL
Chair: Elisabeth McGraw

1550 - 1610	Telschow A.: *Wolbachia* destabilizes mosquito population dynamics
1610 - 1630	Rasgon J.: *Wolbachia* can enhance pathogen infection in mosquito vectors
1630 - 1650	Martinez J.: Frequency of antiviral protection and correlation with cytoplasmic incompatibility: insights from a comparative analysis of *Wolbachia* strains
1650 - 1710	Bourtzis K.: Medfly - gut microbiota - *Wolbachia* tripartite symbiosis: assessing effects on fitness, mating behavior and pest control
1710 - 1900	Poster session
1900 - 2030	Dinner
2030 - 2200	Social hour

Sunday, 8 June 2014

0801 - 0845	Wakeup call: Adriano Banchieri (1576-1634) Barca di Venezia per Padova
0845 - 0900	Announcements

Symposium CELL BIOLOGY
Chair: Laura Serbus

0900 - 0920	Frydman H.: An integrated approach to dissect the mechanisms of *Wolbachia* tropism
0920 - 0940	Kamath A.: A novel *Wolbachia* tropism: targeting of polar cells in the *Drosophila* follicular epithelium
0940 - 1000	Toomey M.: Mechanisms of *Wolbachia* tropism to the stem cell niche in the *Drosophila* testis
1000 - 1020	Malone C.: Assessing *Wolbachia* dynamics in the *Drosophila* ovary
1020 - 1050	Coffee break

Symposium CELL BIOLOGY
Chair: Horacio Frydman

1050 - 1110	Miller W.: *Wolbachia* in the mind – a mutualistic puppet master orchestrating proper host sexual behaviour
1110 - 1130	Serbus L.: The impact of dietary nutrition on intracellular *Wolbachia* titer
1130 - 1150	Newton I.: Necessity is the mother of invention: actin manipulations by the reproductive parasite *Wolbachia pipientis*

Symposium ECOLOGY, DIVERSITY, DYNAMICS
Chair: Seth Bordenstein

1150 - 1210	Hunter M.: Fitness benefits of a facultative symbiont are influenced by host nuclear genotype
1210 - 1230	Zchori-Fein E.: Beyond single-bacterium symbiosis: factors correlated with variation in symbiotic communities of insects
1230 - 1400	Lunch

Symposium EVOLUTION
Chair: Seth Bordenstein

1400 - 1420	Gottlieb Y.: *Coxiella* endosymbionts in the *Rhipicephalus sanguineus* brown tick species group
1420 - 1440	Teixera L.: Phylogenomics and evolution of symbiont-mediated protection to pathogens
1440 - 1500	Husnik F.: Host-symbiont interactions at the symbiotic interfaces of obligately blood-sucking insects
1500 - 1520	Zug R.: *Wolbachia*-arthropod mutualisms: some comments on pathogen interference, dependence, tolerance, and resistance
1520 - 1550	Coffee break

Symposium EVOLUTION
Chair: Roman Zug

1550 - 1610	Bordenstein S.: Speciation by symbiosis: what have we learned so far?
1610 - 1630	Hammerstein P.: Dobzhansky-Muller and *Wolbachia*-induced incompatibilities in a diploid genetic system
1630 - 1650	Riegler M.: Turn up the heat and you'll get more sons! Bacterially facilitated temperature dependent sex ratio in an Australian species of thrips
1650 - 1710	Group photo
1710 - 1900	Poster session & informal discussions
1900 - 2030	Dinner
2030 - 2200	Social hour

Monday, 9 June 2014

0802 - 0845	Wakeup call: Motosoma, son of Zeami (1394-1432) Shakkyo
0845 - 0900	Announcements

Symposium DISEASE AND PEST CONTROL
Chair: Jason Rasgon

0900 - 0920	Schneider D.: *Wolbachia* outbreak in tsetse fly hybrids: symbiont titer regulation, bi-directional CI, and overcoming of male hybrid sterility
0920 - 0940	Kaur R.: What can we infer from symbionts titre in their respective *Drosophila* host tissues?
0940 - 1000	Makepeace B.: A worm in bacterial clothing: proteomic analysis supports the hypothesis that *Wolbachia*-driven recruitment of neutrophil antimicrobial proteins protects *Onchocerca ochengi* against eosinophils
1000 - 1020	Ford L.: A•WOL macrofilaricidal drug discovery and development – optimisation of anti-*Wolbachia* efficacy
1020 - 1050	Coffee break

Symposium PHENOTYPES
Chair: Markus Riegler

1050 - 1110	Bouchon D.: Functional analysis of the host immune response in the symbiotic association between the pill-bug *Armadillidium vulgare* and the feminising *Wolbachia*
1110 - 1130	Bertaux J.: A bug may hide another: cryptic *Wolbachia* in unfeminized lineages of *Armadillidium vulgare*
1130 - 1150	Kern P.: A new molecular sexing technique for butterflies – does *Wolbachia* really feminise genetic males in *Eurema*?
1150 - 1210	Wang YF.: *Wolbachia*-induced paternal defect in *Drosophila* is likely by interaction with the juvenile hormone pathway
1210 - 1230	Kaltenpoth M.: Symbiotic bacteria affect cuticular hydrocarbon profiles in tsetse flies (*Glossina m. morsitans*)
1230 - 1400	Lunch
1400 - 1900	Excursions
1900 - 2030	Dinner
2030 - 2200	Social hour

Tuesday, 10 June 2014

0829 - 0845	Wakeup call: Lou Reed (1943-2013) Metal machine music – the amine β ring pt. 1
0845 - 0900	Announcements

Symposium ECOLOGY, DIVERSITY, DYNAMICS
Chair: Molly Hunter

0900 - 0920	White J.: Double trouble: multiple endosymbiont infection and multiple manipulations in a linyphiid spider
0920 - 0940	Simhadri R.: Modulation of microbiome of *Drosophila melanogaster* by *Wolbachia*
0940 - 1000	Valiente Moro C.: Are *Wolbachia* and microbiota linked to the genetic structure of invasive and endemic populations of *Aedes albopictus*?
1000 - 1020	Weill M.: Evolution of the interaction *Culex pipiens* | *Wolbachia*
1020 - 1050	Coffee break

Symposium GENETICS & GENOMICS
Chair: Lisa Klasson

1050 - 1110	Fukatsu T.: Functional genomics of beetle-microbe symbioses
1110 - 1130	Comandatore F.: Phylogenomics and analysis of shared genes suggest a single origin of the main lineages of *Wolbachia* in nematodes
1130 - 1150	Chrostek E.: Link between genotype and phenotype in *Wolbachia*
1150 - 1210	Cordaux R.: Impact of *Wolbachia* endosymbionts on the evolution of sex determination in the isopod *Armadillidium vulgare*
1210 - 1230	Badawi M.: Toward the identification of feminizing genes of the bacterial endosymbiont *Wolbachia*
1230 - 1400	Lunch

Symposium GENETICS & GENOMICS
Chair: Takema Fukatsu

1400 - 1420	Nichols R.: *Wolbachia*'s Burgess Shale: ancient and modern integration in the genome of *Podisma pedestris* (Orthoptera)
1420 - 1440	Hong X.: Identification of *Wolbachia*-responsive microRNAs in the two-spotted spider mite *Tetranychus urticae*
1440 - 1500	Wang GH. Sex-specific transcription of large proportion of genes in the only cryptic WO prophage genome in a fig wasp species
1500 - 1520	Harumoto T.: *Spiroplasma* infection induces male-specific DNA damage response in *Drosophila melanogaster*
1550 - 1610	Greve P.: *Wolbachia* genome sequencing: phylogenomics, symbiosis-related pan-genome and eukaryote-like proteins involved in host-symbiont interactions
1610 - 1640	Student awards
1640 - 1730	Presentations and discussion about the venue for the Wolbachia Conference 2016
1730 - 1900	Poster session
1900 - 2300	Gala dinner

Wednesday, 11 June 2014

Breakfast at the hotels

Departure

Abstracts of oral presentations

7 June 2014
Morning symposia

Quantifying the *Wolbachia* turnover

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Given its widespread occurrence and often radical phenotypic effects, there is little doubt that *Wolbachia* represents a substantial evolutionary force in arthropods. But the nature and strength of its effects are conditioned by two parameters for which we currently have no estimates: the duration of the typical *Wolbachia* stay in a given host lineage, and the rate at which uninfected lineages acquire a new infection. Assuming the current global incidence of about one third represents a stable equilibrium between transfer and acquisition, one can approximate that infections are lost twice faster than they are acquired. Noting that even closely related host species only rarely share similar, that is, potentially ancestral infections, we can further guess that *Wolbachia* rarely stays more than a few million years in a given host lineage. In an attempt to provide a better-founded estimate of these important parameters, we are currently applying co-phylogenetic methods to estimate the most likely rates of *Wolbachia* acquisition and losses, considering the observed incongruence between *Wolbachia* and arthropod trees. We will present these estimates and discuss their implications regarding the potential evolutionary consequences of *Wolbachia* infections.

Experimental evolution of *Wolbachia* in novel hosts

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Richard Stouthamer
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It has been difficult to undertake detailed studies on new *Wolbachia* infections, because they rarely persist. This is likely due to a lack of co-evolution between host and symbiont. Instead of trying to infect a previously uninfected host with *Wolbachia*, our approach is to manipulate the nuclear genetic background of a host (*Trichogramma* parasitoid wasps) long co-evolved with *Wolbachia*, and then observe how the symbiont adapts to its "new" host background. In *Trichogramma*, unfertilized eggs develop into males, and fertilized eggs develop as female. However, *Wolbachia* induces parthenogenesis, so that unfertilized eggs also develop as female. To challenge *Wolbachia*, recombinant backgrounds were created by exploiting the fact that *Wolbachia*-infected *Trichogramma* will, in rare cases, use sperm from males of uninfected populations to fertilize their eggs. Each recombinant *Wolbachia*-infected daughter can be used to start a unique homozygous colony. This leaves no diversity in the host for selection to act upon. In contrast, *Wolbachia*, with its high replication rate, is free to adapt to the newly created recombinant hosts. We created 10 recombinant lines of *Trichogramma* using this method. At generation three, the *Wolbachia* performance of each line relative to the original infected population, was determined by measuring symbiont titer and host sex ratio. In recombinant lines, we observed lower levels of parthenogenesis and the production of intersex offspring. After 26 generations, we saw improved *Wolbachia* performance, marked by higher proportions of females, and lower proportions of intersex. We conclude that the paternal genome, not originally associated with *Wolbachia*, inhibits the *Wolbachia* phenotype. Over a short period of time, *Wolbachia* will adapt to this new nuclear background, so as to more effectively induce parthenogenesis. This system will be used to identify key physiological and genomic changes occurring during adaptation of *Wolbachia* to a novel host.

Phylogenomic analyses of *Wolbachia* supergroup relationships

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The ubiquitous bacterial intracellular endosymbiont *Wolbachia* is known from many arthropods but is also found in filarial nematodes. Considerable differences in *Wolbachia*'s biology have led to the recognition of various supergroups. Nematodes have evolved mutualistic relationships with *Wolbachia* (supergroups C and D) over evolutionary timescales whereas arthropod *Wolbachia* (supergroups A and B) are generally opportunistic, switch hosts frequently and may generate reproductive modifications in their hosts. Further distinct lineages exist, supergroup F being the only strain that was detected in both arthropods and nematodes. Although many *Wolbachia* – host interactions have been studied extensively, the intergroup relationships are only poorly understood. Therefore, *Wolbachia*'s evolutionary history, in particular the number and direction of major host and lifestyle transitions, remains elusive. As yet, attempts to reconstruct *Wolbachia*'s phylogeny were impeded by insufficient taxon or gene sampling and by long branch artifacts originating from distant outgroups. Here, we attempt to overcome these problems by incorporating whole genome data from so far unsampled supergroups into a large scaled phylogenomic analysis of *Wolbachia* strains. Multiple phylogenetic approaches yielded highly congruent results, placing supergroup F as sistergroup to supergroup C and supergroups E and H at the basis of the *Wolbachia* tree. This suggests that *Wolbachia* has evolved from a specialized ancestor and that major host switches have occurred at least twice. Various genes are shared by all arthropod *Wolbachia* supergroups, implying that the reduced genomes of supergroups C and D have evolved independently. These results will serve as framework for future investigations on *Wolbachia* evolution.

Understanding codivergence of hosts and their associated bacteria: *Wolbachia* infection in the *Chorthippus parallelus* hybrid zone

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Departamento de Biología, Universidad Autónoma de Madrid, Spain
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Several studies indicate that codivergence of *Wolbachia* strains with their hosts is less common than horizontal transmission from other taxa. However, codivergence processes are of great value in understanding essential aspects of *Wolbachia* biology, including host adaptation, immune system suppression and coevolution.

In this study, we describe the case of the hybrid zone of *Chorthippus parallelus*, a grasshopper extensively studied for evolutionary purposes. Phylogenetic and phylogeographic studies based on previously established host phylogenies and taking a bacterial MLST approach show that the two subspecies of the grasshopper and a number of *Wolbachia* strains codiverged during the last glaciation period. In addition, extrapolation of the divergence time between bacterial strains leads us to propose that *Wolbachia* infected *C. parallelus* during the divergence of the two subspecies that formed the Pyrenean hybrid zone.

This, combined with the cytoplasmic incompatibility induced by this bacterium in natural populations of *C. parallelus*, suggests that the codivergence between bacterium and host partially explains the current status of the hybrid zone and makes it a good model for studying coevolutionary processes.

Investigations into a case of *Wolbachia* and an endangered butterfly population

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Can population augmentation between populations such as captive rearing and release effectively conserve imperiled populations of insect pollinators, or are these methods too risky for some populations? Augmentation is accomplished by supplementing small unstable populations with individuals of the same species from the same population or from a larger, more stable population. The latter type of augmentation may bring populations together that have historically occurred allopatrically. While augmentation programs are very responsible about not using visibly infected individuals to supplement recipient populations, movement between populations has the potential to spread pathogens. Unknown or unidentified symbiotic bacteria can be introduced when the source population is infected but the recipient population is uninfected or differently infected prior to supplementation. Here we examine the temporal sequence of infection and potential consequences of infection to a butterfly undergoing population supplementation between populations that are infected with symbiotic bacteria, *Wolbachia*, that can decrease population viability.

Despite management actions including habitat restoration, habitat enhancement, and captive rearing and release, some of these populations remain severely susceptible to extirpation. This compelled us to investigate whether CI-inducing *Wolbachia* served as a potential additional variable contributing to the attrition of *Speyeria zerene hippolyta* populations. This study investigates 1) whether the donor populations had different strains of *Wolbachia* from the recipient ones suggesting that the captive rearing and release program accidently introduced new strains; 2) whether there are differences in reproductive success between infected and uninfected females; and 3) the potential ramifications that introduction of novel *Wolbachia* infections has on populations of pollinator species of conservation concern.

Maternal inheritance of long-established laboratory *Drosophila melanogaster* stocks

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Wolbachia is widespread in field populations of *Drosophila melanogaster*, however, there is little detailed information on *Wolbachia* infection among long-established laboratory *D. melanogaster* stocks. There have been studied four hundreds of mutant and wild type lines mainly from the collection of the Laboratory of Populations Genetics, Institute of Cytology and Genetics, Novosibirsk, Russia. The stocks are characterized by M-, S- mitotypes, and infection status: a genotype of *Wolbachia* or no infection. The diversity of maternal inheritance can be expressed in cytotypes: M-MEL, M-w–, S-CS, and S-w–.

In scrutinizing the stocks' records genealogy and our results, we observed some cases of bacteria loss in maternal lineages. However, in most cases the cytotypes proved to be inherited stable, with no *Wolbachia* or mitochondria parental transmissions.

In analyzing one-name wide-used stocks from different Stock Centers and Labs by cytotype, mtDNA sequences and mobile element patterns we have revealed facts of great genetic differences that indicate different origin of certain lines.

Endosymbionts-induced microbiome disruption in *Vollenhovia emeryi* (Hymenoptera: Myrmicinae)

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The *Vollenhovia emeryi* ant can be categorized by its wing morphology, i.e. long-winged and short-winged. A phylogenetic analysis indicates that short-winged colonies are derived from long-winged colonies in *V. emeryi* suggesting that the short-winged is somehow cured of *Wolbachia* and the host genetic background of the morph may have evolved resistance to *Wolbachia* infection.

This raises a question whether the potentially resistant host genetic background and *Wolbachia* infection affect the bacterial symbiont community diversity in the ant species. For answering the question, the high throughput sequencing method was employed to examine the bacterial community diversity of the long- and the short-winged colonies. The specific aims of this study are (1) to investigate the influence of *Wolbachia* infection and the host genetic background to diversity of bacterial symbionts of the two morphs of the ant, (2) to compare bacterial diversity at the caste level.

We find that there are about 180 bacterial symbionts in the short-winged morph. On the other hand, the long-winged morph harbors only about 20 bacterial symbionts. The bacterial community diversity may be influenced by the existence of endosymbionts including *Wolbachia* but not much, if any, by the host genetic background. The bacterial community comparison among castes within the morph shows that the queen caste has rather dissimilar bacterial community most probably due to food resources. Furthermore, a bacterial strain diversity among castes indicates that there may be labor division even between queens. However, masking by dominant species is a known issue in this method. In this presentation, future research plan to tackle it will also be discussed.

Diversity of *Wolbachia* in African Cassava whiteflies

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Bemisia tabaci, the insect vector of major plant virus diseases across the world, hosts many secondary endosymbionts such as *Wolbachia*, *Rickettsia*, *Arsenophonus*, *Hamiltonella*, *Cardinium* and *Fritschea* which play essential roles in their biology and behaviour. This study was aimed at understanding the prevalence and diversity of endosymbionts associated with *B. tabaci* populations from east and West Africa and the possible role of *Wolbachia* on the biology of its host. Prevalence of secondary endosymbionts in laboratory and field populations of cassava whiteflies from Subsaharan Africa (SSA) collected from 2012 to 2014 and from Cassava mosaic virus disease (CMD) epidemic and non-epidemic areas in Uganda in 1997 were screened by PCR. SSA2 biotype of whiteflies which are associated with CMD epidemic in east Africa collected from epidemic areas in 1997 harboured 100% *Wolbachia* infection. Diversity of *Wolbachia* was determined by multiple locus sequence typing (MLST) which formed two distinct clades in phylogeny of concatenated nucleotide sequences. *Wolbachia* haplotype from SSA1-subgroup2 biotype whiteflies were closer to *Wolbachia* from butterfly species whereas haplotypes from SSA2 and SSA1-subgroup3 whiteflies were clusering together. Quantification of *Wolbachia* from whiteflies by qPCR showed significant variable rates of multiplication of *Wolbachia* in different strains of whiteflies. Whiteflies from SSA2 and SSA1-subgroup2 had high quantities of *Wolbachia*, whereas SSA1-subgroup3 and subgroup1 had intermediate and lower quantities respectively. The temporal transition of whitefly population in CMD pandemic areas of east Africa from SSA2 to SSA1-SG1 implicates a possible role of *Wolbachia* in the biology of whiteflies which affects their population dynamics over time.

Ecological and evolutionary impacts of male-killing bacteria

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The effect symbionts have on host individuals are well known, at least at a phenotypic level. In contrast, our understanding of symbiont impact on host populations is less well established, with a contrast between the extent of theoretical predictions and the degree to which these have been tested in natural populations. In this talk, I will outline a case study in ladybirds relating the impact of male-killers on the dynamics of a ladybird disease, and one in butterflies where the evolutionary impact of male-killing is being resolved. I will argue impacts of symbionts on pathogens require us to understand both demographic and protective effects, and that rare events, such as extreme sex ratio bias associated with male-killing, may drive unusual (and interesting) evolutionary changes.

7 June 2014
Afternoon symposia

Application of *Wolbachia* as a pesticide against *Aedes albopictus* (Asian Tiger Mosquito) in the USA

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The Asian tiger mosquito (*Aedes albopictus*) is an invasive species, biting nuisance and vector of medically important pathogens, including dengue and chikungunya). Despite intensive use of conventional pesticides, its range continues to expand in the USA and globally. A proposed autocidal approach for its control is based on *Wolbachia*, an endosymbiotic bacteria that is common in many insect species. Similar to sterile insect technique, the *Wolbachia* approach is based on the release of *Wolbachia* infected males, which cause a form of conditional sterility, known as Cytoplasmic Incompatibility (CI) in the targeted populations. In July 2013, Experimental Use Permit (No. 89668-EUP-1) was awarded by the US Environmental Protection Agency (EPA) to conduct field trial performance tests of a *Wolbachia* biopesticide approach against the Asian tiger mosquito in the continental US. This presentation will summarize regulatory work with the EPA and early experimental work examining male performance, longevity and dispersal in infested neighborhoods of Lexington, Kentucky, USA. Plans for future work in Kentucky and additional states will be summarized.

Cytoplasmic incompatibility patterns between naturally and *w*Pip infected *Aedes albopictus*: implications for safety and long term effectiveness of a suppression strategy

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Aedes albopictus is known to be superinfected with two strains of *Wolbachia* (*w*AlbA and *w*AlbB). In previous works we described the generation of a transinfected *Ae. albopictus* line, named "AR*w*P", by replacing the natural *Wolbachia* double-infection with a single heterologous bacterial strain from *Culex pipiens*. Reported infection data and CI features appeared consistent with a promising use of AR*w*P line as a tool for *Ae. albopictus* suppression strategies. We also demonstrated that in crosses between wild type males and AR*w*P females only *w*AlbA causes full CI, while *w*AlbB and *w*Pip display an asymmetrical CI pattern.

Data from literature indicate that *Wolbachia* density in natural populations varies within a wide range. In particular *w*AlbA strain, besides being variable among individuals, tends to dramatically decrease with male age. In this work, we confirmed these general findings on mosquitoes collected in Northern and Central Italy. In addition, we evaluated the influence of male *w*AlbA and *w*AlbB loads on CI penetrance in crosses with AR*w*P transinfected females. To this aim we combined a quantitative PCR approach with egg hatching data from age controlled crossing experiments. We clearly found that the *w*AlbA "mod+" function towards AR*w*P females is expressed in a density-dependent manner. In fact, young males with very low *w*AlbA load showed a weakened CI, while older males with higher *w*AlbA density were associated to strong CI. Differently, *w*AlbB (a weaker CI inducer towards *w*Pip females) showed neither an evident age-dependent density variation in males nor a significant density-dependent impact on CI in crosses with AR*w*P females.

The results of this work are an unprecedented contribution for evaluating how strong and stable is the bidirectional CI pattern between wild superinfected and AR*w*P mosquitoes. Obtained data are discussed in the perspective of developing a bio-ecologically safe and long-term effective area-wide suppression strategy against *Ae. albopictus*.

Interplay of novel *Wolbachia* strains with *Aedes* and *Anopheles* mosquitoes

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Diseases transmitted by blood-feeding arthropod vectors, such as mosquito-borne malaria and dengue fever, cause 1.5 million human deaths every year. The insufficiency of currently available strategies, including vaccines, drugs and pesticides, has led to an increase in vector-borne diseases, making it urgent to develop novel control methods. Due to its ability to induce both cytoplasmic incompatibility and pathogen interference, *Wolbachia* is now widely recognized for its potential to modify mosquito populations such that they become refractory to the human pathogens they transmit. Toward this goal, we have introduced different *Wolbachia* strains into the major disease vectors, including two dengue vectors *Aedes aegypti* and *Ae. albopictus* and one primary malaria vector *Anopheles stephensi*. This provides us the opportunity to compare the interactions of either the same *Wolbachia* with different hosts, or the same host with different *Wolbachia* strains, in terms of pathogen interference, CI and fitness cost. We found *w*AlbB induced resistance to both dengue and malaria, and conferred certain benefits on both *Ae. aegypti* and *An. stephensi*. But *w*AlbB appeared to cause more fitness cost in female fecundity in *An. stephensi* than in *Ae. aegypti*. Two *Ae. albopictus* lines with different tripe infection were generated, and a difference in CI, antiviral effect and fitness is currently investigated. In all the transinfected lines, mosquito basal immunity was boosted by the novel infection, which contributed to *Wolbachia*-mediated pathogen interference. We also explored the interplay of *Wolbachia* with mosquito innate immunity by taking advantage of the currently available reverse genetic tools and transgenic mosquitoes with immune deficiency. The above results will be discussed in relation to developing *Wolbachia*-based strategies for vector-borne disease control.

Wolbachia infection lengthens extrinsic incubation period in dengue infected mosquitoes

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The *Wolbachia* strain *w*Mel, that has been artificially introduced into the dengue virus mosquito vector *Aedes aegypti*, decreases the susceptibility of mosquitoes to dengue virus. The extrinsic incubation period (EIP), which is the time between when a mosquito takes a dengue infected bloodmeal and the time when the mosquito is capable of infecting a human on a subsequent bite, is one of the key components of disease transmission. We have devised a non-destructive method to repeatedly sample the saliva of mosquitoes to obtain estimates of EIP. We show here that *w*Mel (1) delays EIP in dengue infected mosquitos by 1.1 days, (2) narrows the window of infectivity of infected mosquitoes by around 4 days, (3) reduces the amount of dengue copy number in mosquito saliva by approximately 3-fold and (4) reduces the saliva volume produced across the insect's lifespan. Surprisingly, we also found that (5) *w*Mel lengthened the lifespan of dengue-infected mosquitoes possibly by lessening their dengue viral burden. These findings allow us to measure the vectorial capacity of *Wolbachia*-infected mosquitoes and more accurately estimate the impact of releasing *Wolbachia* infected mosquitoes as a strategy to reduce dengue transmission.

Wolbachia destabilizes mosquito population dynamics

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Mosquitoes are important vectors for disease transmission in humans and livestock, causing worldwide problems for public health and agriculture. Recent efforts in pest control focus on biological control strategies that incapacitate the pathogen inside the vector by altering the vector's microbiome. A prominent example is the release of intracellular bacteria *Wolbachia* into natural mosquito populations with the aim to eliminate dengue fever. However, whether such artificial infections can be a successful strategy to control vector-borne diseases and minimize the risks associated with releasing potential disease vectors is still under debate. Here, we demonstrate that *Wolbachia* can destabilize mosquito population dynamics by contrasting *Wolbachia*-infected versus uninfected cage populations of the Asian tiger mosquito (*Aedes albopictus*). We found that the population variability (measured as coefficient of variation of population size through time) of the infected cages is significantly higher than that of the uninfected cages. The elevated population variability is explained by the increasing nonlinear dynamics, as quantified by nonlinear time series analyses (S-Map analysis, state space reconstruction). In conclusion, the results suggest two potential risks for using *Wolbachia* in biological control programs. First, boom-and-bust of the mosquito population size is more likely to occur in *Wolbachia*-infected than in uninfected populations. Second, *Wolbachia*-infected mosquito populations are more difficult to manage because the higher degree of non-linearity makes their population dynamics less predictable. These findings have important management implications for the use of artificial bacterial infections as control of mosquitoes and vector-borne diseases.

Wolbachia can enhance pathogen infection in mosquito vectors

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Due to its ability to suppress pathogen infection in vector arthropods, laboratory and field investigations are currently ongoing for the use of *Wolbachia* to control arthropod-borne diseases. However, it has become clear that *Wolbachia* infection does not always lead to pathogen suppression in insects. Multiple studies using rodent and avian malaria models suggest that *Wolbachia* may enhance some *Plasmodium* parasites in mosquitoes, but to this point enhancement has not been observed for any human pathogens. We evaluated the effects of *Wolbachia* on infection, dissemination and transmission of West Nile virus (WNV) in the naturally uninfected mosquito *Culex tarsalis*. *Wolbachia* reached high titers and disseminated to numerous tissues including the head, proboscis, thoracic flight muscles, fat body and ovarian follicles. Contrary to other systems, *Wolbachia* did not inhibit WNV in this mosquito. Rather, WNV infection rate was significantly higher in *Wolbachia*-infected mosquitoes compared to controls. Quantitative PCR indicated that REL1 (the activator of the antiviral Toll immune pathway) was downregulated in *Wolbachia*-infected relative to control mosquitoes. This is the first observation of *Wolbachia*-induced enhancement of a human pathogen in mosquitoes, suggesting that caution should be applied before using *Wolbachia* as part of a vector-borne disease control program.

Frequency of antiviral protection and correlation with cytoplasmic incompatibility: insights from a comparative analysis of *Wolbachia* strains

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In the last few years, *Wolbachia* has been shown to play an important role in protecting *Drosophila* and mosquito hosts against viral infection. While the mechanisms remain unclear, it was shown that not all bacterial strains have the ability to confer protection. In order to assess the frequency of such an induced phenotype, we have investigated antiviral protection in 19 *Wolbachia* strains originating from 16 *Drosophila* species after transfer into the same genotype of *D. simulans*. We found that approximately half of the strains protected against two RNA viruses, suggesting that a substantial proportion of insect species may benefit from *Wolbachia*-mediated protection. The level of protection against two distantly related RNA viruses – DCV and FHV – was strongly genetically correlated, which suggests that there is a single mechanism of protection with broad specificity. Furthermore, *Wolbachia* is mainly providing resistance to viral infection, as the increases in survival following infection can be largely explained by reductions in viral titer. Variation in antiviral protection provided by different *Wolbachia* strains is genetically correlated to their density within the host, supporting previous data. Finally, we found no correlation between protection and the ability to induce cytoplasmic incompatibility (CI), showing that the most protective strains do not necessarily have the highest invasive potential by means of CI. Moreover, *Wolbachia* infection was in some cases associated with costs which might represent a limitation for the use of *Wolbachia* as a biocontrol agent to prevent the spread of arboviruses.

Medfly - gut microbiota - *Wolbachia* tripartite symbiosis: assessing effects on fitness, mating behavior and pest control

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The Mediterranean fruit fly (medfly), *Ceratitis capitata*, is one of the most important agricultural pests worldwide. In many countries, the control of this pest is currently based on the development of Genetic Sexing Strains (GSSs) and their application in programs incorporating the Sterile Insect Technique (SIT), an irradiation-induced male sterility approach. *Wolbachia*-infected lines have also been established and express 100% Cytoplasmic Incompatibility (CI) and could potentially be useful for the development of Incompatible Insect Technique (IIT), a *Wolbachia*-induced male sterility approach, and / or the enhancement of SIT programs. In addition, a number of studies have shown that the gut-associated microbiota of medfly plays a significant role on host life history traits. In this study, we will present data about the effect of *Wolbachia* on host fitness and mating behavior as well on the diversity and structure of the gut-associated microbiota. Our results are of major importance for programs which aim to control pests and diseases using exclusively or combining *Wolbachia*-based approaches.

8 June 2014
Morning symposia

An integrated approach to dissect the mechanisms of *Wolbachia* tropism

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Tissue tropism is a fundamental aspect of host-microbe interactions. *Wolbachia* have a peculiar tropism for the microenvironment that supports stem cells in the *Drosophila* ovary, known as stem cell niches. This niche tropism facilitates germline colonization promoting successful infection of the next generation. The cellular and molecular basis for niche tropism are not well understood. We are dissecting *Wolbachia* – host interaction using a combination of cellular, molecular, developmental and computational approaches in *Drosophila* cell lines and whole organism. We have measured the dynamics of *Wolbachia* density and proliferation in the developing host, and found that the *Wolbachia* intracellular accumulation is driven mostly by higher division rates of bacteria that infect niche cells. Towards identifying the host molecular pathways responsible for this tropism, we performed a high-throughput sequencing on *Drosophila melanogaster* cell lines that are stably infected with *Wolbachia* and found approximately 1000 genes statistically significantly modified by infection. Host genes that regulate or are part of the stem cell maintenance, cell cycle, metamorphosis, adhesion, sugar metabolism, immunity, proteolysis and antioxidants are transcriptionally affected. Using bioinformatic tools we determined a set of core pathways with potentially relevant biological roles in *Wolbachia* tropism. These candidate pathways are being tested for their functional significance on *Wolbachia* tropism and replication in the host cells.

A novel *Wolbachia* tropism: targeting of polar cells in the *Drosophila* follicular epithelium

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Wolbachia propagate mainly by vertical transmission via the female oocyte. They are under a high selective pressure to colonize the female germline and infect the next generation. However *Wolbachia* infection is not just limited to the germline cells, but also several somatic cell populations have been reported to have higher *Wolbachia* loads compared to surrounding tissue, including stem cell niches, neurons, trachea and Malpighian tubes. Here we identify a previously overlooked *Wolbachia* tropism to the polar cells (PC) in the *Drosophila* ovary. PCs are a particular cell type within the follicular epithelium of a *Drosophila* egg chamber which have a role in the patterning of the eggshell, oocyte localization and polarity. During *Drosophila* oogenesis, we can easily follow all the stages of the PC development allowing us to investigate the kinetics of the intracellular accumulation of *Wolbachia* in the PCs and the surrounding follicular epithelia. Surprisingly, *Wolbachia* have been found to co-ordinate their intracellular accumulation with specific developmental events during oogenesis. Our results show that PC tropism of *Wolbachia* is acquired through higher division rates in these cells compared to the surrounding epithelia. In this study, we also investigate various host factors which are responsible for the specific accumulation of *Wolbachia* in these cells. PC tropism provides an additional entry point for *Wolbachia* into the oocyte during egg development. This analysis provides us mechanistic insights into the preferential targeting of *Wolbachia* to specific host cell types.

Mechanisms of *Wolbachia* tropism to the stem cell niche in the *Drosophila* testis

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Wolbachia target the stem cell niches in the *Drosophila* ovary to enhance germline colonization and subsequent vertical transmission. This tropism is pervasive across the *Drosophila* genus, with the pattern of targeting being evolutionarily conserved. We have found that tropism to the stem cell niche in the testis, known as the hub, is not evolutionarily conserved. Using hybrid analysis, we have found that both *Wolbachia* and host derived factors are important for tropism in this system. Towards identifying the cellular and molecular mechanisms of cell tropism, we are investigating hub targeting of the closely related *Wolbachia* strains *w*Mel and *w*MelPop, which differ significantly in their frequencies and densities of hub infection. The targeting discrepancy of these two strains of *Wolbachia* indicate that this phenotype is rapidly evolving, as they shared a common ancestor only 8,000 years ago. With the plethora of tools available in *D. melanogaster*, we are using a candidate gene approach to target host proteins enriched in the stem cell niche in the testis for RNAi mediated knockdown in the hub. We have identified *Drosophila* stem cell related signaling pathways that promote *Wolbachia* accumulation. Understanding the cellular and molecular bases of tissue tropism is a fundamental aspect of *Wolbachia*-host interactions.

Assessing *Wolbachia* dynamics in the *Drosophila* ovary

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In order to investigate the molecular relationships underlying *Wolbachia* infection, we have undertaken tissue-specific RNAi screening for host genes that influence *Wolbachia* dynamics. We assayed approximately 1,000 germline RNAi knock-down lines which produce ovarian phenotypes and drove these knock-downs in the presence or absence of *Wolbachia* to ask whether the bacteria would modify the previously determined phenotypes. After measuring egg laying, fertility and ovarian morphology, we observed little difference other than flies laid fewer eggs when infected with *Wolbachia*.

Additionally, we have continued several directed approaches to understand the experience of cells during infection, and how host-cells influence bacterial behavior. We performed gene expression profiling and identified validated numerous host-genes up-regulated during infection. We used this finding to develop a single-molecule RNA FISH assay allowing us to visualize *Wolbachia* transcripts during ovarian development. We have now paired this with tissue-specific gene knock-downs, allowing us to assay bacterial density and gene expression. We have continued selected host-gene knock-downs in immune, metabolic and mitochondrial pathways to assess the dynamics of mitochondrial and bacterial responses within the ovary. Our continued work will be discussed.

Wolbachia in the mind – a mutualistic puppet master orchestrating proper host sexual behaviour

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Wolbachia of the neotropical *Drosophila* species cluster *D. paulistorum* present one of the rare examples of conspecificity and obligate mutualism in arthropods. This neotropical species cluster is currently under incipient speciation in nature, consisting of six reproductively isolated semispecies that each harbour closely-related but reciprocally incompatible *Wolbachia* strains. In their native hosts these endosymbionts have evolved fixed vital interactions by affecting fecundity and viability of flies. In mixed genetic backgrounds of F1 hybrids between semispecies however, mutualistic *Wolbachia* transform into pathogens by overreplication via loss of replication control, and thereby cause embryonic mortality and sterility. As recently shown by our group neotropical *Wolbachia* (*w*Pau) also influence host sexual behaviour by affecting female assortative mating, i.e. by orchestrating proper mate recognition, most likely via pheromone perception and interpretation.

Here we have surveyed via FISH analyses in detail the temporal and spatial titer dynamics and tissue tropism of the mutualist in adult fly brains and found that, in contrast to *w*Mel, the presence of *w*Pau is exclusively restricted to well-defined brain regions which are known for being functionally important for pheromone perception, signal interpretation as well as learning and longterm memory.

The impact of dietary nutrition on intracellular *Wolbachia* titer

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While a number of studies have identified host factor that influence endosymbiont titer, little is known concerning environmental influences on endosymbiont titer. To address this, we examined nutrient impact on maternally transmitted *Wolbachia* endosymbionts in *Drosophila*. We show that feeding either laboratory-reared or wild-caught *Drosophila* a yeast-enriched diet dramatically suppressed *Wolbachia* titer carried within germline-derived oocytes. This yeast-induced titer reduction is mediated in large part by somatic Tor and insulin signaling pathways. Disrupting Tor with the small molecule Rapamycin dramatically increases oocyte *Wolbachia* titer, whereas hyper-activating somatic TORC1 suppresses oocyte titer. Furthermore, ablation of insulin producing cells located in the *Drosophila* brain eliminated yeast impact on oocyte titer. By contrast, dietary yeast had no impact on *Wolbachia* titer in the somatically derived central nervous system. These findings highlight the interactions between *Wolbachia* and germline cells as distinctive in sensitivity to insulin and TORC1-mediated nutrient responses.

Necessity is the mother of invention: actin manipulations by the reproductive parasite *Wolbachia* pipientis

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The obligately intracellular *Wolbachia* pipientis is a ubiquitous α-proteobacterial symbiont of insects and nematodes related to the Rickettsial pathogens *Ehrlichia* and *Anaplasma*. Studies of *Wolbachia* cell biology suggest that this bacterium is capable of manipulating host cortical actin during transmission between somatic cells and the germ line. Here, we identified and characterized *Wolbachia* proteins that localize to or disrupt the eukaryotic actin cytoskeleton. Two of these proteins (WD0830 and WD1171) induce growth defects in yeast upon expression, supporting their role as secreted bacterial effectors. In further support of their interaction with the cytoskeleton, expression of these two proteins abrogates or exacerbates growth defects in the context of the yeast morphogenesis checkpoint kinase mutant (Δswe1). We present an in depth analysis of one of these candidate secreted effectors, WalE1 (WD0830), a protein that promotes actin polymerization through direct interaction with actin and a reduction in the critical concentration of actin. This bacterial protein is unique, having no homology to previously identified formins or actin nucleating domain structures and is the first identification of any *Wolbachia* protein that alters actin polymerization dynamics. Finally, we analyze effects of WalE1 expression on the actin cytoskeleton and the *Wolbachia*-*Drosophila* symbiosis in the context of cell lines and germ line stem cell niche tropism. We suggest these *Wolbachia* proteins, and WalE1 specifically, may be utilized by the bacterium during invasion of the stem cell niche.

Fitness benefits of a facultative symbiont are influenced by host nuclear genotype

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Some arthropod-associated symbionts such as *Wolbachia*, known previously only as reproductive parasites, have recently been shown to benefit their hosts through nutrition and / or defense. Multiple roles of symbionts create more possibilities for genetic conflict among host and symbiont genes. The global whitefly species complex *Bemisia tabaci* has several facultative bacterial symbionts, among which *Rickettsia* sp. nr. *bellii* is common worldwide. *Rickettsia* swept rapidly to near fixation in the invasive *B. tabaci* "B" species in the southwestern USA in the early 2000s. In a laboratory line established from a local population, *Rickettsia* causes fitness benefits such as greater fecundity, developmental success and shorter development times as well as reproductive manipulation in the form of strongly female-biased sex ratios. We found differences in the phenotype of *Rickettsia*, however, in a second whitefly line collected from the same population. While *Rickettsia* appears to increase performance in both lines, the magnitude of benefit varies. We used introgression between the lines to separate the contributions to the *Rickettsia* phenotype of the host cytotype (including the primary symbiont *Portiera*, the fixed symbiont *Hamiltonella*, and mitochondrial genes) from those of the nuclear genotype. Our results suggest whitefly nuclear genotype is more important than cytotype in influencing *Rickettsia* expression. Furthermore, field surveys in Israel show a decline in *Rickettsia* frequency in whiteflies from 2000-2012, where earlier work found few fitness benefits associated with *Rickettsia* infection. In general, our results suggest that differences in the *Rickettsia* phenotype in whiteflies underlie the variable frequencies of *Rickettsia* in whiteflies worldwide, and raise the question: if *Rickettsia* is so good for whiteflies, why is there host control of the *Rickettsia* phenotype?

Beyond single-bacterium symbiosis: factors correlated with variation in symbiotic communities of insects

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The target of natural selection is suggested to be the holobiont – the organism together with its associated symbiotic microorganisms. The well-defined endosymbiotic communities of insects make them a useful model for exploring the role of symbiotic interactions in shaping the functional repertoire of plants and animals. Here, we studied the variations in the symbiotic communities of the whitefly *Bemisia tabaci* by compiling a dataset of over 2,000 individuals derived from several independent screenings. The facultative bacteria harbored by each individual were clustered into entities termed secondary symbiont community (SSCs), each representing a natural assemblage of co-occurring bacterial species. The association of SSCs with whitefly individuals stratified the otherwise homogeneous population into holobiont units. We both identified community structures that are specific to whitefly groups sharing unique genetic backgrounds, and characterized the SSC variations within these groups. The analysis revealed that community variations are typically associated with ecological preferences, and that SSC complexity is positively correlated with both distance from the equator and specificity of the insect host interaction. Our findings point to the role of host-community and intra community interactions in shaping community structure, and encourage further exploration of the functional significance of community variations.

8 June 2014
Afternoon symposia

Coxiella endosymbionts in the *Rhipicephalus sanguineus* brown tick species group

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Obligatory interactions between arthropods and microbial symbionts stem from a stressful environment or restricted resources, such as diet. Ticks, which are pestiferous obligatory blood feeders that have to cope with long periods of starvation between blood meals, are therefore expected to be inhabited primary obligatory symbionts. Although evidence for the presence of obligatory symbiotic bacteria in ticks has accumulated over the years, little is known about specific tick-symbiont interactions. We recently demonstrated a possible obligatory association between a symbiotic bacterium of the genus *Coxiella* and two of the brown tick species group: *Rhipicephalus sanguineus* and *R. turanicus*. *Coxiella* was prevalent in all individuals examined, did not interact quantitatively with other secondary symbionts, was specifically localized to the Malpighian tubules and female gonad tissues, and was maternally transmitted. The genome of the symbiotic *Coxiella*, however, demonstrates genomic characterizations of both primary and secondary known symbionts of insects, as it has a 1.7 Mbp chromosome in which there are only 922 protein-coding genes, and only 48.7% of the genome content is coding. The possible evolution route and function of *Coxiella* in *Rhipicephalus* spp. will be discussed in light of these findings.

Phylogenomics and evolution of symbiont-mediated protection to pathogens

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Wolbachia protect several insects from viral infection. This tripartite interaction was initially described in *Drosophila melanogaster* carrying *w*Mel, its natural *Wolbachia* strain. *w*Mel has been shown to be genetically polymorphic and there has been a recent change in variant frequencies in natural populations.

We have compared the antiviral protection conferred by different *w*Mel variants, their titres and influence on host longevity. The phenotypes cluster the variants into two groups - *w*MelCS-like and *w*Mel-like. *w*MelCS-like variants give stronger protection against viruses, reach higher titres and often shorten the host lifespan. We have sequenced and assembled the genomes of these *Wolbachia*, and shown that the two phenotypic groups are two monophyletic groups. Our results indicate that the more protective *w*MelCS-like variants, which may have a cost, were replaced by the less protective but more benign *w*Mel-like variants in natural populations.

We are currently analyzing how *w*Mel variants frequencies change under experimental evolution on an outbred *D. melanogaster* population infected with *Drosophila* C virus. This will elucidate to what extent selection can act on maternally transmitted symbionts variation when a host phenotype is under selection.

Our work helps to understand the natural variation in *w*Mel and its evolutionary dynamics and informs the use of *Wolbachia* in arthropod-borne disease control.

Host-symbiont interactions at the symbiotic interfaces of obligately blood-sucking insects

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Insects living exclusively on vertebrate blood, such as lice, bedbugs, Triatominae bugs or blood-sucking flies (louse flies, bat flies and tsetse flies), rely on symbiotic bacteria to supplement the nutrients missing from their blood diet (B-vitamins and cofactors). Although these insects are common vectors of pathogens, data on their interactions with the host, as well as the interactions among the mutualistic and parasitic members of the microbiome, are scarce. We characterize a simple but specific symbiotic system within the sheep parasite *Melophagus ovinus* (Diptera: Hippoboscidae). The system is composed of obligatory mutualists *Arsenophonus melophagi* and four additional facultative microorganisms, three bacteria (*Sodalis melophagi*, *Bartonella melophagi* and *Wolbachia*), and one kinetoplastid (*Trypanosoma melophagium*). Using combination of microscopy, genome sequencing and RNA-seq, we untangle interactions within the system and compare it to the functionally convergent, but phylogenetically independent symbiotic systems of other blood-sucking insects.

Wolbachia-arthropod mutualisms: some comments on pathogen interference, dependence, tolerance, and resistance

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Wolbachia are the most abundant bacterial endosymbionts among arthropods. They are notorious for their reproductive parasitism which, although lowering host fitness, ensures their spread. However, even for reproductive parasites it can pay to enhance host fitness. Indeed, there is increasing evidence of *Wolbachia*-associated fitness benefits, suggesting that the symbionts can also behave as mutualists, both of the facultative and obligate type. In particular, many studies report *Wolbachia*-induced anti-pathogenic effects, e.g. against several RNA viruses, different *Plasmodium* species, and bacteria. Here we critically assess the biological relevance of such *Wolbachia*-induced pathogen interference. To this end, it is crucial to check whether pathogen interference does occur in nature and, if yes, whether it is associated with a fitness benefit to the host. Based on a literature survey, we find that, so far, there is only limited support for a fitness-enhancing effect of anti-pathogenic effects in natural *Wolbachia*–host interactions.

In contrast to facultative mutualisms such as protection against pathogens, there are other *Wolbachia*–arthropod interactions where the host strictly depends on symbiont presence for reproduction or survival. We show that, frequently, such cases of host dependence (obligate mutualisms) arise through the evolution of tolerance (or "compensatory evolution") in the host. Originally, tolerance evolved as a means to cope with the harmful effects of *Wolbachia* infection. However, tolerance mechanisms are costly in the absence of infection and thus tend to render the host dependent on *Wolbachia*. Once the host depends on its symbiont, evolution of resistance (another host strategy to cope with infection) is no longer an option. This might explain why host resistance to *Wolbachia* has been found only so rarely, given that selection would act on hosts to suppress reproductive parasites.

Speciation by symbiosis: what have we learned so far?

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In the early 20th century, Ivan Wallin conceived a significant role for microorganisms in the origin of species because he discovered the bacterial nature of mitochondria. The idea was subsequently resurrected and popularized by Lynn Margulis. Since then, several studies on *Wolbachia* helped ground the evidence for speciation by symbiosis and "moved the needle" from a controversy to a reality. I will consolidate this history and discuss how *Wolbachia*-based studies were just a beginning for blending microbial symbionts and nuclear genes, i.e. the hologenome, into a unified theory on the origin of species.

Dobzhansky-Muller and *Wolbachia*-induced incompatibilities in a diploid genetic system

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Genetic incompatibilities are supposed to play an important role in speciation. A general theoretical problem is to explain the persistence of genetic diversity after secondary contact. Previous work has pointed out that Dobzhansky-Muller incompatibilities (DMI) are not stable in the face of migration unless local selection acts on the alleles involved in incompatibility. With local selection, genetic variability exists up to a critical migration rate but is lost when migration exceeds this threshold value. Here, we investigate the effect of intracellular *Wolbachia* on the stability of hybrid zones formed after the Dobzhansky Muller model. *Wolbachia* are known to cause a cytoplasmic incompatibility (CI) within and between species. Incorporating *Wolbachia* into speciation scenarios can lead to a significant increase of critical migration rates and maintenance of divergence, primarily because *Wolbachia*-induced incompatibility acts to reduce frequencies of F1 hybrids. *Wolbachia* infect up to two-thirds of all insect species and it is therefore likely that CI co-occurs with DMI in nature. The results indicate that both isolating mechanisms strengthen each other and under some circumstances act synergistically. Thus they can drive speciation processes more forcefully than either when acting alone. Moving from the abstract to the concrete, however, a number of puzzles remain. While two North American fruit fly species (*Drosophila recens* and *D. subquinaria*) serve as a showcase of CI-aided speciation, this fascinating system remains difficult to grasp theoretically.

Turn up the heat and you'll get more sons! Bacterially facilitated temperature dependent sex ratio in an Australian species of thrips

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Kelly's citrus thrips, *Pezothrips kellyanus* (Thysanoptera: Thripidae), is an economically important pest of citrus in Australasia and the Mediterranean region. We investigated the genetic diversity of *P. kellyanus* populations collected from its current range in order to detect its most likely origin. In this way, we also detected *Cardinium* and *Wolbachia* for the first time and studied their roles in the reproduction of this species.

Based on genetic markers and infection diversity we conclude that *P. kellyanus* is more likely to originate from Australasia. Australian populations are infected with both *Wolbachia* and *Cardinium*. Antibiotic treatment resulted in the establishment of uninfected populations and populations singly infected with *Cardinium*, while establishment of populations singly infected with *Wolbachia* failed. In combination, both infections caused cytoplasmic incompatibility in crosses between uninfected females and infected males. This resulted in embryonic mortality of fertilised diploid females but survival of unfertilised haploid males, and thus in all-male offspring. This is the first evidence of CI in the order Thysanoptera, potentially induced by either *Cardinium* or *Wolbachia*, or both, and we are currently testing their relative roles. We were surprised to find that not all fertilised females delivered the expected all-female offspring, suggesting male fertility problems in the absence of *Cardinium* and *Wolbachia*. Intriguingly, bacterial infections appear to restore fertility in infected individuals at lower temperature, resulting in more female-biased sex ratios at 20°C while at 25°C the sex ratio is comparable to that of uninfected thrips. Such a combined temperature and bacterial effect on sex ratio in *P. kellyanus* is unique, as sex in most insects is genetically determined and not influenced by temperature. This interaction could potentially enable this species to adapt its reproductive output to seasonal temperature changes.

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Wolbachia outbreak in tsetse fly hybrids: symbiont titer regulation, bi-directional CI, and overcoming of male hybrid sterility

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Tsetse flies (*Glossina*) are vectors of *Trypanosoma*, causative agent of Human African Sleeping sickness. They have evolved an intimate relationship with three microbial endosymbionts: *Wigglesworthia glossinida*, *Sodalis glossinidus* and *Wolbachia*. They impact essential host traits like female fertility, larval development, reproduction and parasite immunity. It was suggested earlier to exploit cytoplasmic incompatibility (CI) to induce natural reproductive sterility in tsetse populations and consequently block transmission of *Trypanosoma*. This idea is currently revived as strong unidirectional CI was observed among crosses between *Wolbachia*-infected and antibiotic-treated *Glossina morsitans* group members. Natural hybridization between *Glossina* species has been reported repeatedly, suggesting weak pre-mating barriers to hybrid formation. However, hybrids exhibit reduced female fecundity and male sterility under laboratory conditions.

Here, we show that besides uni-directional CI, *Wolbachia* also trigger bi-directional CI in the *Glossina morsitans* group. We further demonstrate intensive *Wolbachia* increase in tsetse inter-species hybrids and speculate that disturbance of the native host-symbiont equilibrium in hybrids can transform *Wolbachia* into pathogens by loss of replication control. This might consequently trigger hybrid incompatibilities between tsetse flies and thereby host speciation as a side effect. In addition, we discuss the regulation of the other key bacterial endosymbionts *Wigglesworthia* and *Sodalis* in mixed hybrid backgrounds. Finally, we demonstrate that male hybrid sterility can be successfully overcome by knock-down of male *Wolbachia* titer.

What can we infer from symbionts titre in their respective *Drosophila* host tissues?

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The endosymbiotic bacterium *Wolbachia* is considered as a potential biological tool for controlling invasive insect pest populations such as *Drosophila suzukii* (*Dsuz*), which has recently invaded European countries and come out as an emerging threat for fresh fruit cultivation. Recent reports have shown that European *Dsuz* harbors a new *Wolbachia* strain named *w*Suzi, related to the strong CI-causing *w*Ri strain of *D. simulans*. Nothing is known behind this novel host-microbial integrative biology as yet. Thus, to get more insights into this symbiotic relationship, we have examined *Wolbachia* tissue tropism in adult *Dsuz* individuals sampled according to different age status. Quantitative Real Time PCR (qRT-PCR) was used to calculate density of *Wolbachia* in reproductive as well as in different somatic tissues of 1, 7, 14 and 21 days old *Dsuz* flies of both sexes. We detected significant differences in *Wolbachia* titre 1) between reproductive organs of males and females, 2) among different somatic tissues, 3) based on different age groups. Notably, in male gonads, *Wolbachia* density substantially decreases with age, suggesting the possible use of *Wolbachia*-rich young males to evaluate their CI-inducing capability. Results will be further discussed with those obtained from *D. simulans* and *D. melanogaster* harboring their respective *w*Ri and *w*Mel strains. Thereby this approach will help in evaluating the potential use of *w*Suzi to control *Dsuz* population in future.

A worm in bacterial clothing: proteomic analysis supports the hypothesis that *Wolbachia*-driven recruitment of neutrophil antimicrobial proteins protects *Onchocerca ochengi* against eosinophils

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Onchocerciasis is currently controlled by annual mass drug administration of a single anthelminthic, ivermectin. Although this drug is highly effective at reducing disease symptoms, it does not kill the long-lived adult filarial worms (*Onchocerca volvulus*), necessitating repeated treatments for >15 years. Both *O. volvulus* and the closely related bovine parasite *O. ochengi* have a mutualistic relationship with *Wolbachia*. Clearance of this symbiont using tetracycline leads to killing of adult *Onchocerca* spp. approximately one year post-treatment. However, the precise mechanism of action remains unclear. In this study, we treated *O. ochengi*-infected cattle with a short, ineffective oxytetracycline regimen or prolonged, adulticidal therapy. Female worms were removed from nodules in bovine skin at three time-points (0, 12 and 36 weeks post-treatment), and protein extracts were subjected to label-free, quantitative proteomics on an Orbitrap Velos mass spectrometer. Approximately 1,500 proteins were quantifiable per sample, with 30% derived from the worm, 70% from the bovine host, and <1% from *Wolbachia*. Around 100 proteins were differentially regulated, of which the vast majority were host-derived, although *Wolbachia* protein levels were significantly reduced in the prolonged treatment group as expected. Conversely, there was little impact on the expression of worm proteins, except for a glutathione S-transferase and small number of intracellular signalling proteins. The largest group of downregulated bovine proteins were neutrophil-derived antimicrobial proteins, particularly cathelicidins, azurocidin and cathepsin G. In parallel, a bovine homologue of eosinophil major basic protein was upregulated. These data support the hypothesis that *Wolbachia* induces an ineffective neutrophilic response that is disrupted by antibiotic therapy, ultimately leading to immune clearance of the worms by eosinophils.

A•WOL macrofilaricidal drug discovery and development – optimization of anti-*Wolbachia* efficacy

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There is an urgent need to develop a novel treatment for filariasis, and targeting *Wolbachia* provides safe macrofilaricidal activity with superior therapeutic outcomes compared to standard anti-filarial treatments. The Anti-*Wolbachia* (A•WOL) Consortium has developed both in vitro and in vivo assays, to screen chemical libraries for anti-*Wolbachia* activity. The outputs from the A•WOL program are now being pursued as part of A•WOL II Macrofilaricide Drug Discovery & Development programs. Screening of >10,000 compounds from the BioFocus library and chemoinformatic analysis have generated six independent lead series chemotypes with the potential to enter a medicinal chemistry "hit-to-lead" and lead optimization program. A•WOL Drug Discovery is now progressing these lead series through a rigorous lead optimisation and candidate selection process, using iterative cycles of medicinal chemistry and biological testing in order to deliver at least one novel pre-clinical candidate and a chemically distinct back-up, aligned with our target product profiles for an anti-*Wolbachia* macrofilaricide. In addition, ongoing screening of large diversity-based libraries (150 - 500 k compounds) aims to provide additional, chemically diverse hits, with one-order improvement in absolute potency or significant shortening of treatment time, in order to expand the structural diversity of anti-*Wolbachia* chemotypes. A•WOL Drug Development is optimising regimens of anti-*Wolbachia* monotherapy and combination treatment of registered anti-*Wolbachia* and anti-filarial drugs *in vivo* using an adult *Brugia malayi* mouse model. This efficacy testing is driven by a rational PK/PD modelling approach which supports dosage regimens, in order to identify the best treatment regimens to test in field trials.

Functional analysis of the host immune response in the symbiotic association between the pill-bug *Armadillidium vulgare* and the feminising *Wolbachia*

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Today, there is a wide consensus on the essential role of microbial associations to eukaryote evolution. In most cases, the relationship between host and symbiont is so close that the microorganisms cannot be cultured, making them difficult to study. However, high-throughput sequencing has offered new opportunities for symbiosis research.

Due to their widespread association with the *Wolbachia* endosymbionts, terrestrial isopods represent a model system to understand intimate symbioses. *Wolbachia* are vertically transmitted facultative bacteria acting as reproductive parasites in isopods, inducing the feminisation of genetic males in the pill bug *Armadillidium vulgare*. Among the three feminising *Wolbachia* identified in this host, two strains (*w*VulC and *w*VulM) vary in their prevalence and extended phenotypes. *w*VulC, the most prevalent strain exhibiting the strongest feminising effect, is also the most virulent strain inducing various fitness costs.

To decipher the conflicting associations between *w*VulC, *w*VulM and *A. vulgare*, RNA-seq experiments were conducted on cDNA libraries prepared from ovaries and from whole animals challenged by pathogenic intracellular bacteria according to their *Wolbachia* infection status. Reference transcriptome was constructed by *de novo* assembly and annotated. Immune genes were identified and compared to the immune repertoire of *Daphnia pulex*, the only one crustacean genome published today. Identification of differentially expressed immune genes (DE) was then carried out using the R package DESeq. Interestingly the highest number of DE genes was recorded in the animals infected by the less virulent strain *w*VulM. In most treatments, these DE genes could be assigned to GO categories that are underrepresented when *Wolbachia* are on board. This study is part of the widest program ImmunSymbArt granted by the French National Research Agency which aims to determine the symbiotic syndrome in four arthropod systems.

A bug may hide another: cryptic *Wolbachia* in unfeminized lineages of Armadillidium vulgare

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Among the *Wolbachia*-host symbioses a growing number of unexpected low titer infections has been described in bark beetles, *Drosophila paulistorum* complex of species, aphids, *D. simulans*, tse-tse fly and cherry fly. For 30 years our laboratory has bred distinct lineages of the terrestrial isopod *Armadillidium vulgare*, steadily infected or uninfected with *Wolbachia*. However, using Fluorescence In Situ Hybridization (FISH) and nested PCR, we showed that females from laboratory lineages regarded as uninfected were actually infected with cryptic *Wolbachia*. While *Wolbachia* have a feminizing effect in *A. vulgare*, the crypto-infected lineages have an equilibrated sex ratio. Moreover, *Wolbachia* remain undetected with PCR and TEM, the former standard methods to discriminate between infected and uninfected lineages.

Even more surprisingly, we also discovered a serendipitous crypto-*Wolbachia* in males of infected lineages, whereas they are considered as the individuals who escaped from the infection. In laboratory lineages both infected with *Wolbachia* and crypto-infected, males presented an opposite pattern of infection compared to crypto-infected females, with a huge infection in gonads and a low infection in the nerve chord. Additionally, we put in relation *Wolbachia* and crypto-*Wolbachia* localization patterns with the host phenotype, under the hypothesis that *Wolbachia* presence in some specific cells of the nerve chord is required to feminize woodlice.

A new molecular sexing technique for butterflies – does *Wolbachia* really feminise genetic males in *Eurema*?

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The endosymbiont *Wolbachia* can bias sex ratios and modify host sex determination through cytoplasmic incompatibility, parthenogenesis, male-killing or feminisation. Feminisation is the least reported phenotype, mostly detected in terrestrial crustaceans. It also occurs in three insect species, including two closely related butterfly species in the genus *Eurema*. Feminisation cause genetic males to develop into phenotypic and functional females. So far the main evidence of feminised male butterflies has been the absence of the W chromatin body that can be detected in true females. However, its detection is not reliable for all Lepidoptera and a molecular method based on sex chromosomal markers may be better. We developed a quantitative PCR (qPCR) assay, comparing relative gene dose ratios of Z-chromosomal genes with an autosomal gene, to differentiate directly between true genotypic females and *Wolbachia* feminised males. This test correctly genotyped the sex of butterflies that lacked the feminising *Wolbachia* strain *w*Fem. However, all *w*Fem-infected females that were negative in the W chromatin body assay (thus considered feminised males) also had female gene dose ratios. These results conflict with the current model of feminisation of males in *Eurema* and suggest re-assessment of the role of *Wolbachia* as feminising agent in this host.

Wolbachia-induced paternal defect in *Drosophila* is likely by interaction with the juvenile hormone pathway

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Wolbachia are endosymbionts that infect many insect species. They can manipulate the host's reproduction to increase their own maternal transmission. Cytoplasmic incompatibility (CI) is one such manipulation, which is expressed as embryonic lethality when *Wolbachia*-infected males mate with uninfected females. However, matings between males and females carrying the same *Wolbachia* strain result in viable progeny. The molecular mechanisms of CI are currently not clear. We have previously reported that the gene Juvenile hormone-inducible protein 26 (JhI-26) exhibited the highest upregulation in the 3rd instar larval testes of *Drosophila melanogaster* when infected by *Wolbachia*. This is reminiscent of an interaction between *Wolbachia* and juvenile hormone (JH) pathway in flies. Here we first found that the expressions of Jhamt and Met, which play key roles in JH pathway, were significantly increased in the presence of *Wolbachia*, suggesting an interaction of *Wolbachia* with the JH signaling pathway. Then we found that overexpression of JhI-26 in *Wolbachia*-free transgenic male flies caused significantly decrease in hatch rate. Surprisingly, *Wolbachia*-infected females could rescue the egg hatch. Overexpression of JhI-26 caused upregulation of the male accessory gland protein (Acp) gene CG10433, but not vice versa, suggesting that JhI-26 may function at the upstream of CG10433. Likewise, overexpression of CG10433 also led to paternal-effect lethality. Both JhI-26 and CG10433 overexpressing males resulted in nuclear division defects in the early embryos. Finally, *Wolbachia*-infected males decreased the propensity of the mated females to remating, a phenotype also caused by both JhI-26 and CG10433 overexpressing males. Our results provide a working hypothesis whereby *Wolbachia* induce paternal defects in *Drosophila* probably by interaction with the JH pathway via JH response genes JhI-26 and CG10433.

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Symbiotic bacteria affect cuticular hydrocarbon profiles in tsetse flies (*Glossina m. morsitans*)

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Microbial symbionts play important roles for the ecology and evolution of many insects, with their most well-known effects being nutritional supplementation, reproductive manipulation, and defense against antagonists. However, recent studies indicated that symbionts can also alter the chemical profile of the host and thereby affect mate choice decisions, which can ultimately result in reproductive isolation and speciation. Here, we investigated the effect of bacterial symbionts on the host's cuticular hydrocarbon profiles in the multipartite symbiosis between tsetse flies (*Glossina morsitans morsitans*) and their associated microbial symbionts, i.e. *Wigglesworthia*, *Sodalis*, and *Wolbachia*. We manipulated symbiont infection status by treatment with ampicillin (reducing *Wigglesworthia* and to a lesser extent *Sodalis* titers) and tetracycline (reducing titers of all symbionts, i.e. *Wigglesworthia*, *Sodalis* and *Wolbachia*), respectively, and subsequently analyzed CHC profiles of male and female offspring ten days after eclosion. The results revealed that ampicillin treatment did not affect CHC amount or composition in female offspring, but changed the CHC profile of male offspring. Tetracycline treatment, on the other hand, strongly affected CHC composition of both female and male offspring, and it significantly reduced the overall amount of CHCs in males. Furthermore, the relative amount of the females' contact sex pheromone 15,19,23- trimethyl-heptatriacontane decreased upon tetracycline treatment. These results provide evidence for an influence of *Wolbachia* and possibly *Sodalis*, as well as – to a lesser extent – *Wigglesworthia* on the amount and composition of CHC profiles in *G. m. morsitans*, which may have important implications for reproductive success and mate choice in tsetse flies.

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Double trouble: multiple endosymbiont infection and multiple manipulations in a linyphiid spider

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Spiders are host to a plethora of heritable endosymbiotic bacteria, but symbiont function within spider hosts has rarely been determined. We screened an assemblage of 60 linyphiid spiders in the genus *Mermessus*, collected from a single locale, and found 12 different endosymbiotic strains in the genera *Wolbachia*, *Rickettsia*, and *Cardinium*. In an additional screen of a single species, *M. fradeorum*, we detected one *Rickettsia* and two *Wolbachia* strains in multiple combinations. We established laboratory populations of two *M. fradeorum* matrilines: one doubly infected with *Rickettsia* and *Wolbachia* and one singly infected with the other *Wolbachia* strain. The *Rickettsia*-*Wolbachia* infected mothers produced extremely female-biased offspring. Antibiotic treatment successfully eliminated both endosymbionts and restored the sex ratio to the expected 1:1 in subsequent generations. Fitness assays and preliminary chromosomal examination suggest a feminizing mechanism. The other *Wolbachia* strain induced cytoplasmic incompatibility. In a two-way factorial mating assay between *Wolbachia* infected and cured spiders, we found that cured females mated with infected males produced 73% fewer offspring than all other pairings. Interestingly, in a separate assay we also found that the *Rickettsia*-*Wolbachia* females exhibited CI when mated with males with this alternative *Wolbachia*, but at a much lower level, suggesting partial rescue. These results show that populations of *M. fradeorum* are composed of individuals with contrasting reproductive manipulations, and that symbiont infection frequencies are likely quite dynamic, potentially influencing multiple aspects of spider evolution and ecology.

Modulation of microbiome of *Drosophila melanogaster* by *Wolbachia*

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The gut microbiome and *Wolbachia* affect several aspects of their host's biology, including development, fecundity, lifespan, immunity, metabolism, stem cell activity and even mate choice. The gut microbiome has also been recently implicated in altering disease transmitting capabilities of mosquitos. However, the potential interactions of *Wolbachia* with commensal bacteria are mostly unknown. Here we show that *Wolbachia* can alter the composition of the microbiome of the *Drosophila* host. We initially observed the changes in the pH of the food of the flies in a *Wolbachia*-dependent fashion. By 16S ribosomal sequencing, we identified differences in the commensal population of infected and non-infected flies as a potential source of the pH changes in the food. We further characterized the effect of *Wolbachia* on the microbiome composition dynamics of flies differentially infected with *Wolbachia* for several generations by metagenomic sequencing. We hypothesize that *Wolbachia* induced alterations in the immunity, oxidative stress, and metabolism could favor the selection of certain microbial populations by the host. We are testing these hypotheses by using *Drosophila* cell line and whole organism assays. This study raises the possibility that *Wolbachia*-host interactions could contribute to host phenotypes that were previously attributed to *Wolbachia* alone.

Are *Wolbachia* and microbiota linked to the genetic structure of invasive and endemic populations of *Aedes albopictus*?

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Above and beyond their ecological impact, invasive mosquitoes are a source of worldwide concern because of their utmost importance as vectors of a wide range of pathogens to humans and animals. Originated from South-East Asia, *Aedes albopictus* is considered as one of the most invasive species worldwide. As insect associated microbiota is recognized to play a significant role in host biology, we hypothesized that bacterial microbiota could be parts of the puzzle of understanding the driving forces of *Ae. albopictus* adaptation and subsequent invasiveness. In this context, we performed a comparative analysis of genetic (11 microsatellite markers and COI haplotype) and bacterial diversity (metagenomics on V5-V6 region of the 16S rRNA gene) of field-caught mosquito populations from Vietnam (area of origin) and France (recently colonized area). In whole mosquito body, bacterial microbiota was largely dominated by *Wolbachia pipientis* (64 to 89% of the whole bacterial content) with a conserved proportion of both *w*AlbA and *w*AlbB clades. However, analysis of gut microbiota revealed a higher diversity, in terms of species richness and evenness that was dominated by genera *Dysgonomonas* sp. and *Chryseobacterium* sp. Two important bacterial correlation networks were identified revealing possible interactions between these groups. The gut microbiota of French populations displayed a lower diversity in comparison to the Vietnamese ones as well as a lower host genetic diversity estimated through allelic richness. A new cryptic species living in sympatry with *Ae. albopictus* was identified in sylvatic Vietnamese site. Interestingly, this species did not harbor *Wolbachia* but showed a bacterial diversity similar to other ones. Our ecological genomics approach highlighted the presence of a mosquito core microbiota regardless of the geographical origin of the populations or host species and raises the question of its role in the mosquito biology and ecology.

Evolution of the interaction *Culex pipiens* / *Wolbachia*

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Almost all *Culex pipiens* mosquitoes are infected with *w*Pip strains and this interaction exhibits the greatest variation in cytoplasmic incompatibility (CI) crossing types reported in any insect. This diversity appears to be under the control of *Wolbachia* uniquely, since neither the nuclear background nor other bacterial symbionts have been reported to influence the outcome of the crossings.

After developing a specific MLST, we were able to distinguish five distinct *w*Pip genetic groups, which all share a monophyletic origin. An extensive worldwide screening of *w*Pip infections in 118 natural populations of the *C. pipiens* complex evidenced a clear geographic structuration. Each population was found mostly monomorphic for the *w*Pip infection group. However, no clear association between *Wolbachia* and the taxons of the *C. pipiens* complex was demonstrated, showing no implication of *Wolbachia* in their differentiation.

Additionally, we crossed many *C. pipiens* lines from different geographic origins, infected with strains from distinct *w*Pip groups. This large dataset showed that most crosses involving *w*Pip strains from the same genetic group were compatible. The only intragroup CI observed were unidirectional, between *C. pipiens* from different geographic origins. These results support the theoretical studies predicting that only compatible *Wolbachia* strains can stably coexist in panmictic host populations.

Finally, we showed that compatible *Wolbachia* strains from the same *w*Pip group, with identical MLST genotypes, could display distinct CI patterns when crossed with genetically distant lines. Their complete genomes were thus sequenced. A comparative genomic approach revealed surprisingly high genome dynamics at such a small evolutionary scale, essentially driven by repeated elements. These genomic analyses combined with our large dataset of CI relationships in C. pipiens provide a unique opportunity to finally identify the genes responsible for CI.

Functional genomics of beetle-microbe symbioses

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Beetles represent the most species-rich animal order Coleoptera, embracing over 400,000 described species in the world and accounting for almost 25% of all known animal forms. The insects are generally armored with sclerotized elytra and exoskeleton, comprising a considerable portion of the biodiversity in the terrestrial ecosystem, occupying important ecological niches as herbivores, xylovores, fungivores or carnivores, and often regarded as notorious agricultural, forestry or medical pests. Reflecting their diversity, microbial symbiotic partners of the beetles are also diverse: some are bacteria while others are fungi; some are stored in external pouches called mycangia, some are residing in the gut cavity, and others are harbored in specialized cells called bacteriocytes; some are cultured by the host beetles and utilized as food sources, some help digestion of woody materials, some provide protection against natural enemies, and others synthesize essential nutrients. Here we present our recent research progresses in the field of beetle-microbe symbiotic associations, with special emphasis on their genomic and functional aspects.

Phylogenomics and analysis of shared genes suggest a single origin of the main lineages of *Wolbachia* in nematodes

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While in arthropods *Wolbachia* is often associated with distortion of host reproduction, in filarial nematodes it appears to be essential for the survival of the host, and this symbiotic relationship is considered mutualistic. Phylogenetic analyses highlighted that most of the *Wolbachia* strains are symbionts of filarial nematodes belonging to the C and D supergroups. The origin of this mutualistic endosymbiosis is of interest for both basic and applied reasons. To understand how and when the symbiosis between *Wolbachia* and filarial nematodes originated, a robustly rooted tree is required. The genetic distance between *Wolbachia* and the nearest outgroups and the current limited number of *Wolbachia* genomes hamper this objective. To address these issues, we sequenced the genome of the D supergroup *Wolbachia* endosymbiont of *Litomosoides sigmodontis*, thus obtaining two genomes for each of the C and D filarial *Wolbachia* supergroups.

All the high quality *Wolbachia* genomes available in database were retrieved (from A, B, C and D supergroups). A gene database was generated selecting orthologues on the basis of homology and annotation, and removing duplicated or possibly recombined or saturated genes. For these selected genes, and for the respective amino acid sequences, the best evolutionary models were found and then used in Maximum Likelihood and Bayesian phylogenetic analyses, performed on both the nucleotide and amino acid partitioned concatenates. The *Wolbachia* phylogenies obtained were congruent, and presented a highly supported root between a (A + B) clade and a (C + D) clade. This analysis supports a scenario wherein the symbiosis between *Wolbachia* and filarial nematodes originated from a single transition event. This result leaves open many questions about the mechanism of this transition. Comparative genomic analyses are currently being performed, with the goal of trying to elucidate how this transition happened.

Link between genotype and phenotype in *Wolbachia*

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Symbiotic bacteria *Wolbachia* live within the cells of numerous insect species. Our work aims at understanding the interaction of the endosymbionts of *Drosophila melanogaster*, *w*Mel, with their hosts. Although natural *w*Mel variants are mutualistic, the laboratory variant *w*MelPop proliferates massively in the hosts, causing degeneration of tissues which culminates in insets early death. Sequencing and assembly of the genomes of *w*MelPop and very closely related *w*MelCS allowed us to identify genetic differences between the two variants. We demonstrate that the identified differences are responsible for the pathogenic phenotype of *w*MelPop. Therefore we provide the first link between genes and phenotypes in *Wolbachia*.

Impact of *Wolbachia* endosymbionts on the evolution of sex determination in the isopod *Armadillidium vulgare*

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In the isopod *Armadillidium vulgare*, genetic sex determination follows female heterogamety (ZZ males and ZW females). However, many *A. vulgare* populations harbor maternally-inherited *Wolbachia* bacterial endosymbionts which can convert genetic males into phenotypic females, leading to populations with female-biased sex ratios. The W sex chromosome has been lost in lines infected by *Wolbachia* and all individuals are ZZ genetic males. The female sex is determined by the inheritance of *Wolbachia* by the *A. vulgare* individual, thereby leading to a shift from genetic to cytoplasmic sex determination. Surprisingly, some *A. vulgare* lines exhibit female-biased sex ratios despite the lack of *Wolbachia*. In these lines, female individuals are ZZ genetic males carrying an unknown feminizing factor. To elucidate the genetic basis of female sex determination in these lines, we sequenced the genome of a female by Illumina technology. After *de novo* genome assembly, we identified a large piece of the *Wolbachia* genome transferred into the *A. vulgare* nuclear genome. The transferred genomic fragment shows non-Mendelian inheritance and co-segregates perfectly with the female sex in pedigrees, in agreement with observed biased sex ratios. These results suggest that sex determination in these *A. vulgare* lines is under the control of nuclear gene(s) of bacterial origin. Overall, our results indicate that *Wolbachia* bacteria can drive shifts in sex determination mechanisms in *A. vulgare*. More generally, they emphasize that bacterial endosymbionts can be powerful sources of evolutionary novelty for fundamental biological processes in eukaryotes, such as sex determination.

This research is funded by an ERC Starting Grant (EndoSexDet) to RC.

Toward the identification of feminizing genes of the bacterial endosymbiont *Wolbachia*

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Symbiotic interactions are a major driver of evolution. The symbiont genotype is able to alter the host phenotype, and the other way round: it is called "extended phenotype". In this respect, *Wolbachia* endosymbiosis is remarkable. This intracellular bacterium is a well-known reproductive parasite able to induce feminization of genetic males or cytoplasmic incompatibility in its terrestrial isopod crustacean hosts. Currently, no molecular genetic basis of these reproductive manipulations has been described. In order to identify genes involved in feminization, we used a comparative genomics approach of two closely related strains of *Wolbachia*: one inducing feminization of its host *Armadillidium vulgare* (*w*VulC) and the other one inducing cytoplasmic incompatibility in *Cylisticus convexus* (*w*Con). The effect induced by these strains is considered strain-specific as it is conserved when *Wolbachia* is cross-transfected to the other host. First, we sequenced the genome of *w*Con by 454 pyrosequencing. Genome assembly of *w*Con resulted in ~200 contigs for a total genome length of ~2 Mb and ~2,000 predicted genes. Next, a home-made bioinformatics pipeline comparing the genome of *w*Con with the previously sequenced genome of *w*VulC enabled us to determine 326 candidate genes for feminization. Then, we investigated expression patterns of these candidate genes during host sexual differentiation in order to pinpoint genes involved in the manipulation of host sex determination.

This work is funded by an ERC grant (EndoSexDet) to RC, which aims to identify the genetic factors implicated in the sex determination of *A. vulgare*, thereby contributing to evaluate the evolutionary impact of endosymbionts on sex determination of their hosts.

10 June 2014
Afternoon symposia

Wolbachia's Burgess Shale: ancient and modern integration in the genome of *Podisma pedestris* (Orthoptera)

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Evidence is accumulating for lateral gene-transfer of *Wolbachia* into invertebrate host genomes: Approximately one-third of sequenced invertebrate genomes contain *Wolbachia* gene insertions, which range in size from short segments to nearly the entire *Wolbachia* genome. NGS skimming (very low coverage sequencing) of the grasshopper *Podisma pedestris* suggests massive repeated introgression – and a well persevered record of ancient events. We use 454 amplicon sequencing of nuclear inserts to survey the same integrated sequence from several wild populations.

Because *Podisma* has been a model species for hybrid-zone studies, its postglacial colonisation of the French Alps has been worked out in detail. Making use of the known timings for the isolation of different populations, we have begun to draw inferences about the insertions, and the post-insertion fate of integrated sequences.

Identification of *Wolbachia*-responsive microRNAs in the two-spotted spider mite, *Tetranychus urticae*

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The two-spotted spider mite, *Tetranychus urticae* Koch, is infected with *Wolbachia*, which have the ability to manipulate host reproduction. *T. urticae*, like other organisms, produces several microRNAs (miRNAs), which are a type of small RNA (sRNA) that have the ability to regulate genes at the post-transcriptional level. To understand the role of miRNAs in the response of *T. urticae* to *Wolbachia* infection, we constructed sRNA libraries of infected and uninfected *T. urticae* for both sexes (four libraries). Illumina sequencing led to the identification of 83 known miRNAs and 112 novel miRNAs in the four libraries. We identified 91 miRNAs that were differentially expressed between infected and uninfected females and 20 miRNAs that were differentially expressed between infected and uninfected males. The four transcriptomes also provided information on protein-encoding genes that were differentially expressed in response to *Wolbachia* infection. A comparison of the miRNA and the mRNA data suggested that the miRNAs were regulating genes related to amino acid metabolism, sphingolipid metabolism, lysosome function and apoptosis. The differential expression of 14 miRNAs and one target gene were confirmed by qRT-PCR. These findings not only help to understand *Wolbachia*-mediated host miRNA variations but also provide molecular targets for further functional studies.

Sex-specific transcription of large proportion of genes in the only cryptic WO prophage genome in a fig wasp species

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Temperate bacteriophage WO is a model system for studying tripartite interactions among viruses, bacteria, and eukaryotes, especially investigations of the genomic stability of obligate intracellular bacteria. Few WO genomes exist because of the difficulty in isolating viral DNA from eukaryotic hosts, and most reports are by-products of *Wolbachia* sequencing; only one partial genome of a WO phage has been determined directly from isolated particles. We successfully determined the complete genome sequence of prophage WO (WOSol) in *Wolbachia* strain *w*Sol, which is the only *Wolbachia* strain infecting the fig wasp *Ceratosolen solmsi* (Hymenoptera: Chalcidoidea), by high-efficiency thermal asymmetric interlaced PCR. We further used real-time qPCR to prove WOSol that is a cryptic prophage, which are simply regarded as genetic remnants.

Only three open reading frames (ORFs) in cryptic WO prophages have previously been reported to be actively transcribed. We comprehensively examined the transcription of the only cryptic WOSol prophage in *Wolbachia* strain *w*Sol by conventional reverse transcription PCR and real-time qPCR. Our major findings were: i) a high percentage of ORFs are actively transcribed (59%, 17/29); ii) the expression of these ORFs is highly sex-specific, with a strong male bias (three in females, 15 in males); iii) an ank (ankyrin-domain-containing) gene actively transcribed in both wasp sexes is more highly expressed in males. Our results suggest that cryptic WO may play roles in *Wolbachia* biology, particularly through the males.

Spiroplasma infection induces male-specific DNA damage response in *Drosophila melanogaster*

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In *Drosophila*, two bacteria, *Wolbachia* and *Spiroplasma* are known as heritable symbionts. These bacteria are remarkable in their ability to manipulate host reproduction like cytoplasmic incompatibility and male-killing. Molecular mechanisms underlying these symbiont-induced reproductive phenotypes are of great interest but remain poorly understood. Interaction between *Drosophila melanogaster* and its native *Spiroplasma* symbiont strain MSRO is one of the best model systems for exploring how the host's molecular and cellular pathways are involved in the symbiont-induced male-killing. Previous studies showed that *Spiroplasma* infection induces ectopic apoptosis and severe neural defects only in male individuals during embryogenesis. In this study, we performed RNA-seq analysis for further understanding the molecular mechanisms of these pathogenic phenotypes. By analyzing differently expressed genes, we identified a group of genes specifically up-regulated in *Spiroplasma*-infected male embryos. These genes include regulators involved in apoptotic pathway and DNA damage response pathway. In this presentation, we would like to show our detailed molecular genetic analyses on male-specific DNA damage, and discuss the mechanistic insight into how *Spiroplasma* selectively kills male embryos in *Drosophila*.

Wolbachia genome sequencing: phylogenomics, symbiosis-related pan-genome and eukaryote-like proteins involved in host-symbiont interactions

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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 developed a novel targeted-genome enrichment procedure that allows specific isolation of endosymbiotic DNA from host mitochondrial and nuclear DNA. We assayed the robustness of this method on phylogenetically distant *Wolbachia* strains demonstrating that this procedure can be used to enrich target DNA from unculturable microbes over large evolutionary distances.

The combination of this approach with Next Generation Sequencing technologies (Illumina HiSeq and MiSeq) allowed us to successfully sequence six new strains of *Wolbachia* that induce either host feminization or cytoplasmic incompatibility in terrestrial isopods. Comparative genomics were then performed using these newly sequenced genomes together with 16 publically available genomes to establish a pan-genome of *Wolbachia* and phylogenomics studies. We then investigated the composition of this pan-genome in order to look at the insight of the basis of *Wolbachia* symbiosis. A conversed motif recognition analysis using HMMER3 also allowed us to identify eukaryote-like proteins such as ankyrin repeats containing proteins that are known to be involved in host-symbiont interactions, including the virulence of the bacteria.

Abstracts of poster presentations

(in alphabetical order of the presenting author)

Quantitative analysis of proliferation and gene expression of bacteriophages infecting male-killing and non-male-killing spiroplasmas in *Drosophila*

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Spiroplasmas are endosymbiotic bacteria infecting a variety of plants and arthropods. These bacteria cause male-killing in *Drosophila* and are also known as SRO (sex ratio organisms). It was reported that SRO Spiroplasmas are infected with short-tailed bacteriophages, but few studies have been done on the SRO phages, mainly because the SRO spiroplasmas are difficult to culture. In this study, we determined the whole genome sequences of SRO phages infecting *Spiroplasma* strains MSRO (SRO from *D. melanogaster*), NSRO (SRO from *D. nebulosa*) and NSRO-A (non-male-killing variant of NSRO), and then investigated the density dynamics of SRO phages and the expression of phage genes. In general, density dynamics of the three SRO phages in terms of the copy numbers of phage ORFs per host EF1-alpha copy were similar except for a short period immediately after the emergence. However, the density dynamics of three ORFs on NSRO-A phage showed different patterns from the other phages. On the other hand, density dynamics of the SRO phages in terms of phage ORF copies per spiroplasma dnaA copy were different among the *Spiroplasma* strains. Upon adult emergence, densities of all the three SRO phages were similar at 10 - 20 copies per *Spiroplasma*. After that, the densities of MSRO phage were almost constant throughout the adult life. In contrast, the densities of NSRO and NSRO-A phages increased around tenfold during the first week and then kept the same level. However, the densities of the three ORFs mentioned above did not increase so much in NSRO-A infected files. Interestingly, the expression levels of a gene encoded by one of the tree ORFs were about ten times higher in adult flies infected with male-killing MSRO and NSRO than in those infected with non-male-killing NSRO-A. These results represent the confusing and complex nature of NSRO-A phage, which is different from the other two phages infecting male-killing spiroplasmas and may related with the loss of pathogenicity.

Neutralization of a redundant gene involved in homologous recombination caught in the act in *Wolbachia* endosymbionts

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As obligate intracellular bacteria, *Wolbachia* endosymbionts exhibit reduced genomes. This is caused by the process of genomic reduction, occurring through large genomic deletions and the accumulation of mutations. The latter process of genomic reduction involves successive steps including gene neutralization, pseudogenization and gradual erosion until complete loss. While many examples of pseudogenes at various levels of degradation have been reported in endosymbiont genomes, neutralization cases are scarce because of the transient nature of the process. Gene neutralization may occur due to relaxation of selection in non-essential genes, e.g. those involved in redundant functions. In endosymbiont genomes, the homologous recombination (HR) pathway is commonly depleted, whereas it seems intact in some (but not all) *Wolbachia* strains. The HR pathway is implicated in DNA repair and generates genome plasticity. In order to evaluate the evolutionary importance of the HR pathway for *Wolbachia* genomes, we analysed 13 major HR genes. They all have been globally under strong purifying selection during the evolution of *Wolbachia* strains hosted by arthropods. However, we uncovered the recent neutralization of the RuvA gene in a subset of *Wolbachia* strains, which may be related to an ancestral, clade-specific amino acid change that impaired DNA binding activity. Strikingly, RuvA is part of the RuvABC complex involved in branch migration and resolution of Holliday junctions, a function which may be fulfilled by the analagous RecG helicase. While RuvA is experiencing neutralization, RecG is under strong purifying selection. Thus, our high phylogenetic resolution enabled us to characterize a rare example of targeted neutralization of a gene involved in a redundant function in an endosymbiont genome.

Exploring the influence of *Wolbachia* on *Chorthippus parallelus* mating in a Pyrenean hybrid zone

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The Pyrenean hybrid zone between *Chorthippus parallelus parallelus* and the Iberian endemite *Chorthippus parallelus erythropus* (Orthoptera) was originally described as a "secondary contact zone", maintained by the balance between dispersion and the presumed reduced fitness of the natural hybrids. This is supported by the close adherence to Haldane's rule (whereby heterogametic males are sterile) of F1 laboratory hybrids of pure individuals of the two subspecies, their mating behaviour and the homogamy detected in studies of females' sperm preference.

However, recent biogeographical, molecular, cytogenetic and other findings suggest a need to complement this view with data on the influence of *Wolbachia* on various aspects of the biology of these organisms and their hybrid zone. These include cytoplasmic uni- and bidirectional incompatibility, different patterns of infection by the bacterial endosymbiont in distinct populations and areas inside and outside the hybrid zone, and the induction of a higher proportion of abnormal spermatids in natural hybrids.

In a further experiment to evaluate the role of *Wolbachia* in the hybrid zone, we have analysed the infection status of both members of copulating pairs of hybrid individuals collected in the field. Here we discuss the results of a preliminary survey of 79 pairs of hybrids using PCR with primers for *Wolbachia*'s specific wsp gene, and analysed with the Jmating, a program that examines mating frequency data to address sexual selection and sexual isolation.

Incidence of *Wolbachia* sp., *Spiroplasma* sp. and other bacterial endosymbionts in certain parthenogenetic and non-parthenogenetic species of phasmids (Phasmatodea)

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Phasmatodea reproductive biology is partially determined by poorly understood ecological and genetic mechanisms. This order includes standard sexual species, but also many others that display distinct types of parthenogenesis (tychoparthenogenesis, automixis, apomixis, etc.), or both systems facultatively. In a preliminary survey we have analysed *Wolbachia* and *Spiroplasma* infection in close to 250 individuals from around 25 species of stick-insects by bacterial 16S rRNA gene amplification. Our objective was to determine whether some of the bacterial endosymbionts involved in distinct reproductive alterations in other arthropods, including parthenogenesis and male killing, are present in phasmids.

Our results demonstrate that there was no *Wolbachia* infection in any of the phasmid species analysed, but confirm the presence of *Spiroplasma* in some sexual, mixed and asexual species. Phylogenetic analysis identifies these bacterial strains as belonging to the Ixodetis clade. Other bacteria genera were also detected. The possible role of these bacteria in Phasmatodea biology is discussed.

The diversity of bacterial endosymbionts within bees (Anthophila)

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Several evolutionary phenomena in arthropods were shown to be due to the presence of inherited symbionts, e.g., sex-ratio-distortion, cytoplasmic incompatibility, conferring resistance to pathogens, nutritional mutualism and others. All these phenomena may be induced by *Wolbachia*, an alpha-proteobacterium that is estimated to occur in about two thirds of all terrestrial arthropods. Other endosymbionts are comparatively understudied and known only from a few model systems. Although *Wolbachia* is by far the most frequently found endosymbiont in arthropods, detailed screenings for endosymbionts of lower-ranked taxa are lacking. Furthermore, potential interactions of various endosymbionts within one species are not well understood.

In the present study, we screened 307 individuals belonging to 170 bee (Anthophila) species for the presence of the endosymbionts *Wolbachia*, *Rickettsia*, *Spiroplasma*, *Cardinium* and *Arsenophus*. Because *Wolbachia* is present in about 66% of all bees, we tested if species carrying this infection are more or less likely to carry other endosymbionts. Interestingly, we found very different *Wolbachia* infection frequencies across bee families. Furthermore, we find that ecological and phylogenetic host background determines endosymbiont communities in bees.

Detection of *Wolbachia* driven reduction of mitochondrial diversity using RADseq

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Maternally inherited intracellular symbionts can spread within populations and accross closely related species following hybridization events. Because of co-transmission with other cytoplasmic elements, this spread can induce indirect selection on mitochondrial genomes, driving selective sweeps on mitochondria or favoring mitochondrial introgression. Using a large sample of Arthropods from French Polynesia, we aim at estimating the frequency of this phenomenon and the contribution of *Wolbachia*. Selection on mitochondrial genomes can be detected through discordances between nuclear and mitochondrial coalescence times. Mitochondrial data have previously been obtained; we envisage to use RADseq to estimate nuclear diversity within and between specimens.

Estimation of genetic diversity using RADseq data can be hindered by several problems. First, polymorphism on restriction sites may lead to a biased sampling of markers toward shorter coalescent times and to an over-estimation of monomorphic loci in diploïd specimens, both processes inducing an underestimation of genetic diversity. Second, the incomplete sampling of alleles in diploid specimens, when the coverage is limiting, may also induce an over-estimation of monomorphic loci and accentuate the under-estimation of polymorphism.

Here we present an ABC approach to overcome these biases by estimating jointly population parameters and parameters associated with the RADseq experiment. Large sets of sequences are simulated under different population models and RADseq data are derived from these sequences with different coverage parameters. Summary statistics are calculated on each simulated data set corresponding to each parameter set. Then, parameters that would best explain the observed summary statistics are estimated. This ABC approach should allow us to estimate nuclear coalescence time between specimens and thus to detect significantly reduced mitochondrial coalescence times, potentially driven by *Wolbachia*.

Wolbachia shaping symbiotic communities? Insights from the terrestrial isopod microbiome

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Terrestrial isopods represent an excellent model system for multipartite animal-bacteria symbioses due to their well-characterized association with the endosymbiotic reproductive parasite *Wolbachia*. To date, three different feminizing *Wolbachia* strains have been identified in *Armadillidium vulgare*, presumably representing different co-evolutionary histories with their common host. However, the microbiome of terrestrial isopods has never been analysed on a large scale and the role of *Wolbachia* within the bacterial community remains unknown. In order to fill this gap, quantitative PCR and 16S rRNA gene amplicon pyrosequencing were combined to characterize the microbiota of *A. vulgare* on multiple levels: (i) In field vs. lab populations, (ii) in different host tissues, and (iii) depending on *Wolbachia* infection status, i.e. presence/absence of *Wolbachia* as well as infection with different *Wolbachia* strains. This integrative approach allowed us to identify the major factors shaping the microbiota associated with *A. vulgare*. *Wolbachia* infection was an important factor influencing bacterial community structure. Furthermore, *Wolbachia* represented the predominant member of the bacterial community in infected individuals. These findings indicate that *Wolbachia* plays an important role within the terrestrial isopod microbiome. However, *Wolbachia* was not the only major player: *Candidatus* Hepatoplasma crinochetorum, a facultative symbiont previously reported from the midgut caeca, was for the first time observed to be highly abundant in all tested host tissues. The potential interactions of *Wolbachia* and *Ca.* H. crinochetorum constitute an interesting example for symbiont-symbiont relationships between two highly abundant members of a diverse bacterial community.

Stable *Wolbachia* infection rate in the parasitoid wasp *Hyposoter horticola*

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Despite a recent growing interest for the study of *Wolbachia* and its consequences on insect populations, our understanding of its persistence in structured and complex natural systems remains poor. We identified a new *Wolbachia* infection, strain called *w*Hho, present at a low and stable rate in *Hyposoter horticola* the parasitoid wasp of the Glanville fritillary butterfly, *Melitaea cinxia*. Within the last two decades, the Glanville fritillary butterfly has become a model organism for the study of metapopulation. On the Åland archipelago between Finland and Sweden, the butterfly and its parasitoid wasp follow a similar population dynamic, with yearly local population turnover. After describing the new bacterial strain present in the wasp species, we investigated its effects on several life-history traits of its host in order to try and explain its stable persistence at a low prevalence in the Åland metapopulation system.

Evolutionary origin of insect-*Wolbachia* nutritional mutualism

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Obligate insect-bacterium nutritional mutualism is among the most sophisticated forms of symbiosis wherein the host and the symbiont are integrated into a coherent biological entity and unable to survive without the partnership. Originally, however, such obligate symbiotic bacteria must have been derived from free-living bacteria. How highly specialized obligate mutualisms have arisen from less specialized associations is of interest. Here we address this evolutionary issue by focusing on an exceptional insect-*Wolbachia* nutritional mutualism. While *Wolbachia* endosymbionts are ubiquitously found in diverse insects and generally regarded as facultative / parasitic associates for their insect hosts, a *Wolbachia* strain associated with the bedbug *Cimex lectularius*, designated as *w*Cle, was shown to be essential for host's growth and reproduction via provisioning of B vitamins. We determined the 1,250,060 bp genome of *w*Cle, which was generally similar to the genomes of insect-associated facultative *Wolbachia* strains, except for the presence of an operon encoding the complete biotin synthetic pathway that was acquired via lateral gene transfer presumably from a co-infecting endosymbiotic *Cardinium* or *Rickettsia*. Nutritional and physiological experiments, in which *w*Cle-infected and *w*Cle-cured bedbugs of the same genetic background were fed on B vitamins-manipulated blood meals via an artificial feeding system, demonstrated that *w*Cle certainly synthesizes biotin and the *w*Cle-provisioned biotin significantly contributes to the host fitness. These findings strongly suggest that acquisition of a single gene cluster consisting of biotin synthesis genes underlies the bedbug-*Wolbachia* nutritional mutualism, uncovering an evolutionary transition from facultative symbiosis to obligate mutualism facilitated by lateral gene transfer in an endosymbiont lineage.

Cytological mechanism of *Cardinium*-induced cytoplasmic incompatibility in *Encarsia pergandiella* (Hymenoptera: Aphelinidae): preliminary results

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Cytoplasmic incompatibility (CI) between closely related populations occurs frequently in arthropods as a consequence of infections by *Wolbachia* and *Cardinium* endosymbionts. CI causes sterility in matings between infected males and uninfected or differently-infected females. CI-bacteria infect a large number of arthropods and strongly influence the ecology and evolution of their hosts. CI may also affect the invasiveness of alien genotypes and the effectiveness of biocontrol agents. Cytological analyses of *Wolbachia* CI crosses in *Drosophila* and *Nasonia* have provided insights into the mechanism of CI. These studies have shown that incompatible embryos die in the first mitotic division of the zygote, and implicate paternal chromatin remodeling and asynchrony of male and female pronuclei entering the first mitotic division. The delay leads to abnormal division or the exclusion of male chromatids. So far, nothing is known about the sequence of events in the embryogenesis of incompatible CI *Cardinium* crosses.

We conducted a cytological analysis of reproductive incompatibility caused by CI *Cardinium* in *Encarsia pergandiella*. We set up incompatible and control matings, dissected eggs from whitefly hosts, and fixed and stained eggs with DAPI during early embryogenesis. DAPI-stained eggs were imaged with a confocal microscope. We also attempted live-staining with the nucleic acid stain Syto-11 to allow for visualization of embryogenesis in real time. This technique involves dissection in buffer followed by a short incubation before imaging with a deconvolution microscope.

We observed the formation of chromatin bridges after the first mitotic division of the zygote, which is a common feature of *Wolbachia*-induced CI. Our preliminary results suggest a common mechanism of CI induced by the two bacterial lineages. Identifying parallels between CI caused by *Wolbachia* and *Cardinium* will shed light on fundamental mechanisms that underlie this reproductive incompatibility.

Bacterial endosymbionts may induce female-biased sex ratio in the invasive mitochondrial type Q2 of *Bemisia tabaci*

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The whitefly *Bemisia tabaci* (Gennadius) is a complex of cryptic species some of which, namely the Mediterranean (MED) and the Middle East-Asia Minor 1 (MEAM1), are highly invasive, and injurious crop pests worldwide, and able to displace local genotypes. Invasiveness of *B. tabaci* may correlate with the phenotype of inherited bacterial endosymbionts. In a greenhouse crop area of South Italy Q1 and Q2 mitochondrial types of MED, currently the only species found in this area, coexist in the field. Here, the introduction of Q2 (eastern Mediterranean origin) has been very recent and in few years (less than five years) Q2 largely outnumbered Q1 (the indigenous mitotype of western Mediterranean origin). Now 70% of individuals in the field are Q2. Each mitochondrial type is characterized by a specific endosymbiont composition. *Hamiltonella* and *Rickettsia* are at near fixation in Q1 and Q2 respectively; *Arsenophonus*, *Cardinium* and *Wolbachia* infect both types although at different frequencies. Contrarily to Q1, with an even sex ratio, Q2 shows a significant female-biased sex ratio. Although different agro-ecological conditions may have favored the invasion of Q2 in South Italy, the female-biased sex ratio may strongly affect the invasion biology of this mitotype. Endosymbionts (i.e. *Rickettsia*) may have a role in Q2 invasiveness acting as sex-ratio manipulators.

Can *Wolbachia* destabilize the population dynamics of their hosts? – A mathematical analysis

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Understanding the population dynamics of an arthropod species is key to design biological programs that aim to either conserving an endangered species (e.g., butterflies like the Oregon silverspot) or to controlling pests and disease vectors (e.g., mosquitoes of the genus *Aedes*). An important aspect is the stability of the system. For example, destabilized dynamics are typically associated with increased booms and busts of the population size, which could lead (1) to an increased extinction risk of endangered populations, or (2) to unpredictable and severe outbreaks of insect pests. *Wolbachia* is well known for naturally infecting new host species, and also some biological control programs involve artificial infections with *Wolbachia*. It is therefore an important question to what extend *Wolbachia* influences the population dynamics of their host. Here, we extended classical models for insect population dynamics (e.g., Dye model, Ricker model) by incorporating process noise and *Wolbachia*. Each model parameter was tested for its effect on the non-linearity of the dynamics. We found that the non-linear signature is most sensitive to parameters of density dependent regulation. Interestingly, larger variance of the parameters is more likely to destabilize the dynamics, but not their absolute values. These findings suggest that *Wolbachia*-strains, which affect intra-host dynamics, are most likely to destabilize host population dynamics. The results are discussed with respect to the Asian tiger mosquito *Aedes albopictus*, for which *Wolbachia* was shown to affect larval competition.

Habitat-specific fitness dynamics among *Wolbachia* clades in *Drosophila melanogaster*

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A natural *Drosophila melanogaster* population infected with multiple *Wolbachia* clades was allowed to evolve in two different laboratory environments (hot and cold). Pronounced habitat-specific evolutionary dynamics were observed for the different *Wolbachia* clades: the infection spread to fixation in both temperature regimes. One *Wolbachia* clade increased more than 50% in frequency within 15 generations of evolution in the cold environment (10°C / 20°C daily cycles), while the other two clades decreased in frequency. In contrast, the composition of the *Wolbachia* population remained stable over 37 generations in the hot environment (18°C / 28°C daily cycles). This population of *D. melanogaster* has continued evolving in the two environments and I will report results from more advanced generations. The causative factor(s) behind these habitat-specific patterns remain to be elucidated, however. *Wolbachia* is known to increase host fitness through protection against viral infection, improved metabolic processes or by increasing the fertility of the host. The observed patterns may also be a result of cytoplasmic incompatibility (CI) or fecundity differences among the different clades. To test this hypothesis, crosses between individuals carrying different *Wolbachia* clades are being performed among flies from both environments, allowing the direct quantification of CI. I will discuss the CI values obtained from the various crosses carried out.

Host phenotype-associated *Wolbachia* infection polymorphism in *Vollenhovia emeryi* (Hymenoptera: Myrmicinae)

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The ant species, *Vollenhovia emeryi* Wheeler (Hymenoptera: Myrmicinae), is polymorphic in wing morphology of the queen caste; long-winged queen and short-winged queen. Recent studies show that they are clonally reproduced regardless of the wing morphology. Consequently, this study was conducted to investigate the *Wolbachia* infection status of the ant that might potentially be involved in its bizarre reproduction in the ant.

Either individuals or colonies of *V. emeryi* were collected during 2010 - 2013 from 80 locations, encompassing 68 locations in South Korea, 11 in Japan, and one in USA. All of the long-winged morphs were invariably infected with *Wolbachia*. On the other hand, all of the short-winged morphs were devoid of *Wolbachia* collected in South Korea. However, the Japanese short-winged morphs were geographically partially infected. Therefore we tentatively concluded that *Wolbachia* has no role in the host's clonal reproduction. Nevertheless, this may be the first case of the *Wolbachia* infection status linked to host phenotype. The phylogenetic analysis reveals that the short-winged is derived from the long-winged. This suggests the possibility of linkage between the short-wing trait and the evolution of resistance to *Wolbachia* infection in the short-winged.

High Content Imaging: more than a pretty picture

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The A•WOL consortium aims to find a novel macrofilaricidal drug to treat the debilitating diseases lymphatic filariasis and onchocerciasis, through targeting the *Wolbachia* bacteria that reside within these parasitic nematodes. Typically, screening chemical libraries directly against parasitic nematodes is cumbersome, low throughput and relies on animal reservoirs. By focusing on the endosymbiont, we have been able to utilise an insect cell-based screening approach that originally relied upon a quantitative polymerase chain reaction readout, but now employs a High Content Imaging readout running on the Perkin Elmer Operetta® platform. This assay uses texture analysis of cells stained with SYTO®11 (fluorescent DNA stain) as a direct measure of bacterial load and allows the consortium to screen up to 10× 384 well plates per day; a radical increase in throughput from the qPCR screen. Further to its use as a screening tool, the Operetta® is also being used for more fundamental biological investigations including experiments surrounding the infection dynamics within host cells as well as the development of a filarial nematode cell line as part of a Grand Challenges Explorations grant funded by the Bill and Melinda Gates Foundation. The nature of the Anti-*Wolbachia* screening approach will be presented in addition to preliminary data from other High Content Imaging investigations surrounding *Wolbachia* and host cells.

Wolbachia strain *w*AlbB confers both fitness cost and benefit to *Anopheles stephensi*

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Wolbachia is a maternally transmitted intracellular bacterium that infects up to 65% of insect species. However, it is not naturally present in *Anopheles* malaria vectors. This provides an opportunity to develop *Wolbachia*-based strategies for disease control either through population replacement to reduce vector capacity or through population suppression to reduce mosquito population. We previously generated *Anopheles stephensi* carrying a stable *w*AlbB *Wolbachia* infection, and demonstrated its ability to invade into the wild type laboratory populations and confer mosquitoes resistant to *Plasmodium*. The result of the undergoing fitness experiment revealed that infection of *w*AlbB caused a reduction in female fecundity and had a minor negative impact on male mating competiveness. We also observed that *w*AlbB increased the life span of both male and female mosquitoes when they were maintained solely on sugar meals; however, there was no impact on the life span of blood-fed females. In addition, *w*AlbB did not influence immature development and survivorship, and adult body sizes. Additional experiments were conducted to study the interactions of *w*AlbB causing low egg hatch with the blood sources, food nutrition and oxidative stress. We discuss these findings in possible deployment of *Wolbachia*-based strategies for malaria vector control.

Molecular mechanism of *Wolbachia*-induced feminization in Lepidoptera

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Sexual differentiation in the silkworm *Bombyx mori* is controlled by sex-specific splicing of doublesex (dsx). In *B. mori*, the sex-specific splicing of dsx is influenced by the insulin-like growth factor II mRNA-binding protein (Imp). *Wolbachia*-induced feminization is known in the butterfly *Eurema mandarina*. In *E. mandarina*, naturally-occurring double infection of distinct *Wolbachia* strains (*w*CI and *w*Fem) is associated with feminization, while naturally-occurring single infection (*w*CI) is not. Female-specific splicing of dsx manifected in feminized E. mandarina can partially be reverted to male-specific splicing by treating the larvae with tetracycline, suggesting that the target of *w*Fem is located in the upstream of dsx. When *w*CI and *w*Fem were transferred together into cell culture derived from male embryos of *B. mori*, both Imp and dsx showed female-like pattern. On the other hand, *w*CI alone did not affect the expression of both Imp and dsx. These results may suggest that the *w*Fem has a feminizing effect on *B. mori* cells. To gain more insight into the molecular mechanism of *Wolbachia*-induced feminization, transcriptome analysis (RNA-seq) was performed on uninfected cell lines derived from female *B. mori* and uninfected, single (*w*CI) infected and double (*w*CI and *w*Fem) infected cell lines derived from male *B. mori*. Nearly 46,000 mRNAs were surveyed for candidate genes involved in sex determination / differentiation, *Wolbachia*-induced feminization, and / or *Wolbachia* infection / endosymbiosis.

Evidence for horizontal *Wolbachia* transmission within *Eurema* butterflies

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Individuals of Japanese *Eurema hecabe* and *Eurema mandarina* (Lepidoptera: Pieridae) carry either a single *Wolbachia* infection (*w*CI) or a double infection of *w*CI and *w*Fem. *w*CI causes cytoplasmic incompatibility (CI), while *w*Fem has been reported to feminise genetic males into functional females. These two *Wolbachia* strains have distinct wsp and MLST gene sequences and both strains occur in both *Eurema* species. This is probably due to *Wolbachia* introgression through hybridisation, because infected individuals in the different host species share mitochondrial haplotypes. While *E. mandarina* is restricted to temperate regions of eastern Asia, *E. hecabe* is widely distributed in Africa, Asia and Australia. In Australia, it occurs in the tropical and subtropical coastal regions of Queensland and New South Wales, and the tropical top end of the Northern Territory. Besides *E. hecabe*, Australia is home to six other *Eurema* species, which vary in their geographic ranges, life histories and host plant choice.

We sampled and screened populations of six Australian *Eurema* species (including *E. hecabe*) for *Wolbachia*. We found *Wolbachia* in five of the species species, although at different prevalences. The MLST profile was identical to the *w*CI strain in four of the species, and we found a new MLST strain in a fifth species. However, we did not find *w*Fem. We then COI-barcoded infected individuals and did not find any evidence of shared mitochondrial haplotypes between species, suggesting that the shared *Wolbachia* strain has not spread through hybridisation across Australian *Eurema* species. Clear differentiation at mitochondrial and nuclear loci of individuals from different species infected with the same *Wolbachia* strain also argues against *Wolbachia*-host co-divergence. We conclude that horizontal transmission by an as yet unknown route has played a key role in the evolution of *Wolbachia*-host associations in this group of butterflies.

Supergroup J: the less-known supergroup in filarial nematode

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Wolbachia are vertically transmitted intracellular bacteria which exhibit a broad host spectrum, including numerous families of arthropods and only one family of nematodes, the Onchocercidae filariae, including species that cause human filarial diseases, e.g. lymphatic filariasis and onchocerciasis. The different *Wolbachia* lineages have been classified in supergroups which show an asymmetric distribution within the large host range: supergroup A, B, E, H, I and K are commonly found in arthropods; supergroups C, D and J are limited to filariae. Only the supergroup F encompasses arthropod and filarial hosts. The present study focused on the poorly studied supergroup J. This supergroup has been defined on only the lineage found in the filarial nematode *Dipetalonema gracile*. New samples of Onchocercidae species were analyzed using PCR and immunohistochemical staining to detect *Wolbachia* and describe its strains. Here, the supergroup J *Wolbachia* was newly identified in two filaria species isolated from capybaras (Caviidae), e.g. *Yatesia hydrochoerus* and *Cruorifilaria tuberocauda*, and in four Dipetalonema species from monkeys, all originated from South America. In the adult filariae, *Wolbachia* infect the female germline and the hypodermis, with a various density in the different *Dipetalonema* species. The phylogenetic analyses confirmed the monophyly of the clade composed of *Wolbachia* from *Dipetalonema*, *Yatesia* and *Cuorifilaria* genera. The supergroup J was positioned as a sister taxon of supergroup C. Finally, this study pinpoints a specific geographic area for the supergroup J.

Developing and characterizing *Aedes albopictus* with triple *Wolbachia* infections for disease control

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Aedes albopictus is a competent vector for at least 22 arboviruses, notably dengue and Chikungunya virus. The dramatic global expansion of *Ae. albopictus*, displacing *Ae. aegypti* in some areas, has led to increased public health concern that this mosquito would lead to serious outbreaks of arbovirus diseases. This is supported by the observation that *Ae. albopictus* is the primary cause of dengue epidemics in a number of areas including China. The ability of *Wolbachia* to both block virus transmission and spread into mosquito populations has led to an international effort to develop *Wolbachia*-based population replacement to eliminate dengue, with successful demonstrations of its potential for area-wide implementation. Different to *Ae. aegypti*, *Ae. albopictus* is naturally carrying two strains of *Wolbachia*, *w*AlbA and *w*AlbB. In order to utilize the unidirectional cytoplasmic incompatibility (CI) to spread of *Wolbachia* into the population, we introduced the third strain *w*Mel into *Ae. albopictus*, resulting in a stably infected HM line carrying triple *Wolbachia* infections. The HM line is able to maintain *w*Mel at a 100% maternal transmission rate. Cross experiments are conducted to determine the CI pattern between HM, the wild type, and tetracycline treated aposymbiotic lines. We also examined a potential competition among the three strains of *Wolbachia* by comparing their density in different tissues of HM and the wild type mosquito. We will discuss the above results in relation to the HM vector competence for dengue virus and its potential for use in *Wolbachia*-based vector control strategies.

A fast multiplex PCR test for three important insect endosymbionts

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The need to test for *Wolbachia* infections whenever mitochondrial diversity or sex bias in insect populations is assessed is broadly accepted today. In contrast, the contribution of other strictly endosymbiotic, sexual-parasitic proteobacteria to the current mitochondrial landscape of Hexapoda remains heavily understudied. This situation is, to a large part, caused by a lack of routine testing. Here, we present a simple one-tube test for the presence of *Wolbachia*, *Cardinium*, and *Spiroplasma*, the three most prominent bacteria causing sexual alterations in insects. The test is based on a multiplex PCR targeting the IS5 repeat region of *Wolbachia* and specific 16S regions of *Cardinium* and *Spiroplasma*, followed by a High Resolution Melting Analysis. The presence of any of the three endosymbionts will be indicated by a specific peak in the first derivative of the amplicon's melting curve. The test will provide results within two hours, without need of gel electrophoresis or any other post-PCR wetlab step. So far, we have assessed this test system with plasmids derived from host model organisms. A variety of field samples will be used in a next step to verify and fine-tune the test's diagnostic performance.

Investigation of the putative SAM transporter in the REIS Island of *w*Mel

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S-adenosylmethione (SAM) is essential for methylation in all cells. The accepted paradigm for the methylation cycle is that SAM is first produced by S-adenosylmethionine synthetase, which is encoded by metK. Subsequently, the cell utilizes an S-adenosylhomocysteinase, encoded by sahH, to detoxify the S-adenosylhomocysteine (SAH) that is produced after the transfer of the methyl group from SAM to the methylation substrate. However, some obligate intracellular bacteria have lost metK and sahH. These bacteria have replaced these functions with a SAM transporter that imports SAM as it exports the methylation inhibitor SAH.

The *w*Mel genome contains a genetic island that is bounded on both ends by WoMelB prophage sequences. This island encodes for ten ORFs including a putative SAM transporter (WD0621) which appear to have been laterally transferred from a *Rickettsia* Endosymbiont of *Ixodes scapularis* (REIS) like organism. Structurally, SAM transporters share several conserved motifs including an EamA domain, a RhaT domain, and ten transmembrane domains. *In silico* analysis suggests that WD0621 fulfills all these requirements as well as having strong homology to the SAM transporter of *Rickettsia prowazekii* strain Madrid E. (RP076). The *w*Mel genome also encodes a metK (WD0136), however no putative sahH has been identified.

Using a ΔmetK conditionally lethal strain of *E. coli* we analyze the function of WD0621 and determine whether the *w*Mel metK can complement this deletion. We also assess the levels of transcript in *w*Mel for WD0621 and metK.

We finally discuss the implications of SAM transport for an intracellular bacterium, and whether a *Wolbachia* that contains the REIS island has a selective advantage, due to the export of the methylation inhibitor SAH over *Wolbachia* strains that do not contain a SAM transporter.

Low-input transcriptomics of *Wolbachia* and their filarial nematode hosts

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Wolbachia is present in most human parasitic filarial nematodes, where it is an obligate endosymbiotic bacterium. In *Brugia malayi* (a filarial nematode responsible for human lymphatic filariasis, a debilitating disease affecting nearly 100 million people worldwide) depletion of *Wolbachia* through tetracycline antibiotic treatment leads to decreased fertility and eventual death in adult worms. A better understanding of how *Wolbachia* contributes to overall nematode fitness is necessary for the development of enhanced drug targeting of filarial nematodes and their *Wolbachia*. The advent of RNA-Seq has provided a method to investigate this complicated symbiosis. Numerous studies have examined the transcriptomics of filarial nematodes in response to *Wolbachia* depletion however; few have specifically examined changes in gene expression of the bacterial endosymbiont. The preparation of RNA-seq samples using the NEBNext® Ultra RNA Library Prep Kit for Illumina® (New England BioLabs, Inc.) has enabled the use of very low input amounts of total RNA extracted from filarial nematodes to examine gene expression profiles of filarial worms and *Wolbachia* simultaneously. Using this technology, we have been able to identify a number of *w*Bm (*Wolbachia* from *Brugia malayi*) genes that are differentially expressed in response to the critical metabolite, heme. As heme biosynthesis occurs in the symbiont but not in the nematode, these heme-responsive genes in both *Wolbachia* and *Brugia malayi* may be critical for the symbiotic relationship and thus a target for drug discovery.

Genetic basis of *Cardinium*-caused cytoplasmic incompatibility

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Endosymbionts that infect arthropods and cause cytoplasmic incompatibility (CI) manipulate the reproduction of their hosts through "modification" of sperm and "rescue" of affected sperm in symbiont-infected eggs. The recently published genome of *Cardinium hertigii* provided first perspectives on potential CI-candidate genes. In this planned project, i.) additional genomes of *Cardinium* strains from *Encarsia* spp. causing different phenotypes (parthenogenesis, CI and one asymptomatic), will be sequenced. ii.) genes involved in host interactions and CI will be identified, iii.) differences in gene expression of *Cardinium* in male and female wasps will be examined. For i.), genome comparisons of *Cardinium* strains causing different phenotypes will greatly aid in the identification of CI candidate genes. For ii.) and iii.), an RNA extraction protocol yielding sufficient amounts of *Cardinium* RNA was established. RNA will be isolated from 1 - 3 day old male and female parasitoid wasps (*Encarsia pergandiella*). A symbiont-host RNASeq protocol already established for the amoeba symbiont *Amoebophilus asiaticus*, the closest relative of *Cardinium*, will be used. The metatranscriptome of *Cardinium* and *Encarsia pergandiella* will be analyzed, focusing on the expression of potential candidate proteins for CI and eukaryotic cell cycle regulation (e.g. ankyrin repeats, the putative antifeeding prophage secretion system or interference with the host ubiquitin system). We expect that the identification of sex-specific expression of CI-candidate genes and the comparison of *Cardinium* genomes causing different phenotypes will reveal the first insights into the reproductive manipulation of these elusive endosymbionts.

Widespread presence of *Wolbachia* in an Alpine population of the viviparous leaf beetle *Oreina cacaliae* (Coleoptera: Chrysomelidae)

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Oreina cacaliae (Coleoptera: Chrysomelidae) is a rare example of viviparous insect, able to feed on toxic plants and sequester toxic compounds. Here we present the results of a study on the microbiota associated with *O. cacaliae*, based on 16S rRNA bacterial gene sequencing. *Wolbachia* resulted as the dominant bacterium, both in males (100%) and in females (91.9%). Based on multilocus sequence typing, the detected *Wolbachia* was described as a new sequence type (*Wolbachia* Ocac_A_*w*VdO). Phylogenetic analyses assigned *Wolbachia* Ocac_A_*w*VdO to supergroup-A. In situ hybridization and electron microscopy confirmed the presence of *Wolbachia* within *O. cacaliae* oocytes, indicating its transovarial transmission. PCR specific for *Wolbachia* was performed on representatives of six species of *Oreina*; the presence / absence of *Wolbachia* was then mapped on a cladogram representing the phylogeny of the insect host. Finally, since viviparous species of *Oreina* were either infected or non-infected by *Wolbachia*, we cannot derive any conclusion about the possibility that this symbiont played some role in the evolution of viviparity.

Oxidative homeostasis and the evolution of insect / *Wolbachia* symbioses

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Reactive oxygen species (ROS) play a crucial role in insect immune defence. They also contribute to oxidative stress, a state that can lead to severe damages to cell components. Because of such antagonist effects, the oxidative homeostasis has been recently recognized as a key factor underlying trade-offs between life-history traits. Since the immune processes of insects can be triggered against any type of microorganism, a disturbance of oxidative homeostasis is expected to occur following the establishment of a symbiosis. In cases where the symbiont is highly prevalent, this disturbance is likely to constrain the host's life-history and could drive its adaptation to the symbionts (tolerance). An interaction between oxidative stress and symbiosis has been shown in associations involving *Wolbachia*. As a dramatic example, the dependence of *Asobara tabida* (Hymenoptera) to *Wolbachia* for oogenesis may be due to a breakdown of oxidative homeostasis resulting from tolerance evolution. This scenario implies both that the infection is costly for the host, and that a modulation of oxidative homeostasis can lessen this cost. We addressed these questions using *Drosophila melanogaster*, where *Wolbachia* infection is facultative, as a model. In order to assess the phenotypic effects of the infections, we measured the life-history traits of three strains of *D. melanogaster* differing only by their infection status: uninfected or infected by either *w*Mel or *w*MelPop. Experimental manipulations of the oxidative environment differentially affect the life-history traits of the different strains, suggesting that the phenotypic effects of *Wolbachia* can be altered by a modulation of oxidative homeostasis. *Wolbachia* density measurements by qPCR suggest that this alteration is achieved by two independent means, one, but not the other, being related to symbiont density. These results suggest that oxidative homeostasis may often be a target of selection following symbiosis establishment.

Transinfection of *Aedes vexans* with *w*AlbB *Wolbachia* by adult microinjection

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Aedes (*Aedimorphus*) *vexans* (Meigen) is a mosquito species widely distributed all over the northern hemisphere but also present in various parts of Africa, Central America, Australia and the Pacific. In Europe, it can be considered one of the most common mosquito species. It is a known vector of various viruses among which West Nile, Rift Valley Fever, Eastern and Western Equine Encephalitis, Tahyna. Common to other species of *Aedes*, *Ae. vexans* may play a significant role in virus enzootic cycles, due to the ascertained capacity to transmit transovarially certain viruses, maintaining them in nature during inter-epidemic periods. West Nile and RVFV have already shown their potential to establish and spread in newly infected areas. Due its abundance and high potential as vector, *Ae. vexans* could be a key species in determining new outbreaks outside the present geographical range of these important viruses.

Certain *Wolbachia* strains are known to interfere with pathogen transmission causing the viremia titer in the infected hosts to not reach levels compatible with the infection of potential vectors.

Wolbachia transinfection is considered a valuable tool to attempt the production in laboratory of lines with a desired phenotype and having the potential to spread in the wild populations thanks to the driving force of the CI phenomenon. This strategy may lead to the replacement of vector populations with populations with reduced vector competence reducing the risk of epidemics of important diseases. Unfortunately, not all the vector species are susceptible to *Wolbachia* transinfection.

In this work, we report about the use of three different *Wolbachia* strains, *w*AlbA and *w*AlbB from *Aedes albopictus* (Skuse) and *w*Mel from *Drosophila melanogaster* (Meigen) to transinfect *Ae. vexans* (naturally uninfected), by embryonic and adult transinfection. Transinfected adults were obtained by the latter method. *w*AlbB infection was transmitted vertically and established.

Symbiont gene responsible for pest status of insect host

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Understanding insect genotypes that increase or decrease pest status against agricultural plants is not only of evolutionary interest but also of applied importance. Stinkbugs of the *Megacopta cribraria-punctatissima* species complex, which are widely distributed across the East Asian region and recently invading the North America, naturally live on kudzu plants *Pueraria* spp. and often infest and damage soybean plants. Our previous study showed that *M. punctatissima* distributed in the mainland Japan successfully utilizes soybean plants whereas *M. cribraria* distributed in south-western islands of Japan does not perform well on soybean plants. Symbiont exchange experiments revealed that their gut symbiotic bacteria *Ishikawaella*, rather than the insects themselves, determine the different pest status of the insect hosts on soybean. Here we report comparative analyses of *Ishikawaella* genomes associated with these two species to determine symbiont genes relevant to the pest status. We found that these symbiont genomes are quite similar: in total, 173 site differences were identified, which consisted of base substitutions and small indels. Notably, open reading frames for two bacterial genes encoding Ary (arylesterase) and Era were disrupted only in the *Ishikawaella* genome associated with *M. cribraria*. When these two symbiont genes were genotyped in geographical populations of *M. cribraria-punctatissima* complex across the Japanese Archipelago, three types were identified: both Ary and Era are intact; Ary is intact and Era is disrupted; and both Ary and Era are disrupted. When insects from six Japanese populations, whose symbionts exhibit these different genotypes, were reared on soybean plants, their performance was correlated with intact / disrupted Ary genotype. Our finding provides insights into the mechanism and the evolutionary origin of insect pests, potentially leading to novel applications.

Wolbachia diversity between host phenotype and phylogenetic incongruence between *Wolbachia* and WO phage in *Vollenhovia emeryi*

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The *Vollenhovia emeryi* ant, widely distributed in Far East, shows queen polymorphism associated with *Wolbachia* infection status. The phylogenetic analysis of the ant's mtDNA reveals derivation of the *Wolbachia*-free short-winged from the *Wolbachia*-infected long-winged. However, intriguingly, some Japanese short-winged colonies harbor *Wolbachia*. *Wolbachia* specific bacteriophage (WO) is also detected in more than half of the infected colonies with no clear distribution pattern across the host insect lineage. We hypothesized that 1) the infected Japanese short-winged is in the intermediate stage to complete loss of *Wolbachia* and 2) the phage invaded the host after the host insect diverged. To test the hypotheses, we studied the strain diversity using the multi-locus sequence typing (MLST) of five ant colonies; three long-winged colonies from Korea and one long-winged colony and one short-winged colony from Japan. Both Korean and Japanese *V. emeryi* colonies show unexpectedly high levels of *Wolbachia* strain diversity. However, the diversity is not significantly different between the long-winged and the short-winged against our first hypothesis. Phylogenies of *Wolbachia* show Korean strains and Japanese strains are largely monophyletic indicating prior infection before the host divergence. The strain diversity of the phage is also surprisingly high. Phylogenies of orf2 and orf7 genes are incongruent to that of *Wolbachia* and geographically distinct. This indicates that the phage is spatially static and the current infection pattern may be the consequence of local repeated gain and loss of the phage.

What does the influence of *Wolbachia* on the testis stem cell niche reveal on the impact of the bacteria on the *Drosophila melanogaster* male biology?

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Stem cells and their niches are in charge of tissue homeostasis and as such constitute sensitive sentinels of the physiology of an organism. In order to study with precision how physiology affects a stem cell niche, we have developed a quantitative mathematical strategy based on the characterization of the niche cellular composition at the single niche level. By applying this strategy to the testis stem cell niche, we have discovered that infection of *D. melanogaster* with *w*MelCS affected significantly the testis stem cell niche homeostasis in just eclosed adult males. *Wolbachia* having a particular tropism for the gonad stem cell niches in several *Drosophila* species, its impact on these niches might be local. We provide evidences suggesting that local impact is unlikely to fully explain our observations in young *D. melanogaster* males. Indeed, simple manipulations of the social environment of *Wolbachia*-infected males during their metamorphosis are sufficient to restore a testis niche homeostasis comparable to the one observed in control males. In contrast, restoration of the niche homeostasis is precluded in olfactive dead mutant males infected with *w*MelCS. Finally, we show that the influence of social environment on *Wolbachia*-infected males during their metamorphosis is not restricted to the testis tissue and has systemic consequences, both at the eclosion time and the fertility levels.

The history and ongoing invasion of *Wolbachia* in European *Rhagoletis cerasi* populations

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Wolbachia is a widespread reproductive parasite of insects that can impact host ecology and evolution. The European cherry fruit fly, *Rhagoletis cerasi*, is infected by up to five distinct *Wolbachia* strains. The most prevalent strains are *w*Cer1, fixed in all populations, and *w*Cer2, currently invading European populations by inducing cytoplasmatic incompatibility.

Here we analyze the spatial distribution of these two *Wolbachia* strains in European *R. cerasi* populations and compare it with the mitochondrial and nuclear genotypes of its host. We found heavily reduced host mitochondrial diversity, indicative for two sequential selective sweeps caused by the current *w*Cer2 and the former *w*Cer1 invasion. While both infections are associated with specific mitochondrial haplotypes, the analysis of seven microsatellite loci showed no *Wolbachia* linked nuclear structure. Detailed population data over a time period of ten years suggests that *w*Cer2 is rarely lost from infected populations; occasional intraspecific horizontal transmission events were detected in the transition zone in central Germany, however, without disrupting the linkage of *Wolbachia* and mitochondrial haplotypes outside this region. Based on our combined dataset we discuss the evolutionary history of *Wolbachia* in *R. cerasi*.

Wolbachia as incompatibility factor between *Rhagoletis* populations from North America and Mexico

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Wolbachia is probably the most widespread endosymbiont, infecting a broad range of arthropod species. This bacterium can have significant influences in the biology of its host by influencing its reproduction. The most common mechanism is the induction of a cytoplasmic incompatibility that can lead to a postzygotic reproductive isolation among different *Wolbachia* infected populations.

Rhagoletis is a serious pest in orchards infesting various different fruits mainly in North America and Mexico. The most famous member of this genus is the apple maggot *Rhagoletis pomonella*. Naturally infesting hawthorns, a population shifted to domesticated apples as a new host. This resulted in the formation of an ecologically and genetically distinct host race, which acts as a textbook example of sympatric speciation. Additionally, recent studies have shown that *R. pomonella* formed at least two genetically isolated populations in Mexico.

In contrast, speciation in the *R. cingulata* species group appears to have formed by a variety of different modes: cherry-infesting *R. cingulata* and *R. indifferens* are diverging by classic allopatric (geographic) speciation, while *R. osmanthi* and *R. chionanthi* formed sympatrically, by adapting to different hosts. Similar to *R. pomonella*, *R. cingulata* also has isolated populations in Mexico.

Mating studies crossing North American *R. pomonella* and *R. cingulata* populations with flies from Northern and Central Mexico have revealed a degree of postzygotic reproductive isolation. In this presentation, we examine the cause of the incompatibility by studying their *Wolbachia* infections and discuss the possible influence of this bacteria on the evolution of reproductive isolation for *Rhagoletis* flies.

Pharmacokinetic / pharmacodynamic modelling of *Wolbachia* growth dynamics under antibiotic drug pressure in a lymphatic filariasis murine infection model

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Wolbachia is an essential endosymbiont of the filarial nematodes *Wuchereria bancrofti*, *Brugia malayi* and *Onchocerca volvulus* which are the causative agents of lymphatic filariasis and onchocerciasis. An estimated 120 million are infected by lymphatic filariasis and 17.7 million infected by onchocerciasis throughout the tropics leading to a profound public health and socio-economic burden in severely affected communities.

Targeting of *Wolbachia* in these filarial nematodes using antibiotic chemotherapy has been shown to deliver a safe macrofilaricidal activity and reduce filarial burden, morbidity and transmission. The current gold standard anti-*Wolbachia* drug regimen is a 100 - 200 mg/day doxycycline dose given for four to six weeks. The A•WOL consortium funded by a grant awarded to Liverpool School of Tropical Medicine by the Bill & Melinda Gates Foundation aims to reduce the current treatment time to seven days or less as aligned with the Target Product Profiles for an anti-*Wolbachia* macrofilaricide.

To achieve a rapid seven-day or less kill rate of *Wolbachia*, a number of antibiotic combinations will be optimised. These include the tetracyclines (doxycycline and minocycline), rifamycins (rifampicin and rifapentine), and a fluoroquinolone (moxifloxacin) as well as combinations with anti-helminthic drugs. Our rational approach involves identifying optimal treatments in *in-vivo* models and translation from the lab into clinical field trials.

We have initially studied the dynamics of *Wolbachia* under drug pressure with doxycycline, minocycline, moxifloxacin, and rifampicin in the AWOL validated mouse *Brugia malayi* infection model. Using experimental pharmacokinetic (PK) and pharmacodynamic (PD) data we have constructed a series of PK-PD models and simulations to further dissect and quantify the dynamics of *Wolbachia* growth.

This data analysis displays the power of PK-PD modelling in quantifying the dynamics of *Wolbachia* growth under antibiotic drug pressure.

Investigation of a candidate *Wolbachia* type IV effector from strain *w*Ana provides insight into a mechanism for host manipulation

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Wolbachia comprises a group of phylogenetically diverse, obligately intracellular bacteria famous for manipulating a broad range of invertebrate hosts. The molecular tools that are used by these bacteria in order to control host cell biology are currently unknown. From genomic sequencing of various *Wolbachia* strains, we know that the bacteria encode a complete, type IV secretion system that likely secretes 'effectors' into host cells during infection. We utilized a combination of high-throughput screening in yeast and bioinformatics to identify and characterize a potential effector from *Wolbachia* that infects *Drosophila ananassae*. We focused on this protein because our analysis revealed that it contains a domain homologous to those found in plant apoptosis machinery. Transcripts of the protein were found in infected, but not uninfected, *D. ananassae* ovaries. Using a custom antibody targeting the putative *Wolbachia* effector, we were able to show that the full-length protein specifically expressed across developmental stages of infected flies. Additionally, we investigated the presence of the protein in cytosolic fractions of infected host cells, a property necessary for secreted effectors. We conducted a yeast-two-hybrid screen to identify interaction partners revealing interactions with important members of the host's oxidative stress response pathways. Because *Wolbachia* are known to manipulate ROS levels in their hosts, we predict that these secreted proteins may be involved in altering host cell redox state.

In vitro and *in vivo* assays for the assessment of cytoplasmic competence of *w*Di from the Asian citrus psyllid, *Diaphorina citri*, with other *Wolbachia* strains

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To assess how different strains of *Wolbachia* compete when occupying the same cells, superinfections of various *Wolbachia* strains were established in the same cultured cells and insects. The most prevalent *Wolbachia* strain of the Asian citrus psyllid, *Diaphorina citri*, *w*Di (Supergroup B) was transinfected into the *Bombyx mori* Bm5 cell line starting with newly laid eggs. *Wolbachia* from the Caribbean fruit fly, *Anastrepha suspensa*, *w*Asu (Supergroup A), the Mediterranean flour moth, *Ephestia kuehniella*, *w*Eku (Supergroup A), *w*Mel (Supergroup A) and *w*AlbB (Supergroup B) were also established in Bm5 cells. To test interactions between *w*Di and other *Wolbachia* strains, dual-infections between Bm5/*w*Di and the other *Wolbachia* strains were made. The dynamics of the dual-infections were followed using qRT-PCR to measure the copy number of each strain relative to the Bm5 host genome. Dual-infections between *w*Di and the other strains resulted in extensive Bm5 cell death within seven days.

Purified *Wolbachia* strains from the cultured cell lines were used to establish single infections in *Drosophila simulans*. Establishment of the *Wolbachia* infection was confirmed by direct PCR using strain specific primers. The single infected *D. simulans w*Di strain was cross-mated with each of the other single infected *D. simulans* strains to establish superinfections to assess cytoplasmic incompatibility (CI) effects. The ratio of the co-infecting *Wolbachia* was monitored by qRT-PCR. Studies established CI effects in cross-mated flies with each singular *Wolbachia* infected strain. We discuss the potential of utilizing other *Wolbachia* strains to provide CI biological drive in the Asian citrus psyllid.

Wolbachia mediated protection against pathogenic bacteria in two isopod models

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Symbionts largely affect their hosts' life history traits and fitness. Previous studies showed that vertically transmitted symbionts can protect the host against pathogens. Among this symbionts, the *Wolbachia* have recently been shown to confer protection to their hosts against a wide range of pathogens, especially viruses. The present study aims to test whether resident *Wolbachia* could confer protection to terrestrial isopods against invasive intracellular bacteria (*Salmonella typhimurium*, *Listeria ivanovii* but also the pathogenic *Wolbachia* strain *w*VulC). To do so, we assessed the survival during bacterial infections of *Armadillidium vulgare* and *Porcellio d. dilatatus* isopods when they are symbiotically associated or not with resident *Wolbachia*. We showed that when feminizing and CI *Wolbachia* have an effect on the survival of their hosts during the bacterial infection, this effect is always beneficial to the host. However, the intensity of the "anti-pathogenic" effect is clearly dependent on the *Wolbachia* strain and the genetic background of the host.

Insights into the evolution of *Cardinium*: the development of a Multi Locus Sequence Typing system

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Cardinium is an intracellular reproductive manipulator in the phylum Bacteroidetes that is found in many different species of arthropods. This independently-evolved symbiont lineage exhibits three of the four reproductive phenotypes attributed to *Wolbachia*, including cytoplasmic incompatibility, long thought to be unique to *Wolbachia*-infected hosts. Genetic and phylogenetic analyses of *Cardinium* in comparison with *Wolbachia* offer an opportunity to determine functional equivalents and differences between the two lineages. Placing individual strains of *Cardinium* within a larger evolutionary context is currently challenging because only two genes (16S rDNA and Gyrase B) have been used to generate phylogenetic trees, and consequently, the relationship of these different strains has only been elucidated in its roughest form. The development of a Multi Locus Sequence Typing (MLST) system would provide the research community with new primers for detecting Cardinium, unambiguous methods of delineating strains, and more informative phylogenetic trees. I am in the process of designing universal *Cardinium* PCR primers for four different genes: Translation Elongation Factor G (EF-G), Heat Shock Protein (groEL), Iron Sulfur Cluster Assembly Protein (sufB), and Gyrase B (gyrB). These will be used to identify, describe, and place strains of *Cardinium* into a phylogenetic tree. Based on my preliminary tree, *Cardinium* appears to display more host affinity than *Wolbachia*. However, similar to *Wolbachia*, the reproductive manipulations are dispersed across the tree, and thus have likely evolved independently multiple times. To date, this is the most robust tree available for investigating *Cardinium* evolution.

The role of the host in *Wolbachia*-virus interactions

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There is growing empirical evidence that *Wolbachia* (*WB*) directly interact with viruses inside the arthropod host, sometimes resulting in low or no virus replication. Previous theoretical studies showed that this direct effect of *WB* can result in reduced virus prevalence (within the population), suggesting that *WB* could be used in the biological control of vector-borne diseases (e.g., dengue fever). However, most of the models focus on *WB*-virus interactions, whereas the potential roles of the host are overlooked. In order to fill this gap, we investigated two questions through mathematical modeling. (1) How is host resistance against the virus affected by *WB*? We investigated the tripartite interactions between *WB*, virus, and host by a model that incorporates (i) horizontal virus- and vertical *WB*-transmission, (ii) cytoplasmic incompatibility (CI), (iii) *WB* resistance against virus, and (iv) host resistance against virus. Our main finding is that the spread of *WB* causes the loss of costly host resistance alleles. We discuss implications of this loss of genetic diversity with respect to vector-borne diseases. (2) How does the host's life history affect *WB*-virus codynamics? We consider a scenario, in which *WB* do not affect virus replication inside coinfected hosts. However, *WB* might indirectly affect the virus because reproductive phenotypes (CI or male killing) increase larval mortality of hosts and thus alter virus dynamics. Our analysis revealed that this indirect effect depends strongly on the host's life history, and can result in two opposing outcomes: (i) reduced virus prevalence and virus invasion ability, and (ii) increased virus prevalence and virus invasion ability. The former occurs for host species with larval competition and undercompensation, the latter for hosts with either adult competition or larval competition and overcompensation. These findings suggest that the effect of *WB* on a specific virus is sensitive to the host's life history.

A male-killing *Wolbachia* affects the dosage compensation of its lepidopteran host?

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Wolbachia, a group of endosymbiotic bacteria harbored by a wide range of insects, is known for various manipulations of host reproduction to expedite their own propagation. *Wolbachia* (*w*Sca) infecting the Adzuki bean borer moth *Ostrinia scapulalis* (Lepidoptera: Crambidae) causes male killing, in which males (genotype ZZ) selectively die during embryonic and early larval development, whereas females (genotype ZW), in turn, selectively die when cured of infection.

In a previous study, we analyzed phenotypic sex (sex determination gene expression pattern, male-type or female type) and genetic sex (sex chromosome type, ZZ or ZW) of the embryos and larvae of normal, *w*Sca-infected, and infected-and-cured *O. scapulalis*. As the results, it was observed that the female-type sex-determination gene was expressed in the infected genetic male (ZZ) progenies destined to die, whereas the male-type sex-determination gene was expressed in the cured genetic female (ZW) progenies destined to die. This result suggested the discordance of the genetic and phenotypic sexes underlie the sex-specific death, but the mechanism of death is unresolved. One plausible explanation for sex-dependent lethality may be discordance in dosage compensation, i.e. sex-dependent adjustment of the expression levels of genes on the sex chromosomes.

So, we focused on the dosage compensation and analyzed the Z chromosome-linked gene expression by quantitative PCR. For this conference, we will describe the result of comparison experiment of Z chromosome-linked gene expression in the embryos and larvae of normal, *w*Sca-infected, and infected-and-cured *O. scapulalis*.

Reproductive parasitism and gene flow modification

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The term reproductive parasitism is commonly used to describe infective agents that manipulate the reproductive system of their host to their own advantage. Among the reproductive manipulations are feminization, male killing, and cytoplasmic incompatibility. Well-studied reproductive parasites belong to the bacterial groups *Wolbachia*, *Rickettsia*, *Cardinia*, *Arsenophonus*, and *Spiroplasma*. The wide distribution of these bacterial groups among arthropods makes the study of reproductive parasitism an important topic in evolution. The present (theoretical) study is motivated by the (experimental) findings that different host populations often differ with respect to their infection state, i.e. some populations are infected while others are not or with a different strain. Based on these findings we constructed several population genetic models in order to investigate how such infection polymorphism affect the gene flow between populations. To measure gene flow we make use of the concept of effective migration rate. Our analysis revealed that, generally, infection with reproductive parasites modify host gene flow. Interestingly, populations with highest degree of infection are thereby converted into population genetic sinks. This is true for all forms of reproductive parasitism. These results give general insights to the evolution of selfish genetic elements. We discuss the implications with respect to host evolution and host-parasite coevolution, especially speciation and local adaptation.

Host plant specialization matters in the epidemiology of *Wolbachia* across phytophagous wasps (Hymenoptera: Torymidae)

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Among eukaryotes, sexual reproduction is by far the most predominant mode of reproduction. However, some systems maintaining sexuality appear particularly labile and raise intriguing questions on the evolutionary routes to asexuality. Thelytokous parthenogenesis is a form of spontaneous loss of sexuality leading to strong distortion of sex ratio towards females and resulting from mutation, hybridization or infection by bacterial endosymbionts. We investigated whether ecological specialization is a likely mechanism of spread of thelytoky within insect communities. Focusing on the highly-specialized genus *Megastigmus* (Hymenoptera: Torymidae), we first performed a large literature survey to examine the distribution of thelytoky in these wasps across their respective obligate host plant families. Second, we tested for thelytoky caused by endosymbionts by screening in 15 arrhenotokous and 10 thelytokous species for *Wolbachia*, *Cardinium*, *Arsenophonus* and *Rickettsia* endosymbionts and by performing antibiotic treatments. Finally, we performed phylogenetic reconstructions using multilocus sequence typing (MLST) to examine the evolution of endosymbiont-mediated thelytoky in *Megastigmus* and its possible connections to host plant specialization. We demonstrate that thelytoky evolved from ancestral arrhenotoky through the horizontal transmission and the fixation of the parthenogenesis-inducing *Wolbachia*. We find that ecological specialization in *Wolbachia*'s hosts was probably a critical driving force for *Wolbachia* infection and spread of thelytoky, but also a constraint. Our work further reinforces the hypothesis that community structure of insects is a major driver of the epidemiology of endosymbionts and that competitive interactions among closely related species may facilitate their horizontal transmission.

Wolbachia infection in Polish *Liophloeodes* (Coleoptera:Curculionidae) populations: pathways of transfer and influence on host reproduction

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Wolbachia routes of transmission are one of most widely discussed problem applying to this bacterium. Although significant role of vertical transfer in spreading of *Wolbachia* is confirmed, frequent incongruence between phylogenies of the bacterium and host indicates that also horizontal transmission is important. Good way to find out routes of *Wolbachia* movement among hosts from particular systematic group is to compare phylogeny of endosymbiont with host phylogeny (similarities pinpoints vertical transfer) and host ecology and geography (similarities suggest horizontal transfer).

About 40% of European weevil species are estimated to be infected with *Wolbachia*. Among them are two species from *Liophloeodes* subgenus *L. lentus* and *L. gibbus*. Both of them are Carpathian subendemic species with similar ecological preferences. In Poland there are many populations of these weevils and there are potential contact zones between divergent populations, that could enable vertical transfer.

The main goal of this research was to discover possible pathways of *Wolbachia* transfer among Polish populations of these two species. Weevils from 15 populations were tested. Their phylogeny (based on EF1-alpha gene) and geographical distribution were compared with phylogeny of their *Wolbachia* endosymbionts (based on ftsZ and wsp genes).

Wolbachia infections and vertical transfer cause many changes in host reproduction. These abnormalities often lead to discordances between mitochondrial and nuclear DNA phylogeny. In second part of the research, nDNA (EF1-alpha and ITS2) and mtDNA (COI and CytB) phylogenies of 20 Polish *Liophloeodes* populations were compared. There was made attempt to discover discordances that would indicate significance of vertical transfer.

Serious incongruence between mtDNA and nDNA phylogeny was found out, however *Wolbachia* phylogeny indicates great role of horizontal transfer among studied populations.

Combining Sterile Insect Technique with *Wolbachia*-based approaches: assessing effects on fitness of triple and double *Wolbachia*-infected strains of *Aedes albopictus*

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Together with other control methods, the Sterile Insect Technique (SIT) and the Incompatible Insect Technique (IIT) are currently being considered, separately or in combination, as potential approaches for the suppression of populations of the major vector mosquito species *Aedes albopictus* in Guangzhou, China. Natural populations of *Ae. albopictus* are double-infected with the *Wolbachia* strains *w*AlbA and *w*AlbB. By embryonic microinjections, a new triple *Wolbachia* infected line (*w*AlbA, *w*AlbB, and *w*Pip), HC line, has been developed which expresses strong Cytoplasmic Incompatibility (CI) in matings between the transinfected males and wild type females. In this study, we present the comparative analysis of the life history traits of three *Ae. albopictus* lines (triple-infected, double-infected and uninfected), all of them with the same ("Guangzhou") genomic background. Under the same rearing conditions, the egg-hatching rate, survivorship of pupae and adults, sex ratio, duration of larval stage (L1 to pupae stage), duration of adult stage (L1 to adult emergence), female fecundity and male longevity were investigated. Our results suggest a minimal effect of *Wolbachia* on the fitness of these populations. Based on this evidence, the triple-infected line is currently considered for mass rearing and the application of a combined SIT-IIT strategy to control *Ae. albopictus* populations in the Guangzhou area in China.

Knockdown of ATPsyn-b caused larval growth defect and male infertility in *Drosophila*

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ATPsyn-b gene is encoded *Drosophila* mitochondrial membrane F0F1-type ATPase F0 b subunit, involved in the process of oxidative phosphorylation and ATP synthesis, thus providing energy for *Drosophila* life activities. In this study, firstly Gal4-UAS system was adopt for acquiring flies lines with ATPsyn-b knock-down both in testis and extensive body respectively. Then, males embryo hatching were detected and showed they are infertile when ATPsyn-b knock-down occur in testis. Further study showed this male sterility was caused by abnormal spermatogenesis, such as sperm nuclei in cyst of mutant males were completely decentralized, thus failed to form normal sperm bundles. Moreover, individualized mature sperm were absent in seminal vesicles near the end of testis. Additionally, ATPsyn-b widely knock-down flies showed developmental stagnation just in the second instar larvae while the control larvae had developed into the third instar. Remarkably, ATPsyn-b knock-down individual were still remain as the size of the second instar larvae until control adult had been all emerged, and died finally. Combining the result of qRT-PCR, that proved the phenotype of arrested development was indeed caused by ATPsyn-b knock-down, which also show cell volume failed to increase proved by Brdu incorporation experiments. In short, our results proved that ATPsyn-b gene played an important role on both *Drosophila* spermatogenesis and larval development, which may help offer a new direction and clue for studying insect male infertility. Further deeper study on the mechanism of infertility were also needed.

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